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CHRONOLOGICAL LIST OF SESSIONS

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388. History of the brain. L. MARSHALL	1326
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1. Ion channels in neurobiology: an evolving molecular perspective. R. L. BARCHI	No abstract
MONDAY	
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3. Cerebral processes and conscious functions. *Chaired by:* B. LIBET	1
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187. Logistics of brain development. P. RAKIC	No abstract

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Theme A: Development and Plasticity

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2.	**Actions of nerve growth factor in the central nervous system**	**Symp.**	**Mon AM**
337.	Autonomic nervous system	Poster	Thu PM
177.	Biochemical and pharmacological correlates of development I	Poster	Tue PM
328.	Biochemical and pharmacological correlates of development II	Slide	Thu PM
354.	Biochemical and pharmacological correlates of development III	Poster	Thu PM
193.	Cell lineage: differentiation and development I	Slide	Wed AM
236.	Cell lineage: differentiation and development II	Slide	Wed PM
309.	Cell lineage: differentiation and development III	Poster	Thu AM
214.	Development and plasticity I: aging	Poster	Wed AM
261.	Development and plasticity II: aging	Poster	Wed PM
376.	Development and plasticity: motor systems	Poster	Fri AM
67.	Development and plasticity: normal and transplanted visual system	Poster	Mon PM
133.	Development and plasticity: olfactory system	Poster	Tue AM
8.	Development and plasticity: retina and optic nerve	Slide	Mon AM
134.	Development and plasticity: sensory system I	Poster	Tue AM
135.	Development and plasticity: sensory system II	Poster	Tue AM
141.	Development and plasticity: visual pathways I	Slide	Tue PM
235.	Development and plasticity: visual pathways II	Slide	Wed PM
298.	Development and plasticity: visual system plasticity	Poster	Thu AM
267.	Development of invertebrates I	Poster	Wed PM
282.	Development of invertebrates II	Slide	Thu AM
120.	Developmental disorders I	Poster	Tue AM
366.	Developmental disorders II	Slide	Fri AM
161.	Endocrine control of development I	Poster	Tue PM
262.	Endocrine control of development II	Poster	Wed PM
52.	Endocrine effects on development and behavior	Slide	Mon PM
388.	History of the brain	Poster	Mon AM
117.	Limbic system: development and plasticity I	Poster	Tue AM
151.	Limbic system: development and plasticity II	Slide	Tue PM
91.	**Molecular synaptogenesis: transition from growth cone to synapse**	**Symp.**	**Tue AM**
178.	Morphogenesis and pattern formation I	Poster	Tue PM
292.	Morphogenesis and pattern formation II	Poster	Thu AM
180.	Neural plasticity in adult animals I	Poster	Tue PM
225.	Neural plasticity in adult animals II	Poster	Wed AM
269.	Neural plasticity in adult animals III	Poster	Wed PM
284.	Neural plasticity in adult animals IV	Slide	Thu AM
318.	Neural plasticity in adult animals V	Poster	Thu AM
75.	Neuronal death	Poster	Mon PM
50.	Neurotoxicity I	Slide	Mon PM
159.	Neurotoxicity II	Poster	Tue PM
293.	Neurotoxicity III	Poster	Thu AM
350.	Neurotoxicity IV	Poster	Thu PM
333.	Neurotransmitters: phenotypic plasticity	Slide	Thu PM

23.	Nutritional and prenatal factors	Poster	Mon AM
57.	Process outgrowth: growth cones	Slide	Mon PM
102.	Process outgrowth: guidance mechanisms	Slide	Tue AM
174.	Process outgrowth: guidance mechanisms, growth cones	Poster	Tue PM
221.	Process outgrowth: molecular mechanisms	Poster	Wed AM
287.	Regeneration I	Slide	Thu AM
367.	Regeneration II	Slide	Fri AM
125.	Regeneration: neuronal and glial responses	Poster	Tue AM
175.	Regeneration: patterns and responses	Poster	Tue PM
74.	Regeneration: transplantation and modulatory factors	Poster	Mon PM
22.	Specificity of synaptic connections I	Poster	Mon AM
288.	Specificity of synaptic connections II	Slide	Thu AM
266.	Sprouting and sprouting mechanisms	Poster	Wed PM
31.	Synapse elimination and competition	Poster	Mon AM
30.	Synaptogenesis and synapse elimination I	Poster	Mon AM
51.	Synaptogenesis and synapse elimination II	Slide	Mon PM
200.	Transmitters: phenotypic plasticity	Slide	Wed AM
197.	Trophic agents I	Slide	Wed AM
271.	Trophic agents II	Poster	Wed PM
272.	Trophic agents: nerve growth factor	Poster	Wed PM
313.	Trophic agents: neurotrophic factors	Poster	Thu AM
279.	Trophic interactions I	Slide	Thu AM
335.	Trophic interactions II	Poster	Thu PM

Theme B: Cell Biology

Session Number	Session Title	Type	Day and Time
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381.	Axonal transport II	Poster	Fri AM
230.	**Biology of isolated brain capillary endothelia: an emerging field in neuroscience**	**Symp.**	**Wed PM**
202.	Blood brain barrier I	Slide	Wed AM
246.	Blood brain barrier II	Poster	Wed PM
319.	Cell surface macromolecules	Poster	Thu AM
4.	Cellular aspects of disease: CNS proteins	Slide	Mon AM
380.	Cellular aspects of disease: nerve, muscle	Poster	Fri AM
26.	Functions of glia I	Poster	Mon AM
119.	Functions of glia II	Poster	Tue AM
291.	Functions of glia III	Slide	Thu AM
224.	Glial and neuronal membrane composition I	Poster	Wed AM
331.	Glial and neuronal membrane composition II	Slide	Thu PM
118.	Metabolic studies	Poster	Tue AM
46.	Molecular biology of gene expression and nucleic acids I	Slide	Mon PM
108.	Molecular biology of gene expression and nucleic acids II	Poster	Tue AM
233.	Molecular biology of gene expression and nucleic acids III	Slide	Wed PM
316.	Molecular biology of gene expression and nucleic acids IV	Poster	Thu AM
324.	Molecular biology of gene expression and nucleic acids V	Slide	Thu PM
130.	Staining and tracing techniques	Poster	Tue AM
77.	Structure and function of identified cells I	Poster	Mon PM
184.	Structure and function of identified cells II	Poster	Tue PM

Theme C: Excitable Membranes, Transduction and Synaptic Transmission

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48.	Action potentials and ion channels I	Slide	Mon PM
95.	Action potentials and ion channels II	Slide	Tue AM
158.	Action potentials and ion channels III	Poster	Tue PM
226.	Action potentials and ion channels IV	Poster	Wed AM
227.	Action potentials and ion channels V	Poster	Wed AM
231.	Action potentials and ion channels VI	Slide	Wed PM
281.	Action potentials and ion channels VII	Slide	Thu AM
346.	Action potentials and ion channels VIII	Poster	Thu PM
148.	CNS neurons: vertebrate and invertebrate I	Slide	Tue PM
155.	CNS neurons: vertebrate and invertebrate II	Poster	Tue PM
176.	Drug effects on receptors	Poster	Tue PM
49.	Pharmacology of synaptic transmission I	Slide	Mon PM
247.	Pharmacology of synaptic transmission II	Poster	Wed PM
295.	Pharmacology of synaptic transmission III	Poster	Thu AM
142.	Postsynaptic mechanisms I	Slide	Tue PM
249.	Postsynaptic mechanisms II	Poster	Wed PM
250.	Postsynaptic mechanisms III	Poster	Wed PM
13.	Presynaptic mechanisms I	Slide	Mon AM
157.	Presynaptic mechanisms II	Poster	Tue PM
248.	Presynaptic mechanisms III	Poster	Wed PM
329.	Sensory transduction	Slide	Thu PM
88.	Synaptic structure and function I	Poster	Mon PM
192.	Synaptic structure and function II	Slide	Wed AM

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Session Number	Session Title	Type	Day and Time
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289.	Acetylcholine II	Slide	Thu AM
360.	Acetylcholine III	Poster	Thu PM
317.	Acetylcholine: receptors	Poster	Thu AM
44.	**Behavioral functions of cholecystokinin in the central nervous system**	**Symp.**	**Mon PM**
348.	Behavioral pharmacology: adrenergic and histaminergic systems	Poster	Thu PM
201.	Behavioral pharmacology: aminergic systems	Slide	Wed AM
378.	Behavioral pharmacology: cholinergic systems	Poster	Fri AM
377.	Behavioral pharmacology: GABA and anxiolytics	Poster	Fri AM
349.	Behavioral pharmacology: neuroleptics and dopamine	Poster	Thu PM
374.	Behavioral pharmacology: phencyclidine and opiates	Poster	Fri AM
347.	Behavioral pharmacology: serotonin	Poster	Thu PM
243.	Catecholamines	Slide	Wed PM
139.	Catecholamines: dopamine receptors I	Slide	Tue PM
211.	Catecholamines: dopamine receptors II	Poster	Wed AM
260.	Catecholamines: dopamine receptors III	Poster	Wed PM
127.	Catecholamines: effects of MPTP	Poster	Tue AM
312.	Catecholamines: electrophysiology	Poster	Thu AM
311.	Catecholamines: morphology and electrophysiology	Poster	Thu AM
94.	Catecholamines: receptors I	Slide	Tue AM
305.	Catecholamines: receptors II	Poster	Thu AM

153.	Catecholamines: release of dopamine	Poster	Tue PM
353.	Catecholamines: synthesis and metabolism	Poster	Thu PM
352.	Catecholamines: tyrosine hydroxylase	Poster	Thu PM
28.	Characterization of cholinergic receptors I	Poster	Mon AM
29.	Characterization of cholinergic receptors II	Poster	Mon AM
55.	Characterization of cholinergic receptors III	Slide	Mon PM
195.	Characterization of cholinergic receptors IV	Slide	Wed AM
27.	Coexistence of transmitters	Poster	Mon AM
238.	Cyclic nucleotides I	Slide	Wed PM
315.	Cyclic nucleotides II	Poster	Thu AM
362.	**Estrogen and the nigrostriatal dopamine system**	**Wksh.**	**Fri AM**
241.	Excitatory amino acids: glutamate and glutamate analogs	Slide	Wed PM
32.	Excitatory amino acids: physiology and pharmacology	Poster	Mon AM
33.	Excitatory amino acids: receptor characterization and localization	Poster	Mon AM
6.	GABA and benzodiazepines: cellular and behavioral effects	Slide	Mon AM
81.	GABA and benzodiazepines: electrophysiology	Poster	Mon PM
79.	GABA and benzodiazepines: pharmacology	Poster	Mon PM
80.	GABA and benzodiazepines: receptor characterization and localization I	Poster	Mon PM
326.	GABA and benzodiazepines: receptor characterization and localization II	Slide	Thu PM
82.	GABA and benzodiazepines: release and localization	Poster	Mon PM
104.	Interactions between neurotransmitters I	Slide	Tue AM
170.	Interactions between neurotransmitters II	Poster	Tue PM
217.	Interactions between neurotransmitters III	Poster	Wed AM
131.	Metabolism of transmitters and modulators I	Poster	Tue AM
240.	Metabolism of transmitters and modulators II	Slide	Wed PM
171.	Modulators I	Poster	Tue PM
218.	Modulators II	Poster	Wed AM
277.	**Molecular insights into GABA-receptor function**	**Symp.**	**Thu AM**
43.	Neurotransmitters and receptors in disease	Poster	Mon AM
325.	Neurotransmitters and receptors in disease: MPTP model of Parkinson's disease	Slide	Thu PM
387.	Neurotransmitters and receptors in epilepsy	Poster	Fri AM
42.	Neurotransmitters and receptors in human disease	Poster	Mon AM
336.	Opiates, endorphins and enkephalins: anatomical localization	Poster	Thu PM
116.	Opiates, endorphins and enkephalins: biochemical characterization	Poster	Tue AM
93.	Opioid receptors I	Slide	Tue AM
173.	Opioid receptors II	Poster	Tue PM
220.	Opioid receptors III	Poster	Wed AM
143.	Opioids: physiological studies I	Slide	Tue PM
265.	Opioids: physiological studies II	Poster	Wed PM
310.	Opioids: physiological studies III	Poster	Thu AM
351.	Opioids: physiological studies IV	Poster	Thu PM
18.	Other biogenic amines I	Poster	Mon AM
76.	Other biogenic amines II	Poster	Mon PM
188.	**Peptide-amine interactions in the neural regulation of cell function**	**Symp.**	**Wed AM**
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204.	Peptides: anatomical localization III	Poster	Wed AM
149.	Peptides: biosynthesis and metabolism I	Slide	Tue PM
169.	Peptides: biosynthesis and metabolism II	Poster	Tue PM
7.	Peptides: receptors I	Slide	Mon AM
124.	Peptides: receptors II	Poster	Tue AM
210.	Physiological effects of peptides I	Poster	Wed AM
259.	Physiological effects of peptides II	Poster	Wed PM

285.	Physiological effects of peptides III	Slide	Thu AM
223.	Receptor modulation: up and down regulation I	Poster	Wed AM
237.	Receptor modulation: up and down regulation II	Slide	Wed PM
268.	Receptor modulation: up and down regulation III	Poster	Wed PM
199.	Regional localization of receptors and transmitters I	Slide	Wed AM
263.	Regional localization of receptors and transmitters II	Poster	Wed PM
307.	Regional localization of receptors and transmitters III	Poster	Thu AM
368.	Serotonin	Slide	Fri AM
322.	**Towards a second generation understanding of neural circuits: the role of modulation**	**Symp.**	**Thu PM**
234.	Transmitter cytochemistry and immunohistochemistry I	Slide	Wed PM
253.	Transmitter cytochemistry and immunohistochemistry II	Poster	Wed PM
299.	Transmitter cytochemistry and immunohistochemistry III	Poster	Thu AM
99.	Transmitters in invertebrates I	Slide	Tue AM
147.	Transmitters in invertebrates II	Slide	Tue PM
273.	Transmitters in invertebrates III	Poster	Wed PM
106.	Uptake storage and secretion I	Slide	Tue AM
179.	Uptake storage and secretion II	Poster	Tue PM

Theme E: Endocrine and Autonomic Regulation

Session Number	Session Title	Type	Day and Time
251.	Adrenal medulla	Poster	Wed PM
61.	Cardiovascular regulation	Poster	Mon PM
150.	Cardiovascular regulation: bulbar mechanisms	Slide	Tue PM
242.	Cardiovascular regulation: diencephalic and cortical mechanisms	Slide	Wed PM
15.	Cardiovascular regulation: ganglionic and spinal mechanisms	Slide	Mon AM
294.	Cardiovascular regulation: hypertension	Poster	Thu AM
92.	**Coordination of respiratory and cardiovascular homeostasis**	**Symp.**	**Tue AM**
164.	Endocrine control	Poster	Tue PM
198.	Neural control of immune system I	Slide	Wed AM
252.	Neural control of immune system II	Poster	Wed PM
339.	Pineal gland	Poster	Thu PM
222.	Regulation of autonomic function	Poster	Wed AM
10.	Regulation of pituitary functions I	Slide	Mon AM
109.	Regulation of pituitary functions II	Poster	Tue AM
196.	Regulation of pituitary functions III	Slide	Wed AM
308.	Regulation of pituitary functions IV	Poster	Thu AM
379.	Regulation of pituitary functions V	Poster	Fri AM
334.	Respiratory regulation I	Slide	Thu PM
338.	Respiratory regulation II	Poster	Thu PM
363.	**Sensory mechanisms in sympathetic ganglia**	**Symp.**	**Fri AM**

Theme F: Sensory Systems

Session Number	Session Title	Type	Day and Time
72.	Auditory sensory organs and transduction	Poster	Mon PM
286.	Chemical sensory systems I	Slide	Thu AM
356.	Chemical sensory systems II	Poster	Thu PM
369.	Chemical sensory systems III	Slide	Fri AM
278.	**Effects of behavioral state and motor activity on sensory neuronal processing**	**Symp.**	**Thu AM**
53.	Invertebrate sensory processing	Slide	Mon PM

323.	**Origins of orientation selectivity in the mammalian visual cortex**	**Symp.**	**Thu PM**
41.	Pain modulation: central pathway mechanisms	Poster	Mon AM
38.	Pain modulation: human studies	Poster	Mon AM
83.	Pain modulation: intrathecal studies	Poster	Mon PM
345.	Pain modulation: mechanisms	Poster	Thu PM
39.	Pain modulation: peptides, monoamines	Poster	Mon AM
190.	Pain modulation: stimulation studies	Slide	Wed AM
40.	Pain modulation: stress analgesia	Poster	Mon AM
56.	Pain: central pathways I	Slide	Mon PM
123.	Pain: central pathways II	Poster	Tue AM
172.	Pain: central pathways III	Poster	Tue PM
70.	Retina and retinofugal projections	Poster	Mon PM
71.	Retina I	Poster	Mon PM
103.	Retina II	Slide	Tue AM
145.	Retina III	Slide	Tue PM
355.	Retina IV	Poster	Thu PM
14.	Sensory systems: auditory pathways I	Slide	Mon AM
73.	Sensory systems: auditory pathways II	Poster	Mon PM
215.	Sensory systems: auditory pathways III	Poster	Wed AM
306.	Sensory systems: auditory pathways IV	Poster	Thu AM
69.	Sensory systems: subcortical visual pathways I	Poster	Mon PM
96.	Sensory systems: subcortical visual pathways II	Slide	Tue AM
296.	Sensory systems: subcortical visual pathways III	Poster	Thu AM
9.	Sensory systems: visual cortex I	Slide	Mon AM
68.	Sensory systems: visual cortex II	Poster	Mon PM
194.	Sensory systems: visual cortex III	Slide	Wed AM
297.	Sensory systems: visual cortex IV	Poster	Thu AM
364.	Sensory systems: visual cortex V	Slide	Fri AM
36.	Somatic afferents I	Poster	Mon AM
37.	Somatic afferents II	Poster	Mon AM
35.	Somatic afferents: central pathways	Poster	Mon AM
219.	Somatosensory cortex	Poster	Wed AM
264.	Somatosensory system	Poster	Wed PM
66.	Spinal cord	Poster	Mon PM
11.	Subcortical somatosensory pathways I	Slide	Mon AM
168.	Subcortical somatosensory pathways II	Poster	Tue PM
229.	**Visual motion processing in cerebral cortex**	**Symp.**	**Wed PM**

Theme G: Motor Systems and Sensorimotor Integration

Session Number	Session Title	Type	Day and Time
62.	Basal ganglia: anatomical pathways	Poster	Mon PM
205.	Basal ganglia: electrophysiology and behavior	Poster	Wed AM
63.	Basal ganglia: immunocytochemistry	Poster	Mon PM
110.	Basal ganglia: immunocytochemistry and physiology	Poster	Tue AM
340.	Basal ganglia: Parkinsonism and its models	Poster	Thu PM
365.	Basal ganglia: substantia nigra	Slide	Fri AM
206.	Cerebellum: anatomy, pharmacology and cellular physiology	Poster	Wed AM
303.	Cerebellum: functional physiology	Poster	Thu AM
59.	Cerebellum: olivo-cerebellar circuitry	Slide	Mon PM
100.	Control of movement: arm and wrist	Slide	Tue AM
302.	Control of movement: locomotion	Poster	Thu AM
24.	Control of posture and movement I	Poster	Mon AM
209.	Control of posture and movement II	Poster	Wed AM
341.	Disorders of motor systems and neural prostheses	Poster	Thu PM

156.	Invertebrate motor function	Poster	Tue PM
373.	Motor systems and sensorimotor integration: cortex	Poster	Fri AM
84.	Motor systems: motor and sensory integration	Poster	Mon PM
64.	Muscle I	Poster	Mon PM
122.	Muscle II	Poster	Tue AM
25.	Oculomotor system I	Poster	Mon AM
144.	Oculomotor system II	Slide	Tue PM
304.	Oculomotor system III	Poster	Thu AM
137.	**Physiological and biochemical basis of fatigue in the motor units of mammals**	**Symp.**	**Tue PM**
65.	Reflex function I	Poster	Mon PM
208.	Reflex function II	Poster	Wed AM
12.	Spinal cord and brainstem I	Slide	Mon AM
121.	Spinal cord and brainstem II	Poster	Tue AM
258.	Spinal cord and brainstem III	Poster	Wed PM
301.	Spinal cord and brainstem IV	Poster	Thu AM
342.	Spinal cord injury and disorders	Poster	Thu PM
97.	Vestibular system I	Slide	Tue AM
207.	Vestibular system II	Poster	Wed AM

Theme H: Structure and Function of the CNS

Session Number	Session Title	Type	Day and Time
113.	Brain metabolism I	Poster	Tue AM
314.	Brain metabolism II	Poster	Thu AM
327.	Brain metabolism III	Slide	Thu PM
370.	Brain metabolism IV	Poster	Fri AM
17.	Clinical neurophysiology: epilepsy	Slide	Mon AM
386.	Clinical neurophysiology: epilepsy, kindling	Poster	Fri AM
382.	Comparative neuroanatomy I	Poster	Fri AM
383.	Comparative neuroanatomy II	Poster	Fri AM
54.	Degenerative diseases: Parkinson's and Alzheimer's disease	Slide	Mon PM
384.	Diseases of the nervous system: epilepsy	Poster	Fri AM
385.	Diseases of the nervous system: epilepsy, kindling	Poster	Fri AM
132.	Diseases of the nervous system: genetic diseases, models	Poster	Tue AM
270.	Diseases of the nervous system: immunology, virology	Poster	Wed PM
129.	Diseases of the nervous system: ischemia, trauma	Poster	Tue AM
128.	Electroencephalography and evoked potentials	Poster	Tue AM
357.	Limbic system and hypothalamus I	Poster	Thu PM
358.	Limbic system and hypothalamus II	Poster	Thu PM
359.	Limbic system and hypothalamus III	Poster	Thu PM
138.	**Molecular bases of the immune response to neural antigens**	**Symp.**	**Tue PM**
98.	Structure and function: cortical and subcortical organization I	Slide	Tue AM
154.	Structure and function: cortical and subcortical organization II	Poster	Tue PM
203.	Structure and function: cortical and subcortical organization III	Poster	Wed AM

Theme I: Neural Basis of Behavior

Session Number	Session Title	Type	Day and Time
280.	Aging: behavior and plasticity	Slide	Thu AM
213.	Aging: neural basis of behavior	Poster	Wed AM
115.	Biological rhythms I	Poster	Tue AM

163.	Biological rhythms II	Poster	Tue PM
239.	Biological rhythms III	Slide	Wed PM
332.	Biological rhythms IV	Slide	Thu PM
3.	**Cerebral processes and conscious functions**	**Symp.**	**Mon AM**
146.	Circuitry and pattern generation I	Slide	Tue PM
300.	Circuitry and pattern generation II	Poster	Thu AM
45.	**Computer-assisted analysis of autoradiographs: applications to the 2-deoxyglucose method**	**Wksh.**	**Mon PM**
185.	Effects of chronic drug administration I	Poster	Tue PM
186.	Effects of chronic drug administration II	Poster	Tue PM
20.	Feeding and drinking I	Poster	Mon AM
21.	Feeding and drinking II	Poster	Mon AM
58.	Feeding and drinking III	Slide	Mon PM
105.	Feeding and drinking IV	Slide	Tue AM
167.	Feeding and drinking V	Poster	Tue PM
16.	Feeding and drinking: CCK	Slide	Mon AM
216.	Hormonal control of behavior	Poster	Wed AM
257.	Human behavioral neurobiology I	Poster	Wed PM
283.	Human behavioral neurobiology II	Slide	Thu AM
111.	Invertebrate learning and behavior I	Poster	Tue AM
191.	Invertebrate learning and behavior II	Slide	Wed AM
232.	Invertebrate learning and behavior III	Slide	Wed PM
101.	Learning and memory: anatomy I	Slide	Tue AM
140.	Learning and memory: anatomy II	Slide	Tue PM
244.	Learning and memory: anatomy III	Poster	Wed PM
321.	Learning and memory: anatomy IV	Poster	Thu AM
114.	Learning and memory: pharmacology I	Poster	Tue AM
162.	Learning and memory: pharmacology II	Poster	Tue PM
256.	Learning and memory: pharmacology III	Poster	Wed PM
160.	Learning and memory: physiology I	Poster	Tue PM
245.	Learning and memory: physiology II	Poster	Wed PM
290.	Learning and memory: physiology III	Slide	Thu AM
320.	Learning and memory: physiology IV	Poster	Thu AM
5.	Monoamines and behavior I	Slide	Mon AM
19.	Monoamines and behavior II	Poster	Mon AM
166.	Monoamines and behavior III	Poster	Tue PM
212.	Monoamines and behavior IV	Poster	Wed AM
343.	Motivation and emotion	Poster	Thu PM
344.	Motivation and emotion: reward systems	Poster	Thu PM
85.	Neural basis of behavior: alcohol I	Poster	Mon PM
86.	Neural basis of behavior: alcohol II	Poster	Mon PM
87.	Neural basis of behavior: alcohol III	Poster	Mon PM
254.	Neural basis of behavior: interhemispheric relations I	Poster	Wed PM
255.	Neural basis of behavior: interhemispheric relations II	Poster	Wed PM
189.	**Neural basis of lateralized behavior: from laboratory to clinic**	**Symp.**	**Wed AM**
78.	Neuroethology I	Poster	Mon PM
152.	Neuroethology II	Slide	Tue PM
165.	Neuroethology III	Poster	Tue PM
181.	Neuropeptides and behavior I	Poster	Tue PM
182.	Neuropeptides and behavior II	Poster	Tue PM
183.	Neuropeptides and behavior: vasopressin	Poster	Tue PM
60.	Psychotherapeutic drugs: aminergic systems	Poster	Mon PM
126.	Psychotherapeutic drugs: anxiolytics	Poster	Tue AM
34.	Psychotherapeutic drugs: neuroleptics	Poster	Mon AM
375.	Sleep	Poster	Fri AM
371.	Stress, hormones and autonomic nervous system I	Poster	Fri AM
372.	Stress, hormones and autonomic nervous system II	Poster	Fri AM

2 SYMPOSIUM. ACTIONS OF NERVE GROWTH FACTOR (NGF) IN THE CENTRAL NERVOUS SYSTEM. I.B. BLACK, Cornell University Medical College (Chairperson); H. Thoenen, Max-Planck Inst. for Psychiatry; L. Reichardt, Univ. of California School of Medicine; W.B. Mobley, WRFAIR, Walter Reed Army Medical Center; E. Johnson, Washington Univ. Medical School.

NGF is the prototypical neuronal trophic molecule: it was discovered over 30 years ago, it is the only neurotrophic molecule for which the amino acid sequence is defined, and it has been the molecular model for many concepts of ontogeny and trophic interactions. Until recently, the actions of NGF were thought to be restricted to peripheral neurons of neural crest origin, whereas brain trophic molecules, if any, were unknown. However, within the past 2 years increasing evidence has indicated that NGF regulates development and maintenance of subpopulations of brain neurons. This remarkable realization is the subject of the present symposium.

Hans Thoenen will begin by providing an overview of NGF research before discussing NGF transport in central systems, and the presence of mRNA coding for NGF in the brain. Lou Reichardt will review the central distribution of NGF mRNA, and discuss novel methods for message detection. Bill Mobley will discuss the effects of the trophic factor on developing populations of rat brain neurons in vivo. Ira Black will present work performed with brain neurons in culture, indicating that NGF specifically and selectively enchances growth of cholinergic neurons in rat caudate-putamen and the nucleus basalis complex. Eugene Johnson will discuss recent studies suggesting that the spinal cord dorsal horn contains NGF that regulates development of primary sensory neurons in dorsal root ganglia. The symposium, consequently, is designed to synthesize converging lines of evidence, indicating that NGF plays a critical role in brain development.

3 SYMPOSIUM. CEREBRAL PROCESSES AND CONSCIOUS FUNCTIONS. B. Libet, Univ. of California, San Francisco (Chairperson); J. C. Mazziotta, UCLA; S. A. Hillyard*, Univ. of California, San Diego; G. A. Ojemann, Univ. of Washington, Sch. of Med.; J. C. Eccles, Max Planck Institute, Göttingen.

Conscious, subjective phenomena can be studied directly via introspective reports but only in human subjects. The symposium provides current examples of significant developments in our understanding of this fundamental phenomenon.

Changes in neuronal activity patterns are accompanied by altered rates of metabolism. The latter can be mapped in cerebral cortex both spatially and temporally by the positron emission tomography (PET). Mazziotta will discuss PET findings that describe altered local metabolic patterns in association with conscious sensory responses and motor tasks, in normal and abnormal psychological states.

Certain long-latency event-related brain potentials are reliably associated with the focussing of attention on sensory input channels and the detection of relevant target signals. Hillyard will present evidence suggesting that these electrical waves are manifestations of different aspects of conscious perceptual processing. The P300 wave, in particular, appears to signify the conscious detection of a signal. This relationship can be used to investigate the role of conscious processing in the visually guided behavior of patients with cortical blindness (the "blindsight" phenomenon).

Different aspects of language functions, - in naming, reading, verbal memory, orofacial movements in expression, and phoneme identification -, have been experimentally analysed by Ojemann in cerebral studies on awake neurosurgical patients. Differential mapping of these cortical functions was achieved with electrical stimulation, while electrophysiological field and single neurone recordings enabled some functional analyses.

The initiation of a fully endogenous voluntary act has often been assumed to be a conscious process. Libet reports finding that onset of a cerebral process, the scalp-recorded "readiness-potential" (RP) precedes the appearance of conscious intent by about 400 msec. However, conscious intent precedes the motor act by about 200 msec, and some form of conscious control, for example a veto of actual movement, still remains possible.

The intervention by a non-material mental/conscious event in neural events of the brain can be envisaged within either a monist (e.g., Sperry) or a dualist (e.g., Popper and Eccles) view of mind-brain interaction. Eccles will present a theory of how such an interaction may occur. The theory proposes that a mental event can influence the probabilistic operations of synaptic boutons, by actions in conformity with the physics of probabilistic fields of quantum mechanics.

CELLULAR ASPECTS OF DISEASE: CNS PROTEINS

4.1 **NUCLEUS BASALIS MEYNERT NEURONS CONTAIN THE VITAMIN-D INDUCED CALCIUM BINDING PROTEIN: A CLUE TO ALZHEIMER DISEASE?** M.R. Celio, A.W. Norman*. Anatomisches Institut Universität Zürich, CH-8057 Zürich (Switzerland) and Biochemistry Dept. UCR, Riverside Ca 92521 (USA).

The basal nucleus of Meynert (NBM) comprises clusters of large multipolar neurons in the basal forebrain which are known to provide the major cholinergic input to the cerebral cortex. Central cholinergic pathways are thought to modulate behavioural states and a selective loss of cortical cholinergic markers has been recognized in Alzheimer's disease. We report on the immunohistochemical detection of the 28k Vitamin-D induced calcium-binding protein (CBP) in the neurons of the basal nucleus of Meynert in primates. For this study we perfused squirrel and rhesus monkeys with fixative and cut the brain with a vibrating microtome. The sections were incubated with diluted antisera against the 28k chicken intestinal CBP and processed by the unlabeled antibody method of Sternberger. In the monkey, the magnocellular components of the basal nucleus of Meynert displayed strong immunoreactivity with the antiserum against CBP. The cell bodies (25-30 um in diameter) and the thick diverging stem dendrites were homogeneously stained. In those cortical layers known to receive NBM projections, CBP immunoreactivity was indeed present. The NBM is not the only brain region which contains CBP and CBP is not restricted to cholinergic neurons in the brain. Interestingly, several of the systems containing CBP have been implicated in Alzheimer's disease pathology. For example NBM, Locus coerules, Nucl. Raphe dorsalis, somatostatin containing interneurons of the neocortex, all seem to be affected by the disease process. CBP, therefore, represent a "common denominator" underlying several diverse elements of the pathology of Alzheimer's disease. CBP may play a direct role in the pathogenesis of this neurodegenerative disorder, because it shuttles and buffer Ca^{++}-ions, thus regulating the intracellular calcium distribution and concentration. Malfunctioning or interference with the function of CBP in neurons may render these cells susceptible to intermittent calcium fluctuations and perhaps more prone to accumulate intolerable quantities of calcium. The uncontrolled elevation of the intracellular calcium concentration may lead to protein denaturation and cell death.

4.2 NEURON-SPECIFIC AND NON-NEURONAL ENOLASE (γ AND α SUBUNIT) ISOENZYME IMMUNOREACTIVITY IN CEREBRAL CORTEX OF PATIENTS WITH ALZHEIMER'S DISEASE. D.E. Schmechel (*), P.J. Marangos, B.J. Crain, G.M. Cook (*), A.D. Roses (SPONS: R.P. Erickson). Departments of Medicine, Anatomy and Pathology, Kathleen P. Bryan Alzheimer's Disease Research Laboratory, Duke University Medical Center; Durham VAMC; Durham, NC 27710; NIMH, Bethesda, MD 20205 (PJM).

The cellular localization of the neuron-specific (NSE or γ) and non-neuronal (NNE or α) subunits of the glycolytic enzyme enolase (EC 4.2.1.11) in primate cerebral cortex reveals that neurons are immunoreactive for NSE, but only weakly (+) or non-reactive for NNE. Especially in hippocampus, non-pyramidal neurons (NPN) are strikingly more NSE immunoreactive than most pyramidal neurons. We examined brain tissue from patients with Alzheimer's Disease (AD) to see whether this normal profile might be changed. We used formalin-fixed routine autopsy material (3 elderly controls and 4 patients with pathological diagnosis of AD). Two other patients with clinical AD had rapid autopsies (23 min and 1 hr to brain dissection); tissue from these cases was fixed for 24 hrs in a variety of fixatives in 5 mm slabs (both proved to have AD). Immunocytochemistry for NSE and NNE (human antigens) and somatostatin was carried out by peroxidase methods on 25-50 micron floating sections.

As in normal hippocampus, the hippocampus of all six cases of AD showed strong NSE staining of NPN compared to pyramidal neurons. This distinction is especially clear for NPN located in a loose reticular plate at the oriens-alveus border and in CA4. They remained strongly NSE(+) even in sectors containing pyramidal neurons with neurofibrillary tangles. Other evidence suggests that most of these 'reticular plate' NPN (see Schmechel et al. Neurosci Lett 47: 227, 1984) are somatostatin-containing GABAergic neurons.

The two rapid autopsy cases of AD showed better enolase immunoreactivity similar to surgical specimens of human brain. Both NSE and NNE localization was very consistent even in large sections resembling normal distribution in primates. However, in specific hippocampal subfields, many pyramidal neurons were very weakly NSE (+), but strongly NNE immunoreactive. Such a reversed profile was also seen in sectors of frontal, temporal and parietal lobe, but not in occipital lobe, cerebellum, or surgical samples of normal human temporal lobe.

In summary, the enolase profile of cortical neurons in regions affected by AD may range from normal (ex, preserved strong NSE(+) of 'reticular plate' NPN in hippocampus) to an abnormal pattern reminiscient of immature or axotomized neurons (ex, shift from NSE to NNE of selected hippocampal pyramidal neurons). Since NSE is involved in neuronal glycolysis, the NSE/NNE profile may allow detection of differences in the metabolic state of specific classes of neurons affected by the AD disease process. (DES supported by VA Career Development Award; C. Cox aided technical effort).

*Indicates nonmember of the Society for Neuroscience

PO indicates abstracts that are published only

4.3 A HYDROPHOBIC DOMAIN OF MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP 2) SHARES ANTIGENS WITH ALZHEIMER NEUROFIBRILLARY TANGLES (NFT). K.S Kosik*, D.J. Selkoe and L.K. Duffy* (SPON: C.G. Rasool), Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115

NFT are one of the major neuronal structural changes in Alzheimer's disease. The exact molecular composition of the fibers comprising NFT is unknown. Ultrastructural and immunological observations have implicated neurofilament protein and, most recently, MAP 2 or MAP 2 fragments as possible constitutive elements of NFT. The latter finding (Kosik et al., PNAS, 1984) is based upon a monoclonal antibody, referred to as 5F9, which is highly specific for MAP 2 and also reacts with NFT. Characterization of the MAP 2 epitope with which this antibody reacts may provide information about one protein component of NFT. This report describes the isolation and characterization of a MAP 2 cyanogen bromide (CNBr) cleavage product that contains 5F9 immunoreactivity and has markedly different solution properties than the parent molecule. MAP 2 was obtained from calf brain using the taxol method (Vallee, J. Cell Biol., 1982) to prepare microtubules, followed by salt precipitation of tubulin and heat precipitation of MAP 1. Supernates highly enriched in MAP 2 were digested with CNBr for 48 h and resolved by SDS-PAGE. A Western blot of the CNBr cleavage products revealed a 5F9-immunoreactive protein doublet of M_r ~40,000. The CNBr cleavage products were also resolved by HPLC with an acetonitrile gradient over a Vydac C4 column. In the region of 40% acetonitrile, the elution profile showed the immunoreactive 40Kd doublet, a 65Kd doublet, and a 92Kd protein. The hydrophobicity of the immunoreactive 40Kd fragment was suggested by its relatively prolonged retention time, so that it co-eluted with CNBr fragments of considerably higher molecular weight. A portion of the total CNBr fragments of MAP 2 remains insoluble in aqueous buffers under both acidic and basic conditions. These relatively insoluble fragments were identified as the same three that showed prolonged retention time by HPLC; their insolubility contrasts with the properties of intact MAP 2, which is highly soluble under a wide range of salinities and pH's. A single HPLC fraction containing the 40Kd immunoreactive protein was rechromatographed under identical conditions as described above to purify further this fragment. A single amino acid analysis of a 40Kd-enriched fraction suggests an abundance of non-polar, hydrophobic residues, particularly glycine, in this fragment. Its composition is in contrast to that of the parent molecule, which is unusually low in non-polar residues. The other large molecular weight fragments of MAP 2 appear to be highly enriched in glutamic acid and thus bear some resemblance to the neurofilament carboxy-terminal tail pieces. We conclude that the portion of MAP 2 which contains an epitope shared with NFT is unusual in its hydrophobic nature in contrast to the highly charged domains which constitute the major portion of MAP 2. Supported by NIH grants 5KO7 NS00835 and RO1 AG05538 (KSK).

4.4 COMPARATIVE PROTEIN CHEMICAL STUDIES OF SENILE PLAQUE AMYLOID FIBERS AND PAIRED HELICAL FILAMENTS IN ALZHEIMER'S DISEASE. D. Selkoe, C. Abraham* and L. Duffy*. Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115

During aging of the human brain and particularly in Alzheimer's disease (AD), progressive neuronal loss is associated with the formation of highly stable intra- and extraneuronal protein fibers. Intraneuronally, paired helical filaments (~20 nm in diameter), together with some straight 10-20 nm filaments, accumulate in cell bodies (as neurofibrillary tangles) and in the dystrophic neurites that comprise senile plaques. Extraneuronally, deposits of unpaired ~8 nm filaments form the central cores of many senile plaques. Similar filaments also occur in the walls of some parenchymal and meningeal blood vessels in AD brain. These various fibers have physicochemical properties which allow their classification as amyloid proteins. Taking advantage of the size (5-22 μm) and dense spherical form of isolated senile plaque cores, we have developed a method to purify them to near homogeneity using fluorescence-activated cell sorting. The isolated, purified cores contain 70-130 pg protein each and contain abundant glycine and other non-polar amino acids and very little proline or cysteine. Their amino acid composition is similar to that of highly enriched fractions of PHF. Although both the amyloid cores and the PHF are insoluble in a variety of strong solvents, including SDS, urea and guanidine HCl, treatment with either saturated (7 M) guanidine SCN or concentrated (80%) formic acid causes disappearance of the fibers by light and electron microscopy and releases two major low M_r proteins at ~6,000 and ~12,000 daltons. The proteins released from PHF and from cores co-migrate by gel electrophoresis and by size-exclusion HPLC. These small polypeptides, which appear to be oligomerically related, react with PHF antibodies that do not recognize normal fibrous proteins. Intact, isolated amyloid cores also react with these antibodies, although in situ cores in brain tissue sections are not labeled. Both the purified amyloid core fibers and highly enriched PHF fractions produce an x-ray diffraction pattern consistent with the presence of considerable cross-ß-pleated sheet structure. Thus, intraneuronal PHF and extraneuronal amyloid fibers, although ultrastructurally distinct, share solubility properties, similar amino acid compositions, major constituent proteins, ß-pleated sheet conformation and specific antigenic determinants. Identification of the molecular origin of the proteins comprising these pathological fibers will now require sequence analysis. (Supported by NIH Grants AG01307 and NS20110.)

4.5 IMMUNOCYTOCHEMICAL STUDIES ON NEUROFILAMENT ANTIGENS IN THE NEUROFIBRILLARY PATHOLOGY INDUCED BY ALUMINUM. J. C. Troncoso*, L. A. Sternberger*†, N. H. Sternberger*†, P. N. Hoffman and D. L. Price (SPON: M. R. DeLong). Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; †Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642.

Intrathecal administration of aluminum salts induces accumulation of neurofilaments in axons and perikarya of rabbit motor neurons. This pathology is associated with impaired axonal transport of neurofilaments. Phosphorylation of neurofilaments, in particular the 200-kd sidearm protein, seems to be important for interactions between neurofilaments and between neurofilaments and other cytoskeletal elements. Moreover, sidearms may play a role in axonal transport in the cytoskeleton. Therefore, it is possible that aluminum may produce its effects by altering phosphorylation of neurofilaments. To begin to test this hypothesis, we used antibodies directed against phosphorylated and nonphosphorylated epitopes of neurofilament proteins for immunocytochemical analysis of spinal cord sections from aluminum-injected rabbits. In control animals, phosphorylated neurofilaments were not conspicuous in perikarya of motor neurons. In experimental rabbits, two out of three phosphorylated epitopes were readily demonstrated in perikarya of affected motor neurons. The presence of phosphorylated epitopes of neurofilaments in perikarya of motor neurons is distinctly abnormal and may have important consequences for the organization and function of the cytoskeleton. This inappropriate location of phosphorylated neurofilaments may be associated with impairments in axonal transport. Finally, it is of interest that a similar, but not identical, pattern of neurofilament phosphorylation has recently been described in neurofibrillary tangles in Alzheimer's disease.

4.6 IN SITU DISTRIBUTION OF PHOSPHORYLATED AND NONPHOSPHORYLATED NEUROFILAMENT EPITOPES FOLLOWING AXOTOMY AND NEURECTOMY OF SENSORY NEURONS. J. Rosenfeld, M. E. Dorman*, N. H. Sternberger*†, L. A. Sternberger*†, P. N. Hoffman and D. L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; †Center for Brain Research, University of Rochester Medical Center, Rochester, NY 14642.

Axonal transection results in an alteration of the metabolism of neurofilaments (NF). Evidence suggests that the 200-kd NF protein plays a role in crosslinking NF. Immunocytochemistry using monoclonal antibodies which recognize either phosphorylated or nonphosphorylated epitopes of the 200-kd NF protein demonstrate that NF epitopes have distinct distributions in the cell bodies/axons of normal neurons. We hypothesize that changes in NF metabolism, observed during regeneration, may be associated with altered distributions of NF epitopes within different regions of neurons. In the present study, the distributions of NF epitopes were examined in neurons of the dorsal root ganglia (DRG) using immunocytochemistry in the setting of effective and ineffective regeneration.

An altered pattern of immunoreactivity was observed following axotomy (nerve crush followed by regeneration) but was not observed following neurectomy (permanent axotomy) of sciatic nerve in adult rats. Three weeks following surgery, sections of DRG were stained using antibodies against phosphorylated and nonphosphorylated NF epitopes. In control animals, nonphosphorylated NF proteins were visualized predominantly in cell bodies, dendrites, and proximal axons, while phosphorylated NF proteins were localized primarily in axons and only rarely observed in cell bodies. Following axotomy, 50-90% of the total number of DRG cells showed phosphorylated epitopes in perikarya. Following neurectomy, phosphorylated NF proteins were not visualized in DRG cells. In both axotomy and neurectomy preparations, antibodies directed against nonphosphorylated NF proteins stained cell bodies of DRG.

This study demonstrated a change in staining patterns of NF epitopes in regenerating sensory neurons, i.e.., epitopes of phosphorylated NF proteins appear in the cell body. When regeneration does not occur, e.g., neurectomy, phosphorylated NF epitopes were observed only in axons. Mechanisms leading to abberant patterns of immunoreactivity are unknown. The abnormal distribution of epitopes could arise from increased amounts of phosphorylated NF proteins, altered regulatory mechanism for NF phosphorylation, etc. Abnormal patterns of phosphorylated NF also occur in cell bodies in a model of neurofibrillary pathology (aluminum intoxication) and in neurofibrillary tangles in individuals with Alzheimer's disease.

4.7 CYTOTOXIN I (NAJA NAJA OXIANA) INHIBITS SPECIFIC KINASES FROM SEIZURE SENSITIVE AND RESITANT STRAINS OF GERBILS AND MICE. J.G. Bajorek* and A.V. Delgado-Escueta. Dept. Neurology, Sch. of Med. Univ. Calif. Los Angeles , and V.A. Wadsworth Med. Ctr., Los Angeles, CA 90024.

Cytotoxin I (Naja naja oxiana) was used to evaluate the activity of phospholipid-calcium dependent kinase present in crude fractions (shocked P2) of mouse (C57/6J) and mongolian gerbil (meriones unguculatus) cerebral cortex. We have previously noted that in vitro phosphorylation assays of this fraction detect a difference between seizure sensitive and resistant strains of these mice and gerbils in the phosphorylation of several proteins separated by SDS polyacrylamide electrophoresis. Since the cytotoxin I polypeptide has been reported to be a relatively specific inhibitor of the phospholipid-calcium dependent kinase in purified enzyme preparations (Kuo et al FEBS Lett 153:183 1983), we hoped to use this property to identify substrates predominantly phosphorylated by the kinase and to expose possible alterations of the enzyme in seizure susceptible animals. In C57/6J mice proteins of 16K and 20K molecular weight were inhibited by 15-20% in an incubation mixture containing 25ug toxin, 5mM ATP32, 0.5mM Ca+2, 5mM Mg+2, 1ug/ul total protein in 0.1 ml of Iris buffer. Other proteins at 44K, 48K, 52K, 61K, and 74-79K were inhibited by only 6-8%. In the gerbils, the 16K and 20K proteins were not inhibited at 25ug but stimulated up to 8%. Using levels from 0-100ug, there was an inhibition noted of 20-25% after 100ug of toxin. This biphasic response was seen in three separate experiments. Seizure susceptible and resistant strains of gerbils showed similar biphasic patterns of toxin response, however seizure prone animals exibited a greater inhibition at the 100ug levels. Most other substrates were not inhibited but showed a slight stimulation. One protein at 48K which was stimulated by calmodulin had the calmodulin stimulation inhibited (30-35%) by the toxin. The complexity of this crude fraction was exposed by the addition of the toxin since the biphasic response may have been due to the phosphorylative activity of other kinases on the same substrates or to the presence of other cofactors or phospholipids which can modify the activity of the kinases. However, the toxin partially differentiated some of the substrates and provides a tool for studying these kinases/phosphatases in epileptic models.

4.8 VISUAL DYSFUNCTION ASSOCIATED WITH ANTINEUROFILAMENT ANTIBODIES. S. Kornguth, T. Kalinke*, G. Grunwald* and D. Dahl. University of Wisconsin, Madison, WI 53705; National Institutes of Health, Bethesda, MD 20205; VA Hospital, W. Roxbury, MA 02132.

This report describes the presence of antineurofilament antibodies in the sera of rabbits that were immunized against large ganglion cells from ox retinas. The intravitreal injection into cats, of these immunoglobulins, was shown to cause a selective reduction in the number of large retinal ganglion cells. The electrophysiological properties of Y-type neurons in the lateral geniculate nucleus were also affected. These immunoglobulins were also shown to react with large retinal ganglion cells by immunohistological methods.

Recent experiments on these polyclonal antibodies from rabbits indicate that they react strongly with a 210 kDa protein from bovine brain as shown by Western blot analysis. On 5% polyacrylamide gels, the 210 kDa antigen comigrates with the high molecular weight protein of the neurofilament triplet. This was determined using rabbit polyclonal antibodies that were produced against the 200 kDa neurofilament protein (from Dr. Doris Dahl).

In order to characterize further the relationship between the large ganglion cell antigens and the neurofilament proteins, our laboratory purified the neurofilament proteins. A crude neurofilament fraction was obtained from bovine brain by Triton X-100 extraction followed by centrifugation through sucrose according the method of Tokutake et al. Anal. Biochem. 135, 102-105, 1983. The pellets, which contained neurofilaments, were solubilized either in 8 M urea or 1.5 M sodium thiocyanate buffered with 10 mM sodium phosphate, pH 7.4, containing 1% 2-mercaptoethanol. Further purification of the 210 kDa protein was obtained by ethanol fractionation. The 210 kDa protein remained in solution at 50% saturation with ethanol. The solution was dialyzed against water and lyophilized to recover the 210 kDa protein.

These observations are of interest because our laboratory has shown that patients with small cell carcinoma of the lung who also have a visual paraneoplastic syndrome, contain a high titer of immunoglobulins in their sera that react selectively with a subset of the neurofilament triplet proteins. This suggests that visual dysfunction may be associated with exposure of the retina to antineurofilament (NF) antibodies in the patient with the small cell tumor and in the cat model. These immunoglobulins may be of etiologic significance in the development of retinal dysfunction.

4.9 PRION PROTEINS ACCUMULATE IN THE ROUGH ENDOPLASMIC RETICULUM OF SCRAPIE-INFECTED HAMSTER BRAINS. R. K. Meyer*, M. P. McKinley*, R. A. Barry* and S. B. Prusiner. Department of Neurology, University of California, San Francisco, CA 94143.

Scrapie is a transmissible, degenerative disease of sheep and goats which occurs after a prolonged incubation period. While the scrapie agent or prion is found in most tissues, the highest titers are found in brain. Animals with scrapie exhibit signs of neurologic dysfunction. Experimental studies have been facilitated by transmission to rodents. The scrapie prion contains a protein PrP and little or no nucleic acid. Scrapie PrP is a sialoglycoprotein, has a M_r of 27-30 kd in preparations purified by proteinase K digestion, and aggregates into amyloid rods and filaments. Immunoblots of unpurified fractions from infected and uninfected hamster brains demonstrated PrP proteins of higher M_r 33-35 kd. Similar results were obtained with rabbit antisera raised against PrP 27-30^{Sc} or against a synthetic peptide corresponding to the N-terminus of PrP 27-30^{Sc}. Proteinase K digestion of infected fractions converted PrP 33-35^{Sc} to PrP 27-30^{Sc}; whereas, the cellular protein PrP 33-35^{C} was completely degraded. After subcellular fractionation, both PrP 33-35^{Sc} and PrP 33-35^{C} were found in membrane fractions but not in 10^5 x g supernatant fluids. The protease sensitivity of PrP 33-35^{C} as well as the proteolytic conversion of PrP 33-35^{Sc} to PrP 27-30^{Sc} were unaltered by extracting the membranes with 2% sodium dodecyl sarcosinate (Sarkosyl). NaCl (0.1-0.5 M), 5 mM EDTA and osmotic shock failed to release the PrP proteins from microsomal membranes; in constrast 2% Sarkosyl did solubilize the PrP proteins since they were recovered in the supernatant fluid after centrifugation at 100,000 x g for 1 hr. Electron microscopy of these microsomal fractions showed membrane vesicles but not rod-shaped structures. The rods have been found in purified preparations of scrapie prions and are thought to be aggregates of the infectious particles. Sixty days after inoculation with ~10^7 ID_{50} units of prions, the concentration of PrP 33-35^{Sc} in scrapie-infected brain was increased ~10 times over that of PrP 33-35^{C} in uninfected brain. Fractionation of microsomal membranes on sucrose-CsCl gradients allowed separation of smooth endoplasmic reticulum (SER) from rough endoplasmic reticulum (RER) membranes. PrP 33-35^{C} was found primarily in RER fractions. Our observations suggest that PrP proteins are integral membrane proteins and that scrapie infection causes PrP 33-35^{Sc} to accumulate in RER. The transport and/or processing of PrP appears to be impaired during scrapie infection. How this alteration in cellular membranes relates to dysfunction of the central nervous system remains to be established.

4.10 SUSTAINED REACTIVE SPROUTING FOLLOWING MINOR HEAD INJURY. J.T. Povlishock, Dept. of Anatomy, Med. Coll. of Va., Virginia Commonwealth Univ., Richmond, VA 23298.

In previous communications we have observed that following minor head injury various forms of reactive axonal change occur. Typically such reactive change entails focal axonal swelling which over a 21 day post-traumatic course persists unchanged, degenerates or gives rise to multiple reactive [regenerative] sprouts. Since such sprout-containing swellings persist over a 21 day period in contrast to other reports of CNS regeneration which have characterized reactive sprouting as rapidly abortive, it seemed appropriate to better characterize this response and to follow it over a more prolonged post-trauamtic course. To this end, anesthetized cats were subjected to minor fluid-percussion brain injury. Following their complete and uneventful recovery from the traumatic insult , the animals were allowed to survive for periods ranging from 3 to 60 days. To aid in the recognition of the reactive axonal sprouting , the anterograde axonal transport of horseradish peroxidase [HRP] was utilized based upon our previous experience that such HRP passage was a sensitive probe for detecting focal reactive axonal change. At the designated survival times, the animals were anesthetized, perfused with aldehydes and prepared for LM and TEM analyses. Within 3 days of the traumatic episode multiple reactive sprouts were observed originating from the traumatically induced axonal swellings . These contained numerous lucent and dense core vesicles and such sprouts ranged from 0.5 to 2 μm in diameter and 5 to 20 μm in length . By the second post-traumatic week numerous sprout-containing swellings were recognized and in addition to these reactive sprouts , multiple robust processes, reminiscent of growth cones, were now also observed. With continued post-trauamtic survival both sprout and robust process-containing swellings were identified. Typically both the sprouts and robust processes had advanced beyond the swelling to parallel the distended myelin sheath and when the myelin sheath was either fragmented or totally lost, such processes could be observed coursing into the substance of the surrounding brain parenchyma which in the case of minor head injury was unaltered. Although with continued survival macrophages approximated such sprout and robust process-containing swellings, they did not appear to be related to the onset of any degenerative change. Such sprout and process extension was recognized to continue over a 60 day post-trauamtic course and , as such , appeared remarkable. As minor head injury causes only limited focal axonal change with sparing of the surrounding brain parenchyma and vasculature, it is conceivable that the retention of a realtively unaltered brain microenvironment contributed to such a dramatic and sustained regenerative response. Supported by NS-20193.

4.11 ECTOPIC AXON HILLOCK-ASSOCIATED NEURITE GROWTH IS MAINTAINED IN METABOLICALLY REVERSED SWAINSONINE-INDUCED NEURONAL STORAGE DISEASE. S.U.Walkley, S.Wurzelmann*, and D.A.Siegel*, Dept. Neuroscience, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, N.Y.10461.

Select populations of neurons in a variety of neuronal storage disorders have been shown to be characterized by growth of ectopic neurites at axon hillock regions. Ultrastructurally these neurites have been shown to resemble small dendrites and to possess spines and synapses. Axon hillock enlargements ("meganeurites") often occur on the same neurons and these unusual changes in neuronal geometry and connectivity have been hypothesized to be major contributors to neuronal dysfunction in such diseases.

Recently, a model of induced neuronal storage disease has been developed using the reversible α-mannosidase inhibitor, swainsonine, found in locoweed plants (Astragalus spp.). This model has been shown to be characterized by induced neuritogenesis and synaptogenesis qualitatively identical to that seen in inherited α-mannosidosis and other storage diseases. The goal of the present study was to use this model to determine the reversibility of axon hillock-associated neurite growth following metabolic correction (inhibitor withdrawal). Three littermate kittens were used. Two animals received identical, measured quantities of swainsonine per os daily, beginning at 1 mo. of age, while the third animal served as untreated control. Neurological deficits appeared in both treatment animals after 3 mos. Swainsonine administration was ended for one animal after 7 mos. and a cerebral biopsy was taken to confirm storage-induced morphological changes. This metabolically "reversed" animal showed slight clinical improvement following cessation of treatment, whereas the animal maintained on inhibitor displayed increased deficits. At 14 mos. of age all three animals were killed and tissues processed for LM and EM.

Combined Golgi-EM studies of pyramidal neurons in the cerebral biopsy taken at the time of swainsonine withdrawal revealed the development of characteristic cytoplasmic vacuolation, axon hillock neurite growth, and neurite-associated synapses. At autopsy, routine EM of cerebral cortex of this animal revealed that storage vacuoles had disappeared but Golgi studies demonstrated the persistence of axon hillock neurite growth which was indistinguishable from that of the continuously treated animal. Combined Golgi-EM studies of these neurite-bearing pyramidal cells in the corrected animal revealed the presence of large numbers of lipofuscin-like inclusions. These preliminary studies suggest that although storage vacuoles undergo modification and reduction in the metabolically corrected disease, ectopic axon hillock-associated neurites do not appear to undergo a similar process of reversal. (Supported by NS-18804 and NS-03356).

4.12 THROMBOXANE/PROSTACYLIN RATIO CHANGES FOLLOWING EXPERIMENTAL SPINAL CORD TRAUMA IN THE RABBIT. T.P. Jacobs, G. Feuerstein, A.I. Faden. Dept. of Neurology, Uniformed Services University of the Health Sciences, Bethesda, Md. 20814 and Neurology Service, San Francisco Veteran's Admisistration Medical Center, San Francisco, California 94121.

Morphologic damage caused by traumatic injuries to the spinal cord result in part from secondary changes developing over a period of several hours post trauma. These secondary alterations, which may contribute to paralysis, appear to be due to release of endogenous substances that may lead to a progressive reduction in spinal cord blood flow (ischemia). Prostaglandins (PGs), which have potent vasoactive properties have been found in increased concentrations in brain tissue after ischemic injury. Recent studies suggested that normal tissue perfusion may depend on a balanced interaction between thromboxane A2 (TXA2) and prostaglandin I2 (PGI2) at the blood-endothelial interface. The present study was designed to evaluate the rate of synthesis of TXB2 and 6-keto-PGF1α after traumatic lumbar spinal cord injury. Thirty-eight rabbits anesthetized with sodium pentobarbital were subjected to a 200g-cm impact injury in the lumbar spinal cord utilizing a modification of the Allen method. At 5 min, 30 min and 24 hrs post trauma, the spinal cord was removed and 1.0mm slices from the injury area were incubated in oxygenated Krebs solution for 45 min. Prostacyclin (measured as 6-keto-PGF1α) and thromboxane A2 (measured as TXB2) released from injured spinal cord slices and noninjured spinal cord were determined by radioimmunoassay (RIA). The rate of release of the PGs expressed as the TXB2/6-keto-PGF1α ratio were as follows: control=0.87; 5 min=1.52; 30 min=1.24 and 24 hrs=0.74. The change in TXA2/PGI2 ratio in the injured zone of the spinal cord was due only to an increase in TXB2 since the release of 6-keto-PGF1α was not changed by injury. These data suggest that there may be a preferential increase in TXB2 synthesis immediately following trauma. TXA2, which is a potent vasoconstrictor and platelet aggregator, may therefore contribute to the reduction of spinal cord blood flow and neurological dysfunction observed after trauma. The results of this study are consistent with previous findings that combined treatment of indomethacin, PGI2 and heparin improve neurological recovery in cats after spinal trauma. (Hallenbeck, J.M. et.al., J Neurosurg 58:749-54, 1983).

5.1 Alpha-2 Adrenergic Receptor Involvement in Adult Male Squirrel Monkey Vocal Behavior. James C. Harris, M.D.* & John D. Newman, M.D. Laboratory of Comparative Ethology, Bethesda, MD 20205.

The response to imipramine in children with separation anxiety disorder (Gittleman-Klein, R. and Klein, D.F. Arch. Gen. Psychiatry, 25:204-207, 1971) suggests the possible involvement of adrenergic mechanisms. Since a reliable indicator of separation distress in primates, including human infants, is the isolation call (IC) (Newman, J.D. In: Infant Crying: Theoretical and Research Perspectives, Lester, B.M. and Boubydis, C.F.Z. (eds.), Plenum, NY, 1985), we have investigated the mechanism mediating this behavior in the adult squirrel monkey, a species in which isolation calls are produced throughout adult life. Because the imipramine effect is thought to produce its anxiolytic effects via α-adrenergic receptors, clonidine, an agonist at α-adrenergic receptors and yohimbine, a specific α_2-antagonist were chosen to test the involvement of adrenergic mechanisms on isolation calls.

Previous studies indicate that adult male squirrel monkeys acoustically and physically separated from conspecifics reliably emit isolation calls for periods of up to one hour. We used this natural behavioral response to measure the effects of clonidine HCl and yohimbine HCl in changing IC rate over a 15 minute test period of separation from the conspecific group. Tests were performed no closer than at weekly intervals. Animals were administered 0.05 to 0.1 mgs. per kg. of clonidine IM 5 minutes prior to testing. The larger dose consistently reduced production of the IC. Subsequent administration of yohimbine (0.2mg/kg) reliably reversed the IC suppression. In addition, animals given this dose of yohimbine either after clonidine or alone, emitted significant numbers of another vocalization, the twitter, normally produced only rarely in isolation.

These findings support the original hypothesis of a role for α-adrenergic receptors in the vocal component of separation distress in the squirrel monkey. The reversal of clonidine suppression with yohimbine, a drug with high affinity for the α_2-adrenergic receptors, implicates this receptor population in this response. The unexpected production of twitters under yohimbine also suggests that adrenergic mechanisms are involved in other aspects of squirrel monkey vocal behavior.

5.2 COLD WATER STRESS ABOLISHES HYPERACTIVITY PRODUCED BY CORTICAL SUCTION LESIONS WITHOUT ALTERING NORADRENERGIC DEPLETIONS. Robert G. Robinson, Kristy A. Zern , Godfrey D. Pearlson, Timothy H. Moran, and Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Focal suction lesions of the right frontal cortex result in hyperactivity and cortical and subcortical norepinephrine (NE) depletions (Pearlson and Robinson, Brain Res., 218:233, 1981). Previous work from this laboratory has indicated that although the NE depletions are not necessary for the production of the behavioral hyperactivity, such depletions are sufficient to produce this behavioral outcome (Kubos, Moran, Saad, and Robinson, Phar. Biochem. and Behav., 21: 163, 1984). In an effort to further characterize the role of NE depletions in the mediation of post lesion hyperactivity, we examined the effect of postoperative forced cold water (15°C) swimming, a procedure demonstrated to increase NE turnover, on both the catecholamine depletions and behavioral hyperactivity produced by focal right hemispheric cortical suction lesions.

Throughout the thirty day postoperative period, lesion and sham operated control animals received swim tests either four times (i.e. once per week over four weeks), once during the first postoperative week or not at all. The effect of swimming stress was different for lesion and sham operated control groups. In the sham group, swimming stress at weekly intervals produced hyperactivity as measured by daily running wheel revolutions (approximately 135% of preoperative baseline). In the lesion group, either a single swimming stress or swimming at weekly intervals blocked the development of the post-lesion hyperactivity. Activity in the lesion, no swim group was 130% of preoperative baseline, significantly greater than the one swim lesion group activity which was 100% of preoperative baseline and the lesion four swim group activity which was 90% of preoperative baseline ($F_{8,171} = 3.69$, $p < .001$). Although altering the behavioral response to cortical lesions, swimming stress did not change the pattern or magnitude of NE depletions. NE was significantly depleted in the lesion as compared with control group in the frontal cortex ($F_{1,41} = 9.16$, $p < .01$), and posterior cortex ($F_{1,41} = 12.19$, $p < .01$).

These findings suggest that the central catecholaminergic effects of the forced swimming stress may have been interacting with different receptor states in lesion and control groups and support the hypothesis that receptor changes, secondary to the NE depletions may be important in mediating the behavioral hyperactivity following focal suction lesions of the right frontal cortex. This work was supported by NIH grant RSDA MH 00163, NS 15178, NS 18622 and NS 15330.

5.3 6-HYDROXYDOPAMINE (6-OHDA) LESIONS OF THE NUCLEUS ACCUMBENS, BUT NOT CAUDATE NUCLEUS, ATTENUATE ENHANCED RESPONDING FOR CONDITIONED REINFORCEMENT PRODUCED BY INFUSIONS OF INTRA-ACCUMBENS D-AMPHETAMINE. J.B. Taylor* and T.W. Robbins*, Dept. of Experimental Psychology, University of Cambridge, Cambridge, England. (SPON: B.J. Sahakian).

Intra-accumbens d-amphetamine has been shown to enhance responding for reward-related stimuli (conditioned reinforcers), whereas intra-caudate d-amphetamine has been found by comparison to produce weak and variable results (Taylor and Robbins, Psychopharm., 84:405, 1984). The present experiment was designed to examine further the involvement of the nucleus accumbens, and the role of dopamine (DA), in this behavioural effect using infusions of d-amphetamine in rats with 6-OHDA-induced depletion of DA in either the nucleus accumbens or the caudate nucleus, and sham-operated controls.

Thirsty rats were trained to associate a light (CR) with water. After this training, all the subjects were implanted with permanent guide cannulae aimed at the nucleus accumbens and assigned to one of four groups, which received either bilateral 6-OHDA (4 mg/ml free base in 2 ul of 0.9% saline) or sham (vehicle) infusions into the nucleus accumbens or caudate nucleus. In the test phase, water was no longer presented but responding on one of two novel levers produced the light (CR lever), whereas responding on the other lever (NCR lever) had no effect. All four groups of subjects received four counterbalanced intra-accumbens infusions of d-amphetamine (3, 10, 20 ug/2 ul) or vehicle over four test days. On a fifth test day, all subjects were pretreated with apomorphine (0.1 mg/kg).

Intra-accumbens d-amphetamine in both nucleus accumbens and caudate nucleus sham-lesioned groups produced a dose-dependent increase in responding on the CR lever, but no significant change in responding on the NCR lever. No selective dose-dependent increases in responding on either lever were found in animals with 6-OHDA-induced depletion of DA (>80%) in the nucleus accumbens following intra-accumbens d-amphetamine. However, intra-accumbens d-amphetamine in subjects with dopamine depletion of the posterior caudate nucleus (>80%) produced dose-related selective increases in responding on the CR lever, which were similar in magnitude to both the sham- lesioned groups except at the highest dose of d-amphetamine (20 ug). Following apomorphine, only the nucleus accumbens lesioned group continued to respond, preferring the CR lever, suggesting the involvement of 'supersensitive' DA receptors induced by 6-OHDA administration.

These results provide evidence to support the hypothesis that enhanced responding for CR found following psychomotor stimulant drugs is critically dependent upon activation of the nucleus accumbens, rather than the caudate nucleus, and is suggestive of a role for DA in mediating this behavioural effect.

5.4 THE TOPOGRAPHY OF LOCOMOTION: EFFECTS OF HALOPERIDOL. P.R. Sanberg, M.D. Bunsey*, S.H. Hagenmeyer, K.H. Russell*, and M.A. Henault. Behavioral Neuroscience Laboratory, Department of Psychology, Ohio University, Athens, Ohio 45701.

The process of screening neurolepics has long been hampered by inadequate methodology. Most behavioral studies on the effects of neuroleptics have involved measuring either catalepsy or the inhibition of stimulant-induced locomotion. However, tests such as catalepsy are plagued with methodological and procedural problems (e.g. inconsistent results, observer bias, learning effects, and dose insensitivity), while inhibition of stimulant-induced activity does not reveal any information on the spontaneous behavioral effects of neuroleptics. In an attempt to circumvent these problems, the present study examined the use of recently developed automated monitors on the behavioral effects of the neuroleptic, haloperidol.

Male Sprague-Dawley rats were placed into the activity monitors at 19:00 and allowed to habituate for one hr. At 20:00 rats were injected i.p. with haloperidol (HAL) in doses of 0.0, 0.1, 0.5, and 1.0 mg/kg and locomotor behavior was monitored for the next three hrs. The rats received each of the drug dosages over a period of two weeks. Dosages were counterbalanced among subjects and a two-day rest period was provided between injections.

Data analysis indicated a significant dose effect for every variable measured. At 0.1 mg horizontal activity (HA), distance travelled (TD), vertical activity (VA), no. of stereotypy (NS), stereotypy time (ST), and anticlockwise revolutions (AC) were significantly decreased from controls. The 0.5 mg group showed additional decreases from controls in movement time, number of movements (NM), av. distance per movement (AD), vertical time, vertical movements, clockwise revolutions (CL), and an increase in rest time (RT). Furthermore, there were significant decreases from 0.1 mg values in VA, NS, AC, and NM and a significant increase in RT. The 1.0 mg rats displayed the same decreases seen in the 0.5 mg rats with additional effects in HA, TD, ST, and CL from the 0.1 mg values. Average speed (SP) was significantly greater at 0.5 mg than at 1.0 mg HAL. In all locomotor categories the drug effect peaked during the second hour.

This study demonstrated the power of the automated monitor as a research tool. The monitor showed that a sub-cataleptogenic dose of HAL profoundly altered several aspects of nocturnal locomotion. The monitor also detected significant differences between the lower and higher dosages. Furthermore, the system revealed a second hour peak dose effect that was evident across all aspects of activity. Finally, SP was revealed to have a peculiar dose effect showing the least inhibition at 0.5 mg/kg.

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5.5 LEARNING AND ATTENTIONAL IMPAIRMENTS IN HYPERACTIVE ADULT RATS TREATED NEONATALLY WITH 6-OHDA. G. K. Hodge and E. A. Reyes. Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131.

Hyperactive children are impulsive and have difficulty sustaining attention. The sense that their inattention is cause for greater concern than their hyperactivity is reflected by the American Psychiatric Association's classification of their behavior as attention deficit disorder (ADD; *DSM-III*, 1980). Attentional problems persist into adulthood (Whalen & Henker, *Hyperactive Children*. NY: Academic, 1980). Animal models of ADD should therefore reveal early and persistent attentional problems.

In humans, Gordon (*J. Abnorm. Child Psychol.*, 7:317, 1979) has reported that hyperactive children do not perform as well as normal controls on a task involving differential reinforcement of low rates of responding (DRL). Hyperactive children made more errors of commission; i.e., they responded impulsively at inappropriate times. We report similar impairments in DRL performance of rats treated neonatally with 6-hydroxydopamine (6-OHDA).

Following desipramine pretreatment (20 mg/kg), cross-fostered male 5-day-old albino rats were injected intracisternally with either 100 µg/25 µl of 6-OHDA or 25 µl of the ascorbic acid/saline vehicle. Some pups received no treatment of any kind.

Beginning at postpartum Day 30, the animals were placed on an alternate-day 22-h food deprivation schedule. Rats were shaped to bar press for food pellets. Presses were eventually rewarded only when a target light was on. Initially, the target light alternated between being on 10 sec and off 10 sec. A distractor light later replaced the off period. On Day 40 and all subsequent days, presses to the distractor light further delayed onset of the target light by additional 10-sec periods. On Day 80, the target light duration was reduced to 7.5 sec; on Day 100, to 5 sec; and on Day 130, to 3 sec. Sessions ended after 50 successes or 20 min.

Analyses of variance revealed no differences between nontreatment and vehicle groups; they were combined (n = 13) and compared against the 6-OHDA group (n = 8). For all days, the 6-OHDA rats weighed less than controls ($p < .01$). During the two sessions that immediately followed shifts to shorter target light durations, the 6-OHDA animals made more commission errors than controls ($p < .05$). At 7.5 and 5 sec durations, the groups eventually attained comparable performance levels, but at the 3-sec duration, the 6-OHDA rats continued to make more errors through Day 170 ($p < .05$).

The results suggest that although both groups are capable of learning the DRL task, the 6-OHDA group has greater difficulty in detecting and making transitions to more stringent criteria, and at the most difficult criterion (3 sec), they continue to make more commission errors than controls. These errors may reflect attentional impairments and impulsivity.

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5.6 DOPAMINE DEFICIENCY WITH CHRONIC COCAINE ABUSE. M.S. Gold, C.A. Dackis,* T.W. Estroff,* and A. Carter Pottash, Research Facilities, Fair Oaks Hospital, 19 Prospect St., Summit, NJ 07901.

Prolactin secretion is tonically inhibited by infundibular dopamine (DA) neurons. Cocaine increases DA neurotransmission acutely by blocking DA reuptake. The chronic effects of cocaine on DA neurons, however, may differ from acute effects. In order to assess the chronic effects of cocaine on DA neurons, we studied prolactin levels in 18 male cocaine abusers and 20 normal male controls, matched for age. Prolactin levels were determined in duplicate by radioimmunoassay on fasting blood drawn at 8 a.m., within 72 hours after the last cocaine use. Prolactin levels in the cocaine patients (mean ± SD; 35.4 ± 26.9 ng/ml) were significantly greater ($t=4.62$, $df=36$, $p<0.001$) than those of the controls (mean ± SD; 7.0 ± 5.0). Most of the prolactin levels of the cocaine patients (67%) were also greater than the established upper normal range (20 ng/ml) for prolactin in males.

The findings of this controlled study in male cocaine abusers are consistent with DA inhibition by chronic cocaine exposure. DA inhibition by cocaine is supported by reports in the literature of reduced DA levels and increased postsynaptic DA receptor density after the administration of cocaine to animals. DA depletion may result fron increased synaptic metabolism of DA after reuptake blockade. The functional inhibition of DA neurotransmission by cocaine could contribute to the abstinence symptoms seen after the abrupt cessation of chronic, severe cocaine abuse. DA depletion, as indicated by hyperprolactinemia, may also underly cocaine craving states. The duration of neuroendocrine and neurotransmitter disruptions by cocaine requires further study.

5.7 RATS TRAINED TO CIRCLE DO NOT EXHIBIT INCREASES IN DOPAMINE OR ITS METABOLITES IN THE CONTRALATERAL STRIATUM OR NUCLEUS ACCUMBENS. C. Szostak*, A. Jakubovic, A.G. Phillips and H.C. Fibiger, Div. Neurol. Sci., Dept. Psychiat., Univ. British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Yamamoto & Freed (Nature, 298, 1982) have reported that rats trained to circle have increased concentrations of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum (ST) and nucleus accumbens (nA) contralateral to their direction of turning. The reliability of this effect was tested in two experiments.

Rats were trained to circle for water according to a continuous schedule of reinforcement until stable performance was obtained. Following a 20-min test session, rats were decapitated and left and right ST and nA were dissected and prepared for analysis of DA, DOPAC and homovanillic acid (HVA) by HPLC. Circling did not influence levels of DA, DOPAC or HVA in the ST or nA contralateral to direction of turning. Moreover, the concentrations did not differ from non-circling, water-deprived control group values. Since the over-all response rates obtained were lower than those reported by Yamamoto & Freed, additional rats were trained to circle according to an intermittent schedule of reinforcement. Intermittent reinforcement resulted in much higher rates of circling. Despite the higher levels of responding, ST levels of DA, DOPAC and HVA were not influenced by circling. The rate of conversion of DA to HVA, as estimated by the HVA:DA ratios, was elevated bilaterally when compared with control ratios. Circling was found to decrease DA levels in the contralateral nA. However, between group differences were not observed. Relative to controls, HVA was increased bilaterally in the nA. This increase was smaller on the contralateral side.

To further assess the role of DA in circling, changes in DA activity resulting from stimulation-induced circling (SIC) were determined. Three groups were used: stimulated rotators, stimulated non-rotators and non-stimulated controls. Electrodes were implanted into the medial forebrain bundle, at the level of the lateral hypothalamus. Levels of DOPAC and HVA were selectively increased in the ipsilateral ST of stimulated rotators. However, this effect was not obtained in one animal that exhibited consistent rates of circling. Levels of DA, HVA and DOPAC were not influenced in the nA.

The present results indicate that high levels of circling produced by positive reinforcement or electrical stimulation can result in selective neurochemical changes. However, the present results failed to confirm that conditioned circling enhances DA utilization in the ST or nA contralateral to the direction of turning. Moreover, while SIC can produce asymmetrical changes in DOPAC and HVA, such effects are not always obtained.

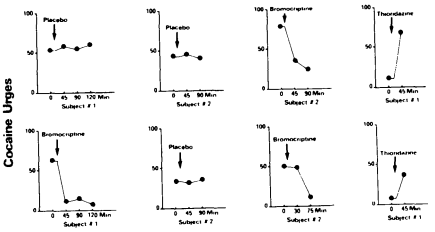
Supported by the Medical Research Council. CS is a NSERC postgraduate scholar.

5.8 MONOAMINES IN THE HIPPOCAMPUS OF RATS FED AN AMINO ACID-IMBALANCED DIET. D.W.Gietzen*,P.M.B.Leung* and Q.R.Rogers. Department of Physiological Sciences: School of Veterinary Medicine and Food Intake Laboratory, University of California, Davis, CA 95616.

Animals will decrease their food intake if prefed a low protein basal diet (BAS) and then offered a ration containing an imbalanced amino acid pattern (IMB). This behavioral response is characterized by a rapid decrease in food intake followed by gradual adaptation to IMB. Previous studies have demonstrated facilitation of the rat's adaptation to IMB feeding in animals bearing bilateral lesions of the hippocampus (Leung, P.M.B. and Rogers, Q.R., Physiol. and Behav.23:129,1979). Since the monoamine neurotransmitter systems may play a role in this behavioral response, we elected to measure the concentrations of several neuroactive amines in hippocampus from rats fed the IMB diet and in controls. Three groups of young male Sprague-Dawley rats were prefed a basal diet. On the day of the experiment they were offered either BAS, IMB or a corrected diet (COR) which differs from IMB only in the addition of 0.5% of the limiting amino acid, isoleucine, to correct the imbalance. The animals were killed after having access to the diet for 3.5 hr. Concentrations of norepinephrine, epinephrine, dopamine, serotonin (5HT) and the serotonin metabolite 5-hydroxyindoleacetic acid (5HIAA) were measured in bilateral sections of the hippocampus by HPLC with electrochemical detection. The concentrations of the monoamines were similar in all three diet-treatment groups. However, the turnover of 5HT, as indicated by the ratio of 5HIAA:5HT, was increased in the hippocampus from the IMB group when compared with either BAS or COR. Since increased turnover is thought to be representative of increased activity in neurons, these results suggest that acute diet-associated increases in the activity of the serotonergic system in the hippocampus may be involved in the animals' later adaptive responses to IMB. (Supported in part by NIH grants AM13252 and AM07355.)

5.9 BROMOCRIPTINE REVERSES COCAINE WITHDRAWAL. C.A. Dackis*and M.S. Gold, Research Facilities, Fair Oaks Hospital, 19 Prospect St., Summit, NJ 07901.

Dopamine (DA) neurons mediate cocaine reward and euphoria. Increased DA neurotransmission after reuptake blockade by cocaine presumably underlies its acute euphoric action. After cocaine abstinence, abusers often experience dysphoria, craving, and other withdrawal symptoms such as anergia, hypersomnia, and irritability. We have previously hypothesized that chronic cocaine abuse is associated with DA depletion, based on hyperprolactinemia in cocaine abusers, and DA disruptions in animals given cocaine. DA depletion may contribute to cocaine withdrawal.[1] In order to test this hypothesis, we administered the DA agonist bromocriptine to two female cocaine abusers (ages 18 & 20) after informed consent. Both patients had hyperprolactinemia (35.7 & 22.9 ng/ml) and intense cocaine craving. They quantified their degree of cocaine craving by marking a 100 mm line anywhere between the two poles, labelled "not at all" and "extremely." Fifteen minutes later, they blindly received either bromocriptine or placebo, and then rated their craving (see figure). The patients reported decreased subjective withdrawal complaints and objective decreases in self rated cocaine craving after bromocriptine but not placebo in all trials. One subject was given thioridazine, a DA antagonist in a similar design and rated a dramatic worsening of cocaine craving. These findings are consistent with DA depletion in chronic cocaine abusers, and indicate that bromocriptine may be an effective treatment for cocaine withdrawal.



[1]Gold MS, Dackis CA: Clinical Therapeutics, 7(1):6-21, 1984

5.10 *COMPARISON OF THERAPEUTIC EFFECTS OF RESERPINE AND ANIONS IN CATION-INDUCED ANXIETY. A.J. Giannini, W.A. Price, M.C. Giannini*, R.H. Loiselle. Dept. of Psychiatry, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Twenty four male volunteers received an exposure of ambient cations at a concentration of 2300 cations/cm^3 for 30 minutes. This was sufficient to induce anxiety in all volunteers as measured by BPRS. Half the volunteers were treated with one dose reserpine 0.2 mg po and the other with ambient anions. Both groups showed a significant reduction in anxiety within one hour. There were no significant differences between the anion- and reserpine-treated group. This indicates that the anxiogenic effects of cations may be mediated by biogenic amines.

GABA AND BENZODIAZEPINES: CELLULAR AND BEHAVIORAL EFFECTS

6.1 NEOCORTICAL PYRAMIDAL CELLS: $GABA_A$ AND $GABA_B$ MEDIATED RESPONSES AND TWO TYPES OF IPSP. B.W. Connors and R.C. Malenka. Depts. of Neurology and Psychiatry, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We have compared the responses to activation of $GABA_A$ and $GABA_B$ receptors with the inhibitory synaptic potentials in neocortex. Experiments were performed on slices of rat sensorimotor cortex in vitro, and intracellular recordings were obtained from physiologically identified pyramidal cells of layer II/III.

Local stimulation elicited a short latency depolarization consisting of a brief EPSP and IPSP followed by a late hyperpolarizing potential (LHP). The early IPSP had a reversal potential of -75 ± 3.2 mV (n=7), which was slightly positive to the resting membrane potential. The reversal potential for the LHP was -91 ± 5.7 mV (n=7). At resting potential, focal application of GABA elicited a depolarization; at potentials positive to rest the response was bi- or triphasic, typically consisting of depolarizing ($GABA_d$) and hyperpolarizing ($GABA_h$) phases that reversed at -51 ± 4.7 mV and -70 ± 4.3 mV (n=11) respectively. We were unable to segregate the two response phases by selective application of GABA to dendritic or somatic regions, although the relative prominence of each phase varied with the application site. Bicuculline and picrotoxin are $GABA_A$ receptor antagonists. Either drug (10 μM) suppressed the early IPSP, and resulted in a large increase in the initial EPSP; the LHP was unchanged or increased slightly in amplitude. Concurrently the $GABA_d$ response was blocked, leaving the $GABA_h$ response unaffected or larger. In the presence of an antagonist the $GABA_h$ reversal potential increased significantly (to -87 ± 2.3 mV, n=5), approaching that of the LHP. When E_{Cl} was shifted positively by decreasing extracellular [Cl], the reversal potentials for both the $GABA_d$ response and the early IPSP also shifted positively, while the LHP and $GABA_h$ response were relatively unaltered. No selective antagonists of $GABA_B$ receptors exist. However baclofen is a selective $GABA_B$ agonist, and its focal application increased membrane conductance and elicited a small, long lasting hyperpolarization that reversed at -91 ± 6.2 mV (n=9). The response to baclofen was resistant to GABA antagonists and changes in E_{Cl}.

The results suggest that $GABA_A$ receptors mediate both the early IPSP and the major part of the neuron's response to exogenous GABA. Both are largely Cl^--dependent, although the complexity of the response to GABA suggests that E_{Cl} may vary on different parts of the same neuron. The similar properties of the LHP, the $GABA_h$ response (in the presence of $GABA_A$ antagonists) and the baclofen response suggest that the LHP may be a $GABA_B$ receptor-mediated IPSP, although its mediation by other transmitters is not ruled out.

Supported by NIH grants NS 12151 and NS 19510.

6.2 PENICILLIN SHORTENS THE MEAN OPEN TIME OF GABA INDUCED MEMBRANE CHANNELS IN CULTURED SPINAL CORD NEURONS. D.A. Mathers* (SPON: E. Puil). Dept. of Physiol., Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Penicillin (PCN) can cause epileptiform seizures when large doses are administered to patients and experimental animals. The convulsant effect of PCN is thought to involve a depression of inhibitory postsynaptic potentials mediated by the neurotransmitter γ-aminobutyric acid (GABA) (Avoli, M., Brain Res. 323: 154, 1984). In the present study, the extracellular patch clamp method was used to resolve currents flowing through single GABA sensitive channels in the membrane of cultured mouse spinal cord neurons. This approach has allowed direct examination of the influence of PCN on the gating of these channels.

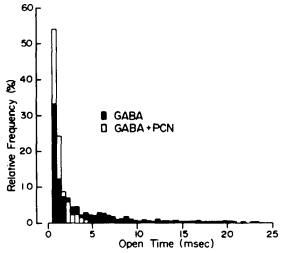


Fig. 1 shows the effect of 2 mM PCN on the open time distribution of GABA induced single channel currents recorded in an outside-out patch voltage-clamped to -60 mV (T=22°C). These distributions were normalized by expressing the number of observations in each time bin as a percentage of the total number of observed events. The first bin was not included in this analysis, since its event frequency was reduced by system bandwidth limitations. A Chi square test indicated tht PCN significantly altered the form of the open time distribution ($P<0.001$). During PCN action, the mean value of the plotted open times decreased from 3.6 msec (570 events) to 1.2 msec (538 events). PCN (2 mM) did not itself display significant agonist action on the GABA sensitive channels in these patches. These data indicate that PCN shortens the mean open time of GABA induced membrane channels, an effect consistent with the convulsant action of this drug.

6.3 ONSET AND SPONTANEOUS REVERSAL OF GABAERGIC SUBSENSITIVITY INDUCED BY CHRONIC DIAZEPAM. S.F. Gonsalves and D.W. Gallager, Dept. of Psychiatry and Neuroanatomy, Yale University School of Medicine and Abraham Ribicoff Research Facilities of the Connecticut Mental Health Center, New Haven, CT 06508

Recent electrophysiological studies have demonstrated that long-term exposure to benzodiazepines attenuates their ability to enhance postsynaptic GABAergic neurotransmission. These findings are consistent with clinical and behavioral evidence that tolerance develops to the pharmacological actions of benzodiazepines after chronic treatment. We have recently reported that slow continuous release of diazepam (DZ) from chronically ($\geq$ 3 weeks) implanted capsules selectively reduces sensitivity to microiontophoretically applied GABA in serotonergic dorsal raphe (DR) neurons (Gallager et al., Brain Res., in press). We now report the time course for the development of GABAergic subsensitivity following DZ capsule implantation and the time course for the reversal of GABAergic subsensitivity following removal of the capsules.

Two silastic capsules containing 90 mg of crystalline diazepam were implanted subcutaneously in male Sprague-Dawley rats under halothane anesthesia. This protocol has previously been shown to release the equivalent of 5 mg/kg of diazepam per day. Control rats received empty capsules. Microiontophoretic sensitivities to 5HT and GABA were determined in serotonergic DR neurons 1, 2 and 3 weeks after the capsules were implanted. Sensitivities were quantified as IxT_{50} values, i.e., the product of iontophoretic current (nA) and time (sec) required to reduce spontaneous firing rate of a recorded cell to 50% of its basal rate. Our results indicated that GABAergic subsensitivity was fully expressed after 2 weeks of exposure to the DZ implants (DZ 2 wk: IxT_{50} 43.0±4.1; DZ 3 wk: IxT_{50}= 46.7±3.1; VEH: IxT_{50}= 30.4 ±1.5, p<.05). GABAergic responsiveness at 1 week was not significantly different from control (DZ 1 wk: IxT_{50}= 29.0±2.8; VEH: IxT_{50}= 30.4 ±1.5, ns). Sensitivity to 5HT was not altered from control at any of the time points tested. In a second group of rats, the time course for spontaneous reversal of GABAergic subsensitivity was estimated after surgical removal of the implants (21 days minimum exposure). Responsiveness to GABA remained depressed for 5 days after the removal of the implants (DZ 5 day withdrawal: IxT_{50}= 44.0± 4.9; VEH: IxT_{50}= 29.4±1.2, p<.005) with control responsiveness returning by 8 days (DZ 8 day withdrawal: IxT_{50} = 33.5±2.9; VEH: IxT_{50}= 29.4±1.2, ns). In contrast, a single injection of the specific benzodiazepine antagonist, Ro15-1788, on the final day of chronic treatment restored GABAergic sensitivity to control levels within 24 hours. (Support: Klingenstein Fund, USPHS MH 14276 & NS 19655, Epilepsy Foundation and the State of Connecticut).

6.4 THE EFFECT OF BENZODIAZEPINES ON CHOLECYSTOKININ-INDUCED EXCITATION OF RAT HIPPOCAMPAL PYRAMIDAL NEURONS APPEARS NOT TO BE MEDIATED BY THE POTENTIATION OF GAMMA-AMINOBUTYRIC ACID. J. Bradwejn and C. de Montigny. Neuroscience Research Center, Université de Montréal, Montréal, Canada.

We have previously shown that benzodiazepine [BZD] receptor agonists, by activating BZD receptors, selectively suppress cholecystokinin (CCK)-induced excitation of rat hippocampal pyramidal neurons (Nature 312: 363, 1984). Since γ-aminobutyric acid (GABA) receptors coexist with BZD receptors, we undertook the present studies to determine whether GABA mediates the antagonism by BZD's of CCK-induced activation.

Male Sprague-Dawley rats (200-300 g) were anesthetized with urethane (1.25 g/kg, i.p.). Five-barrelled micropipettes were used for extracellular unitary recording of CA_3 dorsal hippocampus pyramidal neurons. The following substances were used for microiontophoresis: sulphated CCK octapeptide (10 μM in 200 mM NaCl, pH: 5; Squibb), acetylcholine.HCl [ACh] (20 mM in 200 mM NaCl, pH: 4; Calbiochem), flurazepam.HCl [FLU] (200 mM, pH: 4; Hoffman-Laroche), Met-enkephalin [mENK] (500 M in 200 mM NaCl with BSA 0.01%, pH: 4.6; Sigma), GABA (10 mM in 50 mM NaCl, pH: 4; Calbiochem) and bicuculline (10 mM in 100 mM NaCl, pH: 4; Sigma).

Microiontophoretic application of GABA during the activation of hippocampal pyramidal neurons by CCK, ACh or mENK reduced the firing activity of these neurons to the same extent for the three excitatory neurotransmitters. Activations induced by pulse applications of CCK, ACh or mENK were also reduced to the same extent by background microiontophoresis of GABA. The intravenous administration of picrotoxin (2.5 mg/kg), while completely blocking the effect of microiontophoretically-applied GABA, did not alter the suppression of CCK-induced activation by microiontophoretic application of FLU. Similarly, microiontophoretic application of bicuculline blocked the suppressing effect of GABA, but not that of FLU, on CCK-induced activation.

The results of the first two series of experiments show that, unlike BZD's, GABA does not have any selectivity for CCK-induced activation, as would be expected if a potentiation of GABA were underlying the effect of BZD on CCK-induced activation. The results obtained with picrotoxin and bicuculline show that the ability of FLU to antagonize CCK-induced activation is not affected by GABA receptor blockade. Hence, these results suggest that potentiation of GABA neurotransmission might not be the mechanism through which BZD's selectively suppress CCK-induced activation. This conclusion is consistent with the data of other investigators, suggesting that some of the clinical effects of BZD's, such as anxiolysis, might not be due to the potentiation of GABA neurotransmission.

6.5 INTERACTION OF t-BUTYLBICYCLOPHOSPHOROTHIONATE WITH GABA-GATED CHLORIDE CHANNELS IN CULTURED NEURONS FROM THE CHICK EMBRYO CEREBRUM. M. H. Jalilian Tehrani* and E. M. Barnes, Jr. Dept. of Biochemistry, Baylor Coll. of Med., Houston, TX 77030.

The specific, picrotoxinin-displaceable, binding of [^{35}S]t-butylbicyclophosphorothionate (TBPS) to isolated membranes of chick cerebral neurons, 8 days in culture, was examined by saturation studies at equilibrium. Scatchard plots of these data were curvilinear. Using non-linear regression analysis via a SCAFIT/LIGAND computer program, the best fit was obtained by a model based on two TBPS binding sites which differ in affinity and density: $K_{d(1)}$ = 3.1 nM, $B_{max(1)}$ = 0.031 pmol/mg; $K_{d(2)}$ = 270 nM, $B_{max(2)}$ = 3.9 pmol/mg. The dissociation constants for these two sites were similar to those found in membranes from adult rat and chicken cerebral hemispheres [Tehrani, M.H.J., et al. (1985) J. Neurochem., in press]. As in adult tissues, the specific binding of TBPS to membranes from cultured neurons was displaced by GABA (IC_{50} = 0.6 μM).

The effect of TBPS on $^{36}Cl^-$ uptake by intact cerebral neurons was studied in monolayers of cultured cells. The entry of $^{36}Cl^-$ was resolved into basal (GABA-independent, picrotoxinin-insensitive) and GABA-dependent (picrotoxinin-sensitive) permeabilities. The latter process exhibited a $K_{0.5}$ = 1.5 μM for GABA and an apparent V_{max} of 5.3 nmol Cl^-/sec/mg. This $K_{0.5}$ value was in reasonable agreement with the IC_{50} for GABA displacement of TBPS binding. Kinetic analysis of the GABA-gated Cl^- flux revealed that TBPS is an inhibitor (IC_{50} = 280 nM) whose action is non-competitive (K_i = 140 nM) with respect to GABA. TBPS also blocked the basal Cl^- flux observed in the absence of GABA, but higher concentrations were required (TBPS IC_{50} = 8 μM). Since no inhibition of Cl^- flux was observed at TBPS concentrations near its $K_{d(1)}$ for binding, the high affinity site may represent a physiologically inactive form of the receptor. The TBPS dissociation constant for the binding site of lower affinity, $K_{d(2)}$, agreed well with the IC_{50} and K_i values for inhibition of GABA-gated Cl^- flux, and thus is likely to represent an interaction with active Cl^- channels.

Supported in part by grants AM 17436 and NS 11535 from NIH and DAMD17-84-C-4102 from USAMRDC.

6.6 PHARMACOLOGICAL CHARACTERIZATION OF THE GABA/BENZODIAZEPINE RECEPTOR-CHLORIDE IONOPHORE COMPLEX USING A GABA-INDUCED ^{36}CI INFLUX ASSAY IN CULTURED SPINAL CORD NEURONS. P.F. Lehoullier* and M.K. Ticku (SPON: J. Maas) Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

GABA-mediated inhibition in the nervous system has been shown in electrophysiological experiments to be due to an increase in chloride ion conductance across the neuronal cell membrane. The present study describes a biochemical method of measuring GABA-stimulated ^{36}Cl influx in cultured mouse spinal cord neurons and the effects of various GABA-modulating drugs on this functional assay. The cultures were prepared according to methods described previously (Ransom, et al., J. Neurophysiol., 40:1132, 1977), with the exception that the cells were grown on poly-l-lysine coated plastic coverslips. Following 5 to 6 days in culture, the cells on coverslips were removed from tissue culture medium and incubated at 22°C for 20s in physiological buffer (136 mM NaCl, 5.4 mM KCl, 1.4 mM $MgCl_2$, 1.2 mM $CaCl_2$, 1 mM NaH_2PO_4, 20 mM HEPES, pH 7.4) containing ^{36}Cl in the absence and presence of GABA and/or other drugs. In the absence of any drug the time course of ^{36}Cl influx up to 30s was linear with time. For a 20s incubation, basal ^{36}Cl influx was 6.3 ± 0.9 nmol ^{36}Cl/mg protein and in the presence of 100 μM GABA the influx increased to 12.1 ± 0.9 nmol ^{36}Cl/mg protein. The half-maximal ^{36}Cl influx occurs at approximately 10 μM. GABA-induced ^{36}Cl influx was blocked by (+)bicuculline and picrotoxinin. (+)Bicuculline, in the absence of added GABA, had no effect on basal ^{36}Cl influx, indicating that basal influx is GABA-insensitive. Pentobarbital (25 to 100 μM) potentiated the effect of submaximal concentrations of GABA (20 μM) on ^{36}Cl influx. Pentobarbital in the absence of GABA, at concentrations greater than 100 μM, increased ^{36}Cl influx above basal values. Diazepam (100 nM to 10 μM) also caused a concentration-dependent potentiation of the GABA-induced ^{36}Cl influx. These results are consistent with the electrophysiological evidence and provide us with a functional assay for measuring the properties of the GABA receptor-chloride ionophore, and for characterizing the GABA synaptic pharmacology.

6.7 **IDENTIFICATION OF A DISCRETE SITE IN FOREBRAIN AS A SUBSTRATE FOR THE GENESIS OF CHEMICALLY-INDUCED SEIZURES.** S. Piredda* and K. Gale, (SPON: D. Stoff), Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Although systemic injections of chemoconvulsive agents have been used to create experimental models of generalized epilepsy, to date there has been no identification of a specific locus at which these drugs act to initiate generalized seizures. By directly microinjecting varying doses of chemoconvulsant agents into several brain loci, we have discovered a discrete area in the forebrain from which bilateral motor seizures are elicited after unilateral application of pmol amounts of bicuculline, kainic acid or carbachol, nmol amounts of n-methyl-d-aspartate and physostigmine, and umol amounts of glutamate and aspartate; strychnine was ineffective at this site. This is, therefore, the first identification of a site in the brain from which bilateral motor seizures can be elicited by a single, unilateral and focal microinjection of chemoconvulsants in low doses. This site is located in the deep prepiriform cortex (DPC) and is less than 2.0 mm in diameter. When microinjections of the convulsants were placed in areas adjacent to this active site, no convulsant actions were obtained. The seizures were similar to those evoked by kindling of the limbic system. The bilateral manifestation was evident both behaviorally (forelimb clonus, rearing and falling) and electrographically.

It appears that GABA-mediated inhibition exerts a tonic control of the excitatory mechanisms identified as involved in the origin of these seizures: direct microinjection of muscimol (40 or 80 pmol) prevented seizures induced by the local application of all convulsants examined. In contrast, atropine (150 pmol) prevented only those seizures induced by carbachol, but not those induced by bicuculline and kainic acid. This suggests that the cholinergic system is not crucial for the genesis of seizures from this region. Some other excitatory input must therefore be responsible for the seizures initiated by the local removal of GABAergic inhibition. A specific n-methyl-d-aspartate receptor antagonist, 2-amino-7-phosphonoheptanoic acid (2APH) (100 pmol), was effective in preventing seizures induced by focal application of all convulsants examined, suggesting that excitatory amino acid transmission may be critical for evoking seizures from this locus.

Supported by HHS Grants NS 20576 and DA 02206.

6.8 **ANTICONVULSANT EFFECTS OF GABA AGONISTS AND AN EXCITATORY AMINO ACID ANTAGONIST MICROINJECTED IN THE DEEP PREPIRIFORM CORTEX.** K. Gale, M. Pavlick*, and S. Piredda*. Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

We have recently identified a discrete site in the rat deep prepiriform cortex (DPC) from which bilateral motor and electrographic seizures can be induced by the unilateral microapplication of pmol amounts of bicuculline (Piredda, Lim and Gale, Life Sci. 36:1295-1298, 1985). To determine whether this site plays a crucial role in the development of seizures induced by the systemic administration of bicuculline, we microinjected GABA agonists bilaterally into the deep prepiriform cortex via chronic indwelling cannulas and examined responses to intravenous bicuculline. Generalized seizures induced by bicuculline (.36 mg/kg i.v.) were prevented by direct microinjection of muscimol (5 ng) or gamma-vinyl-GABA (GVG, 5 ug) bilaterally into DPC. Muscimol was effective when microinjected 15 minutes prior to seizure testing; GVG was effective at six and 24 hour after microinjection, consistent with its irreversible inhibition of GABA-transaminase. In addition, 2-amino-7-phosphonoheptanoic acid (2APH, 225 ng), an antagonist of n-methyl-d-aspartate receptors, protected against seizures induced by i.v. bicuculline when the rats were tested 15 minutes following application of 2APH bilaterally into DPC. No obvious behavioral or neurological changes were associated with the drug injections into DPC. These results indicate that a change in the balance between GABA-mediated inhibition and excitatory amino acid transmission in DPC may be a critical mechanism for the initiation of seizures by systemic bicuculline.

In contrast, GVG (10 ug) bilaterally microinjected into DPC was completely ineffective against seizures induced by maximal electroshock (MES) when rats were tested at six or 24 hours after GVG microinjection. These results are consistent with previous studies (Iadarola and Gale, Science 218:1237-1240, 1982) showing that elevation of GABA in substantia nigra and not in any other brain region, confers protection against MES.

Thus enhanced GABA transmission in DPC is selectively effective against chemically-induced seizures. This, taken together with the ability of several chemoconvulsants to induce seizures upon direct application to DPC suggests that the DPC is a likely site of origin of chemically-induced seizures.

Supported by HHS Grants NS 20576 and DA 02206.

6.9 CHRONIC TREATMENT WITH FG 7142 POTENTIATES CONVULSIONS INDUCED BY ISONIAZID AND DMCM. G. Biggio, O. Giorgi, B. Longoni* and M.G. Corda. Institute of Biology, Chair of Pharmacology, University of Cagliari, Italy.

We have previously shown that repeated intraventricular administration of β-CCE (10 μg/twice a day) potentiates, three days after the last administration, the convulsive pattern elicited by isoniazid (1). Here we report that chronic (10 days) intraperitoneal administration of FG 7142 produces a long-lasting (25 days) potentiation of the convulsive effects of isoniazid and DMCM. Rats were treated with FG 7142 (15 mg/kg i.p. twice a day) for 10 consecutive days. Control groups received an equivalent volume of Tween 80 vehicle. A challenge dose of isoniazid (350 mg/kg s.c.) was administered 5,10 or 25 days after the end of the chronic treatment. Control rats showed clonic seizures starting 40 to 90 minutes after the administration of isoniazid. None of them showed more than one convulsive episode. The convulsive effects of isoniazid on FG 7142-treated rats had the same latency as compared with control animals but a two-three fold increase in the number of convulsive episodes per rat was observed. Moreover, the pattern of convulsions displayed by these rats was distinguished by the appearance of severe tonic hindlimb extension. The convulsive action of DMCM (0.33 mg/kg i.v.) was also potentiated by chronic FG 7142. In fact, clonic seizures were observed in 30 and 80% of control and FG 7142 treated rats respectively. The effect of FG 7142 persisted for at least 25 days after the last treatment. The behavioural effcts elicited by FG 7142 were paralleled by a marked decrease in the density of low affinity GABA receptors in different brain areas. The results indicate that chronic administration of β-carbolines induces in the rat brain a persistent down-regulation of GABA receptors which in turn potentiates the convulsive effect of isoniazid and DMCM.

(1) Concas, A., Serra, M., Salis, M., Nurchi, V., Crisponi, G. and Biggio, G., Neuropharmacology, 23(3), 323, 1984).

6.10 CHRONIC FG 7142 ADMINISTRATION INDUCES LONG LASTING PROCONFLICT EFFECT AND GABA RECEPTOR DOWN-REGULATION. M.G. Corda and G. Biggio. Institute of Biology, Chair of Pharmacology, University of Cagliari, Italy

The recent finding that the "proconflict" effect of β-carboline derivatives is potentiated and mimicked by different GABA function inhibitors, suggests that β-carboline-induced anxiety may result from a decrease in the GABAergic activity at the level of the GABA/benzodiazepine receptor complex. To further verify this hypothesis we studied the effect of chronic administration of the β-carboline derivative FG 7142 on an animal model of anxiety and on the kinetic parameters of brain GABA receptors. Adult male rats were injected with FG 7142 (15 mg/kg /twice a day/i.p.) or its Tween 80 vehicle for 10 days. Four and fifteen days after the last injection rats were tested in a modified Vogel's conflict test (PNAS 80: 2072, 1983) to detect proconflict drug action. Punishment-suppressed behaviour was enhanced four days (Control: 24 ± 1.9; FG 7142: 11 ± 1.6 shocks, $P<0.02$) and fifteen days (Control: 27 ± 2.1; FG 7142: 16 ± 1.4 shocks, $P<0.05$) after the last injection. On the other hand the same treatment failed to affect unpunished behaviour. In a parallel series of experiments ^{3}H-GABA binding was measured in different brain areas. Four and fifteen days after the last FG 7142 administration a 25% decrease in the specific binding of ^{3}H-GABA was found in the cortex and cerebellum of FG 7142-treated rats. As revealed by the Scatchard plot analysis the decrease was due to a reduction in the maximum number of binding sites (Bmax) with no change in the apparent dissociation constant (K_D). The results suggest that the long-lasting proconflict effect induced by chronic FG 7142 administration is the consequence of a persistent down-regulation of GABA receptors. This finding may provide useful clues to elucidate the molecular mechanism involved in persistent anxiety states in humans.

6.11 ANTICONVULSANT BUT NOT THE HYPNOTIC EFFECT OF BARBITURATES APPEARS TO BE MEDIATED VIA GABAergic TRANSMISSION. M.K. Ticku and S.K. Rastogi*. Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284 and Lafayette Clinic, Detroit, MI.

To define if the anticonvulsant and/or hypnotic effect of barbiturates is mediated through the GABA system, we compared the behavioral profile of the optical isomers of 1-methyl-5-phenyl-5-propylbarbituric acid (MPPB). R(-)MPPB produced dose-related loss of righing reflex, whereas S(+)MPPB produced dose-related convulsions. Subconvulsive doses of R(+)MPPB were proconvulsant with a single subeffective dose of picrotoxin but not with subeffective dose of strychnine. The seizures induced by S(+)MPPB were blocked by R(-)MPPB and pentobarbital. The loss of righting reflex of R(-)MPPB or pentobarbital was not blocked by the convulsant S(+)MPPB. The radioligand binding studies demonstrated that S(+)MPPB interacts with the picrotoxinin site as studies with [^{35}S]t-butylbicyclophosphorothionate (TBPS) competitively, whereas R(-)MPPB inhibited it noncompetitively. These isomers also give a differential profile on the dissociation of [^{35}S]TBPS; R(-)MPPB accelerating the dissociation of TBPS the most and S(+)MPPB the least (J. Neurochem. 44:480-486, 1985). Pentobarbital also protected against maximal electroshock seizures in rats in a dose-related manner. This protective effect of pentobarbital was blocked by bicuculline. Subprotective doses of pentobarbital in combination with a single subprotective dose of ethanol or diazepam produced a potentiation of the loss of righting reflex and the anticonvulsant effect of pentobarbital. Bicuculline, picrotoxin and RO15-1788 did not block the loss of righting reflex produced by pentobarbital. These results indicate that a) the anticonvulsant but not the hypnotic effect of barbiturates is mediated via GABAergic transmission; and b) anticonvulsant and convulsant barbiturates bind to distinct and/or possibly overlapping sites on the GABA receptor complex.

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PEPTIDES: RECEPTORS I

7.1 BRAIN-HEART PEPTIDES: VISUALIZATION OF ATRIOPEPTIN RECEPTORS IN MAMMALIAN BRAIN. M. Dalpe*, T.V. Dam* and R. Quirion. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill Univ., Verdun, Quebec H4H 1R3 Canada

Recently, we reported on the autoradiographic distribution of [^{125}I]atrial natriuretic factor (ANF) 8-33 binding sites in rat brain (Quirion et al, Peptides, 5, 1167-1172, 1984). We have now further characterized these sites using [^{125}I] rat and human atriopeptin. Male guinea pigs (500 g) were killed by decapitation and brains were rapidly removed and processed for receptor autoradiography (Quirion et al, Peptides, 5, 1167-1172, 1984) or for membrane binding assays. Assays were performed as follows: cerebellum and thalamus-hypothalamus areas were homogenized separately and then centrifuged at 49,000 g for 20 min. Membranes preparations were subsequently incubated for 60 min in 50 mM Tris.HCl buffer, pH 7.4 at 25^{0}C plus 150 mM NaCl, 5 mM $MnCl_2$, .01 mM EDTA. Na_2, 0.5% BSA and various concentrations of either (3-[^{125}I]iodotyrosyl^{28}) rat atrial natriuretic peptide (ANP) or (3-[^{125}I]iodotyrosyl^{28}) human α-atrial natriuretic peptide (α-ANP). Incubations were terminated by rapid filtration under reduced pressure through glass fiber filters (GF/C) presoaked in 0.1% polyethyleneimine. Specific binding of both ligands was determined as the difference in radioactivity bound in presence and absence of 1 μM ANF (8-33). In vitro ANP receptor autoradiography was performed exactly as described for ANF (8-33) by Quirion et al, Peptides, 5, 1167-1172, 1984. Our results indicate that both ANP and α-ANP bind with high affinity (0.02 - 0.08 nM) to a single class of saturable binding sites in guinea pig cerebellum and thalamus-hypothalamus area. Ligand selectivity pattern demonstrates that human α-ANP > ANF (8-33) > atriopeptin II in both tissues. In vitro receptor autoradiography demonstrates that ANP binding sites (in either ANP or α-ANP) are very discretely distributed in guinea pig brain. Moderate to high densities of sites are found in the external plexiform layers of the olfactory bulb, subfornical organ, lateral-medial nucleus of the amygdala, amygdalo-hippocampal area, paraventricular, paratenial, rhomboid and reuniens nuclei of the thalamus, granular cell layers of the dentate gyrus, medial geniculate nucleus and lobules 9 and 10 of the cerebellum. Very low to low densities of ANP sites are present in remaining brain regions. Thus, this study demonstrates the existence of specific ANP binding sites in mammalian brain and suggests the existence of a new family of brain-heart peptides.

7.2 ONTOGENY OF SUBSTANCE P RECEPTORS IN RAT BRAIN. T.V. Dam* and R. Quirion (SPON: P. Gaudreau). Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Quebec H4H 1R3 Canada.

Recent data have demonstrated the existence of high affinity binding sites for substance P (SP) in mammalian brain, including man (Quirion and Dam, in press). SP binding sites are mainly concentrated in forebrain region such as the olfactory bulb, caudate-putamen, septum, nucleus accumbens and hippocampus. In the brainstem, very low to low densities of SP sites are found except in the locus coeruleus, parabrachial nucleus, nucleus ambiguus and inferior olive, all areas enriched in SP binding sites (Shults et al, Peptides, 5, 1097-1128, 1984). We now report on the ontogeny of SP binding sites in rat brain using membrane binding assays as well as in vitro receptor autoradiography. 15 days pregnant female Sprague-Dawley rats were individually housed and had free access to food and water. The development of brain SP binding sites has been studied both pre- and post-natally in -3, -1, +3, +7, +14, +21 days and 3 months old rats of either sexes. Brain membranes were prepared for [^{3}H]SP binding assay as described before (Park et al, Peptides, 5, 833-836, 1984). In vitro SP receptor autoradiography was performed as described by Shults et al (Peptides, 5, 1097-1128, 1984). Our data indicate that SP binding sites reach maximal value one day before birth (B_{max} = 177.0 fmol/mg protein) and then decreased 3 days after birth (88.8 fmol/mg protein) to finally stabilize at adult levels (54.6 fmol/mg protein) fourteen days after birth (59.4 fmol/mg protein). Interestingly, the autoradiographic distribution demonstrates that SP binding sites undergo important redistribution during development. Prenatally (-3 and -1) and early after birth, (+3 and +7), SP binding sites are heavily concentrated in the brainstem with low densities in the forebrain while the opposite is true in adult brain. These data demonstrate the plasticity of SP binding sites and suggest possible roles for SP during brain development.

7.3 RAT BRAIN AND GUINEA-PIG ILEUM TACHYKININ SP-P RECEPTORS ARE REGULATED BY DIVALENT CATIONS AND GUANINE NUCLEOTIDES.
Prem Mohini* and José M. Musacchio. Dept. of Pharmacology, New York Univ. Med. Ctr., New York, NY 10016.

[^{3}H]Physalaemin ([^{3}H]PHY) was used to label the tachykinin SP-P receptor in rat brain and in the guinea-pig ileum. The binding of [^{3}H]PHY is specific, saturable and reversible. In the rat brain, monovalent cations increase the binding of [^{3}H]PHY in an ionic strength dependent manner. Addition of 2.5 mM $MnCl_2$ results in a two fold increase in affinity and a 40 percent increase in B_{max}. Scatchard analysis demonstrates a single population of noninteracting sites with a K_D of 3.3 nM and a B_{max} of 81.3 fmol/mg protein. GTP and Gpp(NH)p decrease the B_{max} by 33-40 percent in the presence of either 125 mM Na_2SO4, 2.5 mM $MnCl_2$ or both. However, guanine nucleotides do not decrease the CNS SP-P receptor affinity for [^{3}H]PHY.

Divalent cations also markedly increase the affinity of Gpp(NH)p for its binding sites as indicated by the increased effectiveness of guanine nucleotides to inhibit binding to the SP-P receptors.

Preincubation of the membranes with N-ethylmaleimide (NEM) or p-chloromercuribenzoate for 30 min irreversibly inhibited the binding of [^{3}H]PHY with an IC_{50} of 1.0 and 0.15 mM respectively. If the SP-P receptors were protected with 10 μM PHY, 3 mM NEM did not inactivate binding, but inhibited the effect of both, divalent cations and guanine nucleotides. These effects could be prevented by reduced glutathione or DTT.

Similar experiments in the guinea-pig ileum also demonstrated that manganese increases the binding of [^{3}H]PHY and that Gpp(NH)p inhibits it. Likewise, if the membranes are pretreated with NEM while the receptors are protected with 10 μM PHY, the effects of divalent cations and guanine nucleotides are blocked.

Since NEM has also been found to inhibit the regulatory effects of guanine nucleotides on adenylate cyclase coupled receptors, our findings indicate that even though the SP-P receptors are not linked to adenylate cyclase, the binding of SP-P agonists may be regulated by a GTP-binding regulatory protein.

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7.4 ANGIOTENSIN II RECEPTOR BINDING IN THE BRAIN OF SPONTANEOUSLY HYPERTENSIVE RATS: AN AUTORADIOGRAPHIC QUANTITATIVE STUDY.
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Angiotensin II (AII) is known to participate in several brain functions including the cardiovascular regulation. We used ^{125}I-[Sar^1,Ile^8]-AII (^{125}I-SI-AII), an AII antagonist, as radioactive ligand, and localized AII receptors in many parts of the central nervous system of the spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats.

The K_d values for ^{125}I-SI-AII binding to AII receptors in the membrane preparation from the septum/anteriorventral part of the third ventricle, dorsal medial brain stem, olfactory bulb and thalamus were around 0.1 nM. The B_{max} values were between 6.95 ± 1.60 to 15.52 ± 4.99 fmol/mg protein. Heavy labelling for AII receptors was recorded in nucleus tractus solitarius (NTS), paraventricular hypothalamic nucleus (PVN), subfornical organ (SFO), suprachiasmatic nucleus (SCN), area postrema, the dorsal motor nucleus of the vagus (DMV), and the nucleus of spinal tract of the trigeminal system (NST).

Among five areas assessed by using quantitative receptor autoradiography in conjunction with ^{125}I-standards, the NTS possesses the highest AII receptor binding. For example, there were 0.85 ± 0.05 fmol/mg protein AII binding in the NTS of 4-week old SHR rats, whereas there were 0.80 ± 0.17 fmol/mg protein AII binding in the NTS of age-matched WKY rats after incubation of 20-μ sections with 90 pM ^{125}I-SI-AII for 60 min. Non-specific binding was determined in the presence of 1 μM non-labeled SI-AII in the above ^{125}I-SI-AII. The heavy labelling of AII antagonist in the PVN, SFO, NTS, DMV, and NST indicates that there are high densities of AII receptors in these areas. One of the most interesting findings of this study is that SHR rats at early hypertensive (7 weeks) and established hypertensive (16 weeks) stages contained significantly higher AII receptor binding in the NST, as compared to age-matched WKY rats. Furthermore, AII receptor binding was significantly higher in the DMV of SHR rats at 16 week established hypertensive stage, but not at 7-week stage. It has been documented that the NST and DMV also contain high densities of opiate receptors. The literature and the present study are therefore collectively in favor of a notion that AII in association with the opiate system in the DMV and NST may play important roles in the development of hypertension in SHR rats. (Supported in part by AHA851334.)

7.5 COMPARISON OF NEUROTENSIN ANALOGS FOR SPECIFIC BINDING TO NEUROBLASTOMA CLONE N1E-115 AND INTRACELLULAR STIMULATION OF CYCLIC GMP.
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Neuroblastoma clone N1E-115 possesses receptors specific for neurotensin (NT), an endogenous tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu). Neurotensin bound to receptors on N1E-115 cells cultured in growth medium containing fetal bovine serum with an equilibrium dissociation constant (K_D) of 11 nM, stimulating the intracellular production of cyclic GMP with a potency (EC_{50}) of 1.5 nM. A number of neurotensin analogs and fragments were compared for their ability to inhibit [^{3}H]neurotensin binding and induce intracellular cyclic GMP formation with intact N1E-115 cells. A direct correlation was found between the EC_{50} for each of these peptides in stimulating cyclic GMP production and its K_D for binding to neurotensin receptors.

The carboxyl-terminal half of neurotensin, NT(8-13), proved to be fifty times as potent as intact neurotensin in stimulating intracellular cyclic GMP formation and 17 fold more active in competing with [^{3}H]neurotensin binding. In contrast, NT(1-6) and NT(1-8) displayed no binding or biochemical activities, and substitutions of amino acids in the amino-terminal half of neurotensin did not significantly alter the biochemical activity of those analogs (e.g., [Gln^4]NT and [D-Pro^7]NT). Modifications in the carboxyl-terminal half of neurotensin produced drastic changes in activity. NT(1-11), NT(1-10), and NT-NHMe were inactive. NT(9-13) had 5 fold less biochemical activity than did native neurotensin and a K_D twenty times larger. [D-Arg^8]NT had the same biochemical potency as did neurotensin with half the binding activity, while substitution of a D-phe in position 11 and a D-pro in position 10 of neurotensin almost completely destroyed the biochemical and binding activities. [D-Trp^{11}]NT and [D-Tyr^{11}]NT retained some functional capabilities at very high concentrations. Replacement of the Tyr^{11} of neurotensin with Phe^{11} had no significant effect on the EC_{50} for stimulating cyclic GMP formation but it increased the K_D almost 7 fold.

All of the neurotensin peptides which were active in stimulating intracellular cyclic GMP production induced maximal responses similar to that of neurotensin itself except for those analogs with D-amino acids in positions 10 or 11, which produced maximal cyclic GMP levels that were routinely lower than that for neurotensin. In addition, [D-Trp^{11}]NT, a reported antagonist for the neurotensin receptor, displayed no ability in the concentration range of 0.01 to 1000 nM to inhibit the stimulation by neurotensin of intracellular cyclic GMP formation. (Supported by Mayo Foundation and USPHS Grant MH 27692).

7.6 NEURONAL CHOLECYSTOKININ RECEPTORS: BINDING CHARACTERISTICS AND EFFECTS ON SECOND MESSENGER SYSTEMS.
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It has previously been reported that cholecystokinin (CCK) recognition sites in the brain differ from those in the pancreas (Science, 208: 1155, 1980). CCK binding sites in membrane preparations from mouse and guinea pig cerebral cortex recognize carboxyl terminal fragments of CCK-8, CCK-4 being the smallest peptide with nanomolar potency. In the pancreas, the IC_{50} of CCK-4 is 1000-fold greater.

To determine further the structural requirements for binding to neuronal CCK receptors, some tetrapeptide analogs were evaluated on ^{125}I-(BH)-CCK-8 binding to mouse cortical membranes, according to published procedures. (Life Sciences 36: 1485, 1985). The results indicated that to retain nanomolar potency:

- The carboxyl terminus must be amidated. (The IC_{50} of CCK-4 was 5.9 nM, whereas the free acid was inactive up to 1.3 μM; Gly extension of the C-terminus reduced activity).
- The amino terminus may be free, but its blockade by t-BOC increased potency more than 20-fold.
- The β-carboxyl of Asp-32 should be free; esterification or amidation of Asp-32 markedly reduced activity.
- Replacement by Gly of each substituent amino acid residue in CCK-4 reduced potency 1000-fold.
- Ser or Gly insertion between Met and Asp abolished activity.

Despite the marked differences between brain and pancreas recognition sites, CCK receptors in both tissues have been reported to be coupled to phosphatidylinositol (PI) hydrolysis (J.B.C., 259: 4346, 1984; Cell Calcium, 3: 413, 1982). However, the effect was not confirmed in brain slices; CCK-8 did not induce inositol phosphate accumulation in ^{3}H-inositol-prelabeled mouse cortical slices under conditions where acetylcholine increased PI hydrolysis 500-600%. Furthermore, although CCK-8 modulates cerebellar cGMP (Steel,Wood and Petrack, this volume), the peptide did not affect basal or dopamine-activated adenylate cyclase in rat brain P_2 membranes or bovine retina.

We conclude that the differences between central and peripheral CCK recognition sites are reflected also in differences in effector coupling mechanisms.

7.7 AUTORADIOGRAPHIC LOCALIZATION OF CHOLECYSTOKININ RECEPTORS IN PRIMATE CORTEX. M.F. Kritzer, R.B. Innis, and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT. 06510.

Cholecystokinin (CCK), a putative peptide neurotransmitter, is found in high concentrations in the cerebral cortex of many species. Although CCK binding sites have been described in several of these species, the highly differentiated primate cerebrum allows a more fine-grained analysis of regional variation in the distribution of cortical receptors. We report here in vitro autoradiographic localization of CCK binding in the cerebral cortex of three macaque monkeys labeled with ^{125}I-Bolton-Hunter CCK-33. Briefly, thaw-mounted cryostat sections (20μ) of unfixed tissue were incubated in 50pM ^{125}I-CCK-33, dried, and placed against LKB Ultrofilm. The addition of 10^{-6}M CCK-8 to the incubation media was used to determine non-specific binding. Binding studies in tissue homogenates and slide-mounted sections showed that ^{125}I-CCK-33 labels a high affinity, saturable site in primate cortex, similar to that of rodent brain.

The laminar organization of CCK binding sites varies among cortical lobes and major cytoarchitectonic fields. In the frontal lobe, the distribution of binding sites is discontinuous: dense radiolabel is seen in the principal and cingulate sulci, while the interposed dorsomedial cortex is only lightly and diffusely labeled. Within all areas of the frontal cortex, radiolabel is distributed with highest concentration in layer IV. The parietal lobe has a similar laminar distribution, and also shows marked regional variation. For example, label is pronounced in layer IV in the rostral somatosensory cortex, but is very light in the intraparietal cortex. In the temporal lobe, from posterior amygdaloid to mid-hippocampal levels, high concentrations of putative CCK receptors are found in layer IV throughout the superior and inferior temporal cortex. A novel finding is the emergence of a bilaminar pattern in the inferior temporal cortex, with dense binding in layer II as well as layer IV. The occipital lobe displays a unique laminar distribution of binding sites which is uniform throughout primary visual striate cortex. In this region, radiolabel is heavy in layers I, V and VI, and, in contrast to other areas, layer IV is more lightly labeled.

Thus, CCK binding is unevenly distributed in primate cerebral cortex. Variations in density of putative CCK receptors across cytoarchitectonic regions and specific laminae in macaque monkey indicate that this peptide may have selective rather than diffuse influences on cortical functioning in primates.

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7.8 THE PEARL MUTATION INDUCES HYPERSENSITIVITY OF THE RETINA TO SOMATOSTATIN-14. H. Suzuki* and L. H. Pinto. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

We recorded action potentials from retinal ganglion cells, in the isolated superfused retinas of wild-type (C57BL/6J), pearl mutant (*pe*/*pe*) and pearl revertant (*pe*$^+$/*pe*$^+$) mice. Somatostatin of either 14 (SS-14) or 28 (SS-28) amino acid composition was introduced into the superfusate while the response of an on-center cell to an optimally-positioned light spot was recorded. The effects of SS-28 upon all genotypes were similar. 10-100 pM of SS-28 enhanced the on-discharge and increased the maintained discharge; 100 pM - 1 nM caused further enhancement of the response in some cells and suppression of the response and maintained discharge in other cells; over 1 nM suppressed in the response and maintained discharge in all cells; and over 10 nM caused long-lived suppression. The effects of SS-14 were similar to the effects of SS-28 in pearl mutants. However, no effect was observed in wild-type or pearl revertant animals for concentrations less than 10 nM. Higher concentrations produced the long-lived suppression seen with high concentrations of SS-28. We conclude that the pearl gene causes hypersensitivity to SS-14. The mechanism might be (a) expression of a normally unexpressed, high-affinity receptor molecule, (b) suppression of an endogenous endopeptidase for SS-14, and (c) mutation of a somatostatin receptor. If the free concentration of SS-14 in pearl mutant retinas were sufficiently high, then this compound might cause the night-blind phenotype observed in intact pearl mutants.

7.9 **ARE ANGIOTENSIN II AND SAR^1, ILE^8-AII BINDING SITES IDENTICAL?** **J.B. Erickson, and J.W. Harding. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520**

Angiotensin derivatives with a sarcosine substitution at the carboxy terminus and an aliphatic amino acid substitution at the amino terminus have been shown to be competitive ligands for the angiotensin "receptor". We have substantiated that data with competition experiments and by analyzing the data using double reciprocal plots. The results show that Angiotensin II and Sar^1,Ile^8-AII are clearly competitive inhibitors in the binding assay. However, a number of observations concerning the binding properties of these two ligands suggest a more complex story. (1) Scatchard plots derived from saturation isotherm data in rat brain membranes and rat and bovine adrenal particulates consistently demonstrate three to five times the number of binding sites for ^{125}I-Sar^1,Ile^8-AII than for ^{125}I-AII in experiments in which the two ligands are run side-by-side in the same tissue preparation. (2) Time course experiments in which dissociation was performed at equilibrium (60 minutes) reveals a very slow off-rate for both ligands, whereas dissociation at a time well before equilibrium (5 minutes) leads to complete dissociation in about 15 minutes. (3) Brain distribution data show binding sites for Sar^1,Ile^8-AII in areas where AII binding is not detectable. The paradox is that two ligands which interact competitively in vivo and in receptor binding studies have distinctly different B_{max} values. An explanation consistent with the above observations is that there are two populations of binding sites, one with fast on-fast off kinetics and a second with slow on-slow off kinetics. The slow on-slow off site may represent a modulatory protein such as a membrane bound angiotensinase associated with the angiotensin receptor. Both ligands competitively interact, but only the degradation resistant ^{125}I-Sar^1, Ile^8-AII analog may be detected at the putative enzyme site since ^{125}I-angiotensin II would be rapidly cleaved and lost. Thus, Sar^1, Ile^8-AII appears to interact with multiple binding sites which include, but are not limited, to the angiotensin receptor.

7.10 INTERACTION BETWEEN CENTRAL ASCENDING DOPAMINERGIC PATHWAYS AND NEUROTENSIN BINDING SITES IN THE RAT BRAIN. W.H. Rostène*, D. Hervé*, J.M. Studler*, P. Kitabgi*, C. Dana*, J.P. Vincent*, J. Glowinski* and J.P. Tassin*. *INSERM U.55, Hôpital Saint-Antoine, 75012 Paris; *INSERM U.114, Collège de France, 75005 Paris, and Centre de Biochimie CNRS, Faculté des Sciences, Parc Valrose, 06030 Nice, France

Several morphological, biochemical and behavioral data suggest an interaction between dopamine (DA) and neurotensin (NT) in both rat and human brains. We thus tested the possibility of NT binding sites regulation by DA pathways. Quantitative autoradiography was used to study the effect of 6OHDA lesion in the ventral mesencephalic tegmentum (VMT) on monoiodo ^{125}I-Tyr$_3$-NT binding on slide-mounted sections from adult male rats. Incubations were carried out with 0.1 nM monoiodo ^{125}I-Tyr$_3$-NT (2000 Ci/mm) for 60 min at 4°C in 50 mM Tris-HCl buffer (pH 7.6) containing 5 mM $MgCl_2$, 0.2% BSA and 20 μM bacitracin. The sections were washed, dried and apposed for 3 weeks on ^{3}H-Ultrofilm. Quantitation of the autoradiograms was carried out by means of ^{125}I-standards.

Correlated to endogenous DA depletions after 6OHDA, ^{125}I-NT binding was decreased in the VMT (-70%), central substantia nigra (-76%) and in the medial and lateral caudate nucleus (-23% and -41%, respectively). In contrast, no decrease was found in the nucleus accumbens and even a significant increase (+45%) of NT binding sites was observed in the prefrontal cortex (PFC) dorsal to the forceps minor, related to a change in the topographical distribution of the binding sites, suggesting a differential DA control of NT binding sites sensitivity in various brain regions.

In order to confirm this hypothesis, DA neurotransmission was chronically blocked by means of administrations (40, 20 and 5 days before sacrifice) of a long-acting neuroleptic, pipotiazine palmitate. This treatment elicited important increases of ^{125}I-NT binding sites in the PFC, nucleus accumbens, entorhinal cortex and medial caudate nucleus (+54%; +34%; +30%; +25%, respectively). No significant change was found in the lateral caudate nucleus, the VMT and in the substantia nigra. Our results confirm the presence of NT binding sites on DA cell bodies and strongly suggest that NT receptors on DA nerve-terminals are localized on two types of sites, one of which, located postsynaptically in limbic and cortical areas, is regulated by DA innervation.

7.11 CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN RAT BRAIN. April V. Applegate*, Michael J. Kuhar and Errol B. De Souza (SPON: K. Dismukes). Dept. of Neuroscience, Johns Hopkins Univ. Sch. Med. and Lab of Neuroscience, Addiction Res. Center, NIDA, Baltimore, MD

CRF, a 41-amino acid peptide originally isolated from ovine hypothalamus, stimulates the release of proopiomelanocortin-derived peptides from the anterior and intermediate lobes of pituitary. In addition, numerous pharmacological and immunohistochemical studies suggest a role for CRF as a neurotransmitter or neuromodulator in the CNS. Further evidence for such a role was provided by our autoradiographic identification and localization of high-affinity binding sites for a radioiodinated analog of ovine CRF (oCRF) in discrete areas of rat brain (De Souza et al., Science 224:1449, 1984). More recently, rat CRF (rCRF) has been isolated and sequenced, and this peptide differs from oCRF by seven amino acid residues. In the present study, we have used a stable iodine-125 labeled ligand (^{125}I-rCRF; New England Nuclear) which is homologous to the endogenous peptide to define the pharmacological characteristics and regional distribution of CRF receptors in rat brain homogenates. The kinetics and pharmacological specificity of ^{125}I-rCRF binding were characterized in membrane preparations of rat cerebral cortex and olfactory bulb. The binding of ^{125}I-rCRF was saturable and of high affinity with an apparent K_d of 1.9 nM and a B_{max} of 129 fmoles/mg protein in the cerebral cortex. Rat CRF, oCRF and the weak receptor antagonist α-helical CRF(9-41)NH_2 inhibited ^{125}I-rCRF binding with IC_{50} values of 1.4, 2.2 and 68 nM, respectively. The relative potencies of various peptides in displacing ^{125}I-rCRF binding were examined; at a 1 uM concentration, the CRF-related peptides oCRF, α-helical CRF, α-helical CRF(9-41)NH_2 and AcoCRF(4-41)NH_2 were equipotent with rCRF, the biological weaker fragment oCRF(1-39)NH_2 displaced 40% of the specific binding, rCRF(1-20)OH, rCRF(21-41) and rCRF(6-33)OH were minimally effective, and the unrelated peptides arginine vasopressin, angiotensin II, vasoactive intestinal peptide and growth hormone releasing factor did not affect ^{125}I-rCRF binding. Regional analysis indicated a heterogenous distribution of ^{125}I-rCRF binding sites within the rat brain and pituitary with highest densities of receptors present in the anterior pituitary and progressively lower concentrations present in the olfactory bulb and cerebral cortex; low but detectable levels of binding were found in the striatum, cerebellum, hippocampus, brain stem and hypothalamus. In summary, these studies using a ligand homologous to endogenous rCRF confirm the presence of specific, high-affinity receptors for CRF in discrete areas of rat brain. These data provide further evidence in support of a role endogenous CRF in regulating CNS function. Supported by NSF Graduate Fellowship and NIMH grants T32MH18030, MH25951 and MH00053.

7.12 COMPARISON OF THE AUTORADIOGRAPHIC DISTRIBUTION OF SUBSTANCE P, SUBSTANCE K, ELEDOISIN, AND NEUROMEDIN K BINDING SITES IN THE RAT CENTRAL NERVOUS SYSTEM. S.H. Buck, E. Burcher*, C.J. Helke, and T.L. O'Donohue. Sect. Biochem. Pharmacol., NHLBI; Exper. Ther. Br., NINCDS; Dept. Pharmacol., USUHS Sch. Medicine, Bethesda, MD; Div. Biol. & Hlth. Sci., Deakin University, Victoria, Australia.

In addition to the putative neurotransmitter, substance P (SP), two novel tachykinin peptides have been identified in the mammalian CNS. These are substance K (SK) (neurokinin α, neurokinin A) and neuromedin K (NK) (neurokinin β, neurokinin B), both similar in structure to the amphibian tachykinin, kassinin (KAS). SP and SK coexist in some neurons since a precursor, β-preprotachykinin, containing one copy of each peptide has been identified. Three distinct tachykinin receptors have been postulated in the CNS and periphery based on bioassay and ligand binding studies: P-type where SP and physalaemin (PHYS) are most potent, E-type where NK, KAS, and eledoisin (E) are most potent, and K-type where SK is most potent. Autoradiographic examination of the binding of ^{125}I-Bolton-Hunter labeled (BH) tachykinins has revealed that BHE, BHSK, and BHKAS binding sites have a different distribution from those for BHSP. However, it is not clear if the former three ligands all label one type (E) of site or E- and K-type sites in the CNS. We have investigated this question by directly comparing the autoradiographic distribution of BHSK, BHE, BHNK, and BHSP binding sites in the rat CNS.

The distribution of BHSK, BHE, and BHNK binding sites was strikingly similar but clearly distinct from sites labeled by BHSP. BHSK, BHE, and BHNK labeled cortical layers 4 and 5, the supraoptic n., paraventricular n., habenula, interpeduncular n., and the periaqueductal gray. Binding sites for BHSP were located in other regions including striatum, septum, dentate gyrus, superior colliculus, certain cerebellar vermi, n. ambiguus, hypoglossal n., and dorsal motor n. of X. Some CNS regions, such as the nucleus of the solitary tract and the dorsal horn of the spinal cord, contained binding sites for all four ligands. In general, BHSP binding sites have a more widespread distribution than those for BHSK, BHE, and BHNK. In the brainstem, BHSP appears to label both sensory and motor nuclei whereas BHSK, BHE, and BHNK label only sensory nuclei.

In crude membrane suspensions from rat cerebral cortex, the high-affinity binding of BHE and of BHNK was inhibited by the tachykinins in the potency rank order of KAS = E ≥ NK > PHYS > SK > SP indicating that both ligands bound to the recently characterized E-type cortical sites.

In conclusion, the results of these studies indicate that BHSP labels the P-type tachykinin receptor while BHSK, BHE, and BHNK all appear to label a distinct type of site, presumably the E-type tachykinin receptor, in the rat CNS.

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DEVELOPMENT AND PLASTICITY: RETINA AND OPTIC NERVE

8.1 OCULAR PIGMENTATION AND THE BOUNDARIES OF DORSAL AND VENTRAL RETINA IN GOLDFISH. A.D. Springer and A.S. Mednick. Department of Anatomy, New York Medical College, Valhalla, NY 10595.

One operational definition used to distinguish dorsal from ventral retinal ganglion cells (RGCs) is to define them in terms of which optic tract their axons enter. Ventral RGC axons enter the dorsal optic tract and dorsal RGC axons enter the ventral optic tract. Dorsal and ventral retina have also been distinguished in terms of a horizontal meridian that passes through the optic disc and is perpendicular to a line that extends from the choroid fissure through the optic disc (vertical meridian). Cobaltous-lysine was applied to retinal slits that were centered on either end of the horizontal meridian and were parallel to the ora serrata. Such slits filled RGC axons in the dorsal optic tract. Thus, the present study found that the horizontal meridian does not accurately predict which optic tract RGC axons enter and it is, therefore, not a reliable indicator as to the boundaries of dorsal and ventral retina. Instead, the goldfish iris contains nasal and temporal pigmentation marks (PMs) that are correlated with the pathways that adjacent RGC axons enter. The PMs varied from fish to fish, but were located approximately 21° above the horizontal meridian. When cobalt was applied to retinal slits above the PMs, RGC axons were filled in the ventral optic tract. When cobalt was applied to retinal slits below the PMs, RGC axons were filled in the dorsal optic tract. In order to obtain converging evidence to prove that the PMs were indicators of the retinal poles, we severed nasal RGC axons along the dorsomedial edge of the optic tectum and retrogradely labeled the RGCs that corresponded to these severed axons. Cobalt-filled RGCs were found ventral to the nasal PM. The present findings indicate that these PMs are useful landmarks in that they reliably predict the nasal and temporal retinal poles. Thus, they could be a means by which to standardize electrophysiological retinotectal maps across studies. In addition, these landmarks would allow reliable intraretinal labeling of RGC axons from defined retinal sectors. Therefore, the PMs will be useful for elucidating the topographical transformations of the RGC axons within the visual pathways. Supported by Grant EY-03552.

8.2 **PHOTORECEPTOR DIFFERENTIATION IN VITRO: DEVELOPMENT AND POLARIZATION OF OPSIN IMMUNOREACTIVE MATERIALS.** R. Adler. Wynn Center, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Retinal photoreceptors are highly polarized cells showing several discrete compartments. Opsin-containing visual pigments, for example, are synthesized in the inner segment and vectorially transported to and selectively accumulated in the outer segment. The mechanisms controlling the development and maintenance of this organization pattern are poorly understood. This laboratory is investigating some of these issues using a recently developed monolayer culture system in which embryonic retinal cells express typical photoreceptor phenotypic traits.

Photoreceptor-containing cultures were prepared as described (Adler et al., J. Cell Biol. 99:1173-1178, 1984) and fixed in paraformaldehyde. Opsin-immunoreactive materials were identified by indirect immunofluorescence using an anti-rhodopsin antibody (courtesy of Dr. D. Papermaster). F-actin was localized with fluorescent phalloidin. These studies showed that opsin-immunoreactive materials: i) are not detectable during the first 2-3 days in vitro; ii) appear first in an apparently random pattern; iii) become selectively concentrated in the photoreceptor apical region including the small outer segment-like process; iv) co-localize with an apical accumulation of phalloidin-stained materials; v) can be detected without plasma membrane permeation; and vi) are not present in neurons. In vivo studies carried out using cryostat sections of chick embryo retinas showed that opsin immunoreactivity is negative through ED15, and becomes detectable in photoreceptor outer segments by ED-16. Phalloidin-positive materials are also abundant in this photoreceptor region.

In summary, these studies show that cells isolated from the embryonic retina before overt differentiation are programmed to express characteristic photoreceptor phenotypic traits such as the polarized distribution of opsin. The co-localization of phalloidin-stained and opsin-immunoreactive materials is compatible with a possible involvement of the cytoskeleton in this phenomenon.

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8.3 **CALCIUM BINDING IN PIGMENTED AND ALBINO EYES.** Ursula C. Dräger. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Pigment defect mutations in mammals are associated with a diverse complex of neurological abnormalities which point to functions of melanin beyond photoprotection. Possible functions discussed include an involvement in redox reactions and scavenger roles for free radicals and potentially harmful heavy metals. The mechanism by which absence of these functions could lead to the defects in hypopigmentation mutants is not clear, however. Here the localization of Ca^{++} binding sites in eyes was determined autoradiographically by extracting endogenous Ca^{++} from tissue sections with EDTA and replacing it with $^{45}Ca^{++}$. The strongest labeling by far was associated with pigmented tissues, due to high concentration of melanin, which was shown to bind Ca^{++} very effectively and in a pH-dependent fashion. The second strongest binding was over the tapetum lucidum of the cat eye, and moderate labeling was associated with eye muscles and inner and outer epithelia of the cornea. The neural retina was generally more lightly labeled than the surrounding tissues of the eye; here the plexiform layers stood out in comparison to the nuclear layers, as did a band located internal to the photoreceptor outer segments.

The Ca^{++} binding to pigmented tissue was not influenced by the presence of Na^{+} or K^{+}; it was relatively insensitive to Mg^{++}, but it was reduced by high concentrations of Ba^{++}, Mn^{++} or Zn^{++}. Ca^{++} and Mg^{++} are probably the major natural ligands of melanin, since they are the only metals with melanin affinity present in more than trace amounts under normal physiological conditions. As binding of an ion of such biological relevance as Ca^{++} is not likely to represent a scavenger function of melanin, the possibility arises that melanin contributes to Ca^{++} regulation and that a defect in Ca^{++} buffering capacity may represent the common denominator of the various neurological defects found in hypopigmentation mutants. Melanin starts to appear in the embryonic eye around the time when the retina is presumably undergoing irreversible specification. At this stage the retinal pigment epithelium may represent a temporary Ca^{++} sink, causing a lowering of Ca^{++} in cells of the adjoining neural retina with which it is transiently connected via gap junctions; this in turn might influence the timing sequence of other processes by slightly delaying the closure of gap junctions; in such case the aberrant optic crossing in albinos may reflect a defect in temporal coordination. In the adult eye the massive Ca^{++} buffering capacity of pigmented tissues behind the retina may influence the Ca^{++} regulation in photoreceptors, and the light sensitivity defect found under scotopic conditions in the hypopigmented mouse pearl (Balkema et al. '81) may reflect a defect in Ca^{++} regulation, which is consistent with a role of Ca^{++} in dark adaptation. Supported by EY 01938 and the American Federation for Aging Research.

8.4 Development of the Outer Plexiform Layer (OPL) of the Cat Retina. Edward H. Polley, Roger P. Zimmerman, and Richard L. Fortney*. University of Illinois, College of Medicine, Department of Anatomy and Ophthalmology, Chicago, IL 60680 and Rush Medical College, Chicago, IL 60612.

The appearance and development of cells contributing processes to the OPL was studied in the fetal and neonate kitten retina. By using techniques of autoradiography after isotope labelling, combined with Lucifer Yellow staining to give a Golgi-like fluorescent representation of single cells, we were able to identify cell "birthdays" and/or classify cells on the basis of their cytoplasmic morphology. In an earlier study of neurogenesis in the inner nuclear layer (INL) (Polley, Walsh, and Hickey, 1982) we had shown that A-type horizontal cell neurogenesis was virtually complete while amacrine cell labelling was just starting at E28. No other cells in the INL are isotope labelled in adult animals injected in utero at E28, although cone photoreceptors are labelled in the outer nuclear layer (ONL).

Serial sacrifice at approximately weekly intervals of single individuals from litters of kittens receiving intravitreal injections clearly demonstrated the development, maturation, and distribution of pulse labelled cells in the neuroretina.

After intravitreal injection of tritiated thymidine in the neonate (postnatal Day 2) the principal population of labelled cells in the INL are the B-type horizontal cell and bipolar cells. Amacrine and Muller cells are rarely labelled although rod photoreceptors in the ONL are frequently labelled. A-type horizontal cells are completely unlabelled.

The differing time course for the production and development of A- and B-type horizontal cells in the INL of the retina is unique to our concepts of the development of laminar organization in the nervous system. There is however, a parallel in the early development of the A-type horizontal cell and associated cone photoreceptors in contrast to the later development of the B-type cell and associated rod photoreceptors. Indeed, the significant increase in the vertical dimension of the OPL after B-type cell birth is probably due to, 1) the developing dendritic processes of B-type horizontal cells in relation to cone pedicles, 2) the developing axonal processes of B-type horizontal cells to rod spherules, 3) the greater than 2:1 ratio of B- to A-type horizontal cells, and 4) the developing processes of newly formed bipolar and rod photoreceptor cells.

The differing time periods of neurogenesis during which these two cell types are produced and their insertion into the changing morphology of the developing retina could account for the reported differences in cell density and position in the adult retina. (Wassle, et al., 1978).

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8.5 STAGES IN THE DEVELOPMENT OF THE INNER PLEXIFORM LAYER OF THE CAT RETINA. Roger P. Zimmerman, Edward H. Polley, and Richard L. Fortney*, Rush Medical College and University of Illinois College of Medicine, Chicago, Illinois 60612

The sequence of development of the inner plexiform layer (IPL) of the cat retina was studied over the two month period centered on the day of birth (E35 to P37) in experiments in which *in vitro* staining with Lucifer Yellow was combined with tritiated thymidine autoradiography or with electron microscopy. *In vitro* staining with Lucifer Yellow yields reliable and consistent Golgi-like staining of individual cells.

Early Fetal Retina (E35): The retina appears to consist primarily of ventricular cells, with a few amacrine and ganglion cells. Cones and horizontal cells have undergone their final mitosis, but are not yet morphologically differentiated. A distinct, unistratified, IPL is made up of the dendrites of several sizes of ganglion cells and processes of amacrine cells with somata lying on both sides of the IPL. Postmitotic cells can be observed crossing the IPL, indicating that the IPL is not a barrier to cell migration at this stage.

Late Fetal Retina (E51): The processes contributing to the IPL are clearly multistratified. Although differentiated cones and horizontal cells, and the beginnings of the outer plexiform layer, are present, no bipolar cell terminals are observed in the IPL.

Neonate Retina (P0 - P37): Bipolar cells are being produced during the first week after birth; their terminals enter the IPL within seven days after the final mitosis. By the end of the first postnatal month, ganglion cell dendrites are stratified in either sublamina a or sublamina b of the IPL, and receive both ribbon and conventional synapses. By P37 the pattern of branching of amacrine and ganglion processes is similar to that found in the adult retina. The laminar distribution of bipolar cell terminals is similar to that of the adult.

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8.6 RELATIONSHIPS BETWEEN GANGLION CELL DENDRITIC FIELD STRUCTURE AND RETINAL TOPOGRAPHY IN THE CAT. J.D. Schall, S.J. Ault* and A.G. Leventhal. Dept. Anat., Univ. Utah Sch. Med., Salt Lake City, UT 84132.

The morphology of retinal ganglion cell dendritic fields varies with distance from the area centralis (Boycott, B.B. & H. Wässle, J. Physiol., 240:397, 1974) and with angle off of the horizontal meridian (Leventhal, A.G. & J.D. Schall, J. Comp. Neurol., 220:465, 1983). The center of the dendritic field of many ganglion cells is displaced laterally from the center of the cell body (Honrubia, F.M. & J.H. Elliott, Arch. Ophtholmol., 84:221, 1970; Wässle, H., L. Peichl & B.B. Boycott, Proc. R. Soc. Lond., B 212:157, 1981). We related the direction of displacement and the orientation of horseradish peroxidase filled retinal ganglion cell dendritic fields to the topography of the adult and developing retina. In our analysis the ganglion cell's principle dendrites primarily determine the displacement of the dendritic field from the cell body, and the orientation of a dendritic field is determined by the spatial distribution of higher order dendritic branches in the inner plexiform layer.

We find that the direction of displacement of the dendritic fields in any spot of retina can be predicted from the direction in which the ganglion cell density falls off most steeply in that spot of retina, i.e., ganglion cell dendritic fields are displaced from their cell bodies down the ganglion cell density gradient.

From approximately embryonic day 50 (of the 65 day gestation) to postnatal day 10 the kitten retina undergoes a period of maturation (reviewed by Rapaport, D.H. & J. Stone, Neurosci., 11:289, 1984). This process begins at the area centralis and spreads over the retina in a horizontally elongated wave. We find that the mean orientation of the dendritic fields in any spot of retina is predicted by the angle through which the wave of maturation passes in that spot of retina; the orientation of a dendritic field does not correlate with the direction of its displacement.

These findings indicate that the displacement of a ganglion cell's dendritic field from its cell body results from mechanisms different from those which are responsible for the orientation of higher order dendrites in the inner plexiform layer.

8.7 EXPERIMENTAL ALTERATION OF CAT RETINAL GANGLION CELL DENDRITIC FIELD STRUCTURE. S.J. Ault*, J.D. Schall and A.G. Leventhal. Dept. Anat., Univ. Utah Sch. Med., Salt Lake City, UT 84132.

Interactions among retinal ganglion cell dendrites (Wässle, H., L. Peichl & B.B. Boycott, Nature, 292:344, 1981; Perry & Linden, Nature, 297:683, 1982) may be responsible for many aspects of dendritic field structure. We have investigated these interactions by studying the effects of reduced ganglion cell density on dendritic field structure.

Areas of retina were depleted of ganglion cells following lesions of the optic tract or pinpoint lesions of the retina near the optic disc in two day old kittens. The dendritic fields of horseradish peroxidase filled ganglion cells which survived within or on the border of the depleted areas were compared with those of their normal counterparts.

While the somas of beta cells which survived within an area of reduced ganglion cell density were not significantly larger than normal, their dendritic fields covered an abnormally large area. To see how this was accomplished, we first related the amount of dendritic material supported by a cell to the size of its cell body. The resulting dendrite/soma ratios were compared for cells in normal and depleted regions of retina. This analysis indicated that the dendrite/soma ratios of beta cells in the depleted areas were not significantly different from normal. Moreover, all types of retinal ganglion cells exhibited similar dendrite/soma ratios; dendrite/soma ratios did not change with retinal eccentricity. A quantitative analysis of dendritic branching indicated that the dendritic fields of cells in depleted regions are abnormally large because their dendrites are distributed more diffusely, branching less than normal.

In agreement with the observations of Perry and Linden (1982) and Eysel, Peichl and Wässle (Soc. Neurosci. Abstr., 9:26, 1983), we have found that the dendritic fields of the alpha and beta cells on the border between normal and depleted regions extend preferentially into the depleted area. This is not accomplished by the growth of an abnormally large amount of dendrite since the dendrite/soma ratios of these cells were normal. Also, the orientations of these dendritic fields were often not the same as the direction of displacement. This provides support for the hypothesis that dendritic field displacement and orientation are determined by different mechanisms.

Based upon our results, we suggest that the size of a ganglion cell's soma determines the amount of dendritic material produced; the local ganglion cell density gradient determines the direction in which the dendritic field grows, and the density of neighboring ganglion cells of the same class determines branching frequency and the area which the dendritic field covers.

8.8 DEVELOPMENTAL CHANGES IN CELL DEATH AND RETINAL GANGLION CELL DISTRIBUTION IN THE POSTNATAL FERRET RETINA Z. Henderson, K. C. Wikler and B. L. Finlay Physiology Laboratory, University of Oxford, Oxford, England and Department of Psychology, Cornell University, Ithaca, NY 14853

The ferret is a highly altricial mammal with large eyes and a marked specialization for central vision which makes it particularly advantageous for studies of the process of formation of retinal specializations. For postnatal days 1 (the day of birth), 2, 3, 6, 10, 24 and adulthood, the spatial distribution of retinal ganglion cells was reconstructed from both paraffin embedded, horizontally sectioned eyes stained with cresylecht violet, and from similarly sectioned material in animals that had received multiple injections of horseradish peroxidase in the thalamus and superior colliculus on postnatal days 1, 2, 5, 9 and at adulthood. Retinae were reacted in the eyecup with cobalt-intensified diaminobenzidine. All retinae were examined for the incidence and spatial distribution of degenerating cells.

The adult ferret retina has an 8 to 1 ratio of cell density from the area of maximum density in the area centralis to the extreme periphery, and a 4 to 1 difference between central temporal retina overall and the remaining retina. On postnatal days 1 through 6, the distribution of all cells and HRP labeled cells is only slightly elevated in superior temporal retina (1.3 to 1). At all days, HRP labelled at maximum a third to half of the cells in the ganglion cell layer, which were always the largest cells. The remaining cells presumably are displaced amacrine cells and glia.

The period of cell degeneration in the retinal ganglion cell layer is quite protracted in the ferret, with large numbers of degenerating cells in evidence at birth, and many still visible by postnatal day 24. In the HRP labeled retinae, HRP reaction product could often be seen in the residual cytoplasm of degenerating cells. Numbers of degenerating cells were at maximum on postnatal day 1. On this day, their spatial distribution was distinctly inhomogeneous. Cell death rates were lowest in the superior temporal retina and temporal margin (15.7 ± 1.48), intermediate in the nasal margin, and highest in the remaining retina (21.5 ± 1.5). The incidence of degenerating cells was particularly high in the inferior retina and in the area immediately surrounding the optic disc in the nasal retina. If the retina is divided into the areas corresponding to the prospective area centralis and the periphery, there is no difference in rates (a.c. = 17.9 ± 1.8; periphery = 19.4 ± 1.6). On later postnatal days, the distribution of degenerating cells appears more uniform, with a suggestion of elevation in the periphery in the latest postnatal days. We suggest that the early inhomogeneity in cell death rates is related to the establishment of projection laterality; specifically, that the elevation of cell death in nasal retina reflects the removal of cells with inappropriate ipsilateral connections. The later cell death may reflect the mechanisms of creation of the area centralis or numerical matching of the retina to its targets.

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8.9 RETINAL TRANSPLANTS INTO NORMAL AND DAMAGED ADULT RETINAS. M. del Cerro, D.M. Gash, G.N. Rao*, M.F. Notter, S.J. Wiegand and C. del Cerro*, Depts. of Anatomy and Ophthalmology and Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

We performed transplants of embryonic (E 13-16) and postnatal (PN 2) retina, neural portion, and pigment epithelium, into the anterior chamber and vitreal cavity of adult rats of the same (Long-Evans) and different strain (Lewis) than that of the donor. The hosts either had normal retinas or retinas selectively damaged by 15 days of continuous exposure to low levels (0.47 mw/cm^2) of fluorescent light. We report herein the successful outcome of these experiments. The fate of the grafts was followed by ophthalmoscopic examination for a period up to 5 weeks. Two to 3 days after transplantation, the grafts became vascularized; light and electron microscopic observations, therefrom, showed histotypical differentiation of the grafts. Although there was variability within different areas, those regions that developed more fully showed formation of all the nuclear and plexiform layers, which were populated by the usual neuronal and glial cell types; the retinal pigment grew and differentiated in the transplants. These results suggest that intraocular retinal transplants may become a valuable tool in determining the extent of neural repair and plasticity possible in the adult retina.

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8.10 GLIAL STRUCTURE IN RELATION TO FIBER ORDER IN THE FERRET'S OPTIC STALK. C. Walsh, S. Price*, & R.W. Guillery. Dept. of Human Anatomy, Univ. of Oxford, Oxford, OX1 3QX, England, and Dept. of Pharmacological & Physiological Sciences, Univ. of Chicago, Chicago, IL 60637, USA.

Retinal fibers in the ferret's optic nerve undergo a systematic sorting in terms of age during their course from the retina to the optic chiasm. Preferential labelling of the oldest retinal fibers shows that older and newer fibers intermingle in the nerve near the eye, but become segregated as the optic nerve leaves the orbit and passes through the optic foramen (Walsh, C., unpublished). In order to determine whether this fiber reordering occurs where the environment of the fibers shows a morphological change, we have studied the developing eye stalk in fetal ferrets from the twenty-first to the thirty-ninth days of gestation (E21-E39) with the light and electron microscope.

In its intraorbital segment, the fetal optic nerve is subdivided into fasicles by early glial cells. Bundles of unmyelinated axons are invaginated into the cytoplasm of these cells rather as fine axon bundles relate to Schwann cells in the peripheral nervous system. The axons lie close to the glial nuclei, and growth cones show no preferential localization within the cross-section of the nerve. In contrast, in the prenatal tract there are few glial cell nuclei, and instead optic axons grow in relation to the peripheral processes and end-feet of radially oriented neuroepithelial cells. This radial glial organization, typical of developing central tracts, is associated in the optic tract with the preferential growth of newer axons near the pial surface (Walsh, C. & Guillery, R.W., J. Neurosci., in press).

The transition from the "peripheral" glial structure to the radial glial, "central" structure occurs near the optic chiasm in the older animals (E30 and on), but the younger animals (E24-E26) show a radial glial structure in the eye stalk between the optic foramen and the optic chiasm, with the point of transition moving progressively nearer the chiasm at later stages. The youngest animals (E21-E24) show a transient pigmentation of the stalk, described previously (Strongin, A.C., & Guillery, R.W., J. Neurosci., 1:1193, 1981; Silver, J. & Sapiro, J., J. Comp. Neurol., 202:521, 1981), which extends from the eye to the optic foramen, the same region that shows the "peripheral" glial structure at slightly later stages. These data suggest that the systematic fiber reordering that occurs near the optic foramen reflects a change in the glial structure in this region.

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9.1 MODULATION OF GABA, SUBSTANCE P AND PROTEIN KINASE IMMUNOREACTIVITIES IN MONKEY STRIATE CORTEX FOLLOWING EYE REMOVAL S.H.C. Hendry, E.G. Jones and M.B. Kennedy
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Neurons displaying immunoreactivity for the inhibitory neurotransmitter, GABA, for the neuropeptide, substance P (SP), or for a calcium/calmodulin-dependent protein kinase (type II CaM kinase) were examined in the striate cortex (area 17) of normal monkeys and of monkeys from which an eye had been removed one to three weeks before sacrifice. In normal monkeys, large numbers of GABA immunoreactive non-pyramidal neurons and punctate profiles are found in area 17. The stained cell bodies and profiles are densest in layers IVA, IVC and VI. SP-positive cells in area 17 are all non-pyramidal and are found mainly in layers II-III and VI. Others in layers IVA and IVC lie among a dense population of stained punctate profiles. These SP positive cell bodies and profiles are found to be also GABA positive in immunofluorescent double-labeling experiments. The CaM kinase immunoreactive neurons are present in all layers of area 17 and include a subpopulation of both pyramidal and non-pyramidal cells. They are densely packed in layers II and IVB and to a lesser extent in layer IVCB and VI. Within each subdivision of layer IV, staining for all three substances is uniform in normal monkeys. By contrast, three weeks following removal of one eye, GABA and SP staining in layers IVA and IVC becomes patchy. Patches of darkly stained cell bodies and terminals in layer IVC alternate with lightly stained patches. Comparison with adjacent sections stained for cytochrome oxidase shows that the patches coincide with ocular dominance columns; darkly stained patches correspond to columns driven by the intact eye and lightly stained patches by the removed eye. One or two weeks following eye removal, the protein kinase stained neurons in layer IVCB also form lightly and darkly stained patches. However, the darkly stained patches here correspond to columns related to the removed eye. Thus, eye removal leads to decreases in GABA and SP immunoreactivities and an increase in kinase immunoreactivity in neurons of removed-eye dominanace columns. The altered immunoreactivities may occur through changes in the concentration of each substance, brought about by a reduction in synaptic activity.
Supported by NIH Grants NS 21377 and NS 17660.

9.2 COMPARATIVE EFFECTS OF IMPULSE BLOCKAGE ON CYTOCHROME OXIDASE ACTIVITY IN THE VISUAL SYSTEMS OF MACACA MULATTA, FASCICULARIS AND SAIMIRI SCIUREUS. E.W. Carroll* and M. Wong-Riley. (SPON: D.A. Riley). Dept. of Anatomy, Med. Coll. of Wis., Milwaukee, WI 53226.

Intravitreal injections of tetrodotoxin (TTX) can reversibly alter the levels of cytochrome oxidase (C.O.) in the visual system (Wong-Riley and Riley, '83; Wong-Riley and Carroll, '84). We sought to compare our previous results in Macaca mulatta (Mm) with M. fascicularis (Mf) and Saimiri sciureus (Ss). In Mf, C.O. activity was also contrasted with monocularly enucleated (ME) animals. 19ug TTX in 10ul sterile saline were injected monocularly every 3-4 days, and survival times were 2, 4, 8, and 12 wks for Ss and 2 wks for Mf (both TTX and enucleation). Perfused tissues were then processed for C.O. histochemistry. Areal measurements of the C.O. rich "puffs" in laminae 2-3 were made using computer-assisted digitization. In the normal retina of each species, similar patterns of C.O. activity were observed: the large ganglion cells tended to be more reactive for C.O. than the smaller ones, and sublamina b of the inner plexiform layer (IPL) was more reactive than sublamina a. In both Mf and Ss, the difference between sublamina a and b was not as pronounced as in Mm. Horizontal cells, cone pedicles, photoreceptor inner segments and the outer plexiform layer were highly reactive. In all three species, slight decreases in C.O. activity (due to TTX) were observed in the ganglion cell layer and in the IPL. The pattern of C.O. activity in the normal dLGN (dorsal lateral geniculate) of Mf and Ss was similar to that of Mm (Wong-Riley and Carroll, '84). Magnocellular layers (1 and 2) were more reactive than the parvocellular layers, layer 6 was more reactive than 5, which in turn was more reactive than layers 3 and 4. In all three species, dramatic decreases in C.O. activity were observed after TTX treatment, the ipsilateral side being more affected than the contralateral. Both ME and TTX were equally effective in decreasing the amount of C.O. activity. In the visual cortex of Ss, TTX injections had little effect on the level of C.O. activity; however, one animal (4 wks) exhibited a slight banding pattern in lamina 4C in a limited region of area 17. In Mf (both TTX and ME), the decrease in C.O. activity produced the typical banding pattern (ocular dominance columns, ODC) in lam 4C, and shrinkage of the puffs overlying the affected ODC. On the average, there was no significant difference in areal decreases of affected puffs between Mm and Mf, and no difference between TTX injections and ME in Mf. However, in both Mm and Mf it appeared that lam 4Cα (normally 4.7% higher in optical density than 4Cβ) decreased by 9.7%, while lam 4Cβ decreased by 21.9%. These results indicate that even in the adult monkey, neurons in the retina, dorsal lateral geniculate nucleus and cortex (primarily in the Macaque) remain sensitive to impulse blockage at the retinal level. [Supported by NIH EY05439 (MWR) and IF32NS07664 (EWC)].

9.3 DEOXYGLUCOSE LABELING OF MACAQUES WITH MONOCULAR ENUCLEATION SHOWS STRIPES, NOT SPOTS, OUTSIDE OF LAYER 4 OF STRIATE CORTEX. S.J.Schein#, F.M.de Monasterio#, C.Kennedy+ & L.Sokoloff+
#Section on Visual Processing, National Eye Institute, NIH and +Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD. 20205.

Activity labeling with deoxglucose of macaques visually stimulated by normal viewing of the room produces a pattern in striate cortex of rows of dense spots in layers 2, 3 and less prominently, 5 and 6 (Kennedy et al. PNAS 1976). Layer 4, which is most heavily labeled, is uniform. Cytochrome oxidase (CO) histochemistry replicates this pattern.

If one eye is occluded, the pattern of 2DG in the ocular dominance "slabs" corresponding to the open eye is apparently unchanged, including the presence of rows of spots outside of layer 4. In the alternating slabs, no spots can be seen. Due to uniform reduction of label in layer 4 of the occluded eye's slabs, stripes appear in this layer. Others have reported this result, and we have observed it in 10 animals (including one with occlusion for 3 months) with only one exception.

Change in CO activity occurs slowly, so the CO pattern just after occlusion is normal. However, 2 months after enucleation of one eye, the CO pattern shows rows of dense spots outside of layer 4 and dense stripes in layer 4, alternating with rows of light spots and light stripes. The only difference between this pattern and the 2DG pattern found in animals with occlusion of one eye is the presence of the light spots in the former, perhaps due to insufficient passage of time. It would thus seem that (1) CO activity regulates according to chronic levels of metabolic activity, in parallel with the acute picture afforded by labeling with 2DG; (2) Removal of the input from one eye has little or no effect on the activity in the ocular dominance slabs corresponding to the other eye.

We report here, however, that the 2DG pattern after enucleation of one eye is quite different from that described above, in that all layers show stripes. Since activity of the interspot zones of the dense (seeing) slabs increases, no spots are found. This result has been obtained in 3 out of 3 unilaterally enucleated animals (and in the above-noted unilaterally occluded animal). In 2 animals, enucleation was performed on the morning of the 2DG procedure; in 1 animal, enucleation was performed several weeks earlier. We speculate that in normal macaques, activity in the interspot zones is reduced by binocular inhibition, which is released by removal of the other eye. We also suggest that the two conclusions listed above should be reexamined.

9.4 2DG EVIDENCE FOR A BINOCULAR BORDER ENHANCEMENT IN LAYERS 2+3 OF MACAQUE STRIATE CORTEX. +Tootell, R.B.H., +Hamilton, S.L.* and #Switkes, E.* +Dept. Psychol., U. Calif., Berkeley, CA and the #Dept. Psychobiology, U. Calif., Santa Cruz, CA

In the course of an extensive series of 2DG experiments in anesthesized, paralyzed macaques, we have discovered (quite serendipidously) a significant 2DG effect which appears to indicate a striate mechanism for extracting binocular object borders from more complex images in the monocular striate inputs. This 2DG "border enhancement" is a diffuse swath of obviously higher 2DG uptake (about 2 mm wide) which appears at the striate representation of certain stimulus borders (described below). Two aspects of the border enhancement are especially significant. First, it occurs only in striate layers 1-3: it is completely absent from the geniculate input layers 4A, 4C and 6, as well as from 4B and 5. Secondly, it appears only following binocular (but not monocular) viewing conditions.

An enhancement is produced at the striate border between the representations of: 1) two (or more) adjacent patterned stimulus fields which differ from each other in orientation, spatial frequency, or texture; or 2) one such patterned field and an adjacent unpatterned (diffuse) grey field of the same mean luminance. An enhancement does not appear at the representation of borders between two unpatterned grey fields of different mean luminance: this and other evidence indicate that the border enhancement is not an artifact of mean luminance per se. The enhancement is robust at the borders of regions of high (but not low) spatial frequency gratings. When the binocular convergence of the border is slightly misaligned, the border enhancement occurs between the diverged border representations, as well as on the border representations themselves. Surprisingly, binocular misalignment of up to a degree has no obvious effect on 2DG results within the representation of a given screen region, in any layer.

It is conceivable that the 2DG border enhancement is the result of feedback interaction from extrastriate areas, perhaps from V2. However, this seems unlikely because: 1) certain aspects of the striate border enhancement are retinotopically more discrete than any known extrastriate 2DG pattern, and 2) the border enhancement is faint in striate layer 1, where much of the projection from extrastriate cortex terminates.

Since the border enhancement appears to be computed de novo within striate layers which are largely binocular, this 2DG mechanism may provide a significant insight into the striate disparity architecture. Because the 2DG border enhancement occurs in layers 2+3 (which project to V2) but not in layers 4B and 6 (which project to MT and perhaps V3), it appears that at least some aspects of binocular coding are completely segregated within the V1-V2 (rather than the V1-MT) stream of visual information processing. Supported by PHS EY-00014 and NSF BNS 82-02275 to R. L. De Valois.

9.5 BINOCULAR CORRELATION SYSTEM IN MONKEY VISUAL CORTEX. G.F.Poggio, F.Gonzalez* and F.Krause*. Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The activity of cortical visual neurons during binocular vision reflects the interaction between the inputs from the two eyes and its characteristics are greatly influenced by the spatial and temporal correlation of the afferent signals. In order to investigate the neural substrate for binocular correlation, we have studied the effects of dynamic random dot patterns with different dot correspondence on the activity of single neurons.

Rhesus monkeys were trained on a fixation/detection task under conditions of normal binocular vision. Visual patterns irrelevant to the behavioral task were presented during the periods of eye fixation and the associated neural response observed. The response properties of the neuron, including its disparity sensitivity to contour stereograms and to dynamic random-dot stereograms, were first assessed. Subsequently, correlation sensitivity was tested with dynamic random-dot correlograms (RDC) generated on a square field (5^0 or 10^0) of random dots and displayed over the neuron's receptive field. All neurons were tested with fields of 1^0-4^0 50% dot density in which the dot pattern of a smaller (1^0-4^0) central region alternated from binocularly identical (correlation) to binocularly complementary (uncorrelation or negative correlation).

Whereas a majority of cortical neurons do not signal binocular correlation, others respond in opposite ways to correlated and uncorrelated patterns. One third of the neurons we studied in V1 (297) responded to uncorrelation either with an increase of their discharge (60) or with suppression (46); for both types the reciprocal effect was evoked by correlation which reduced the discharge of the former and increases that of the latter. Similar effects were found for smaller samples from areas V2 and V3.

A majority of neurons activated by binocular uncorrelation also responded to dynamic random-dot stereograms. Nearly all these neurons had complex receptive field properties, whereas complex and simple neurons occured in nearly equal proportion among the neurons suppressed by or insensitive to binocular correlation.

Although no relation was found between the neuron's disparity sensitivity for 'local' features and its sensitivity to binocular correlation, a particular association was observed for neurons whose activity was suppressed by decorrelation for, if disparity sensitive, they all had tuned excitatory response profiles.

These observations provide some support to the conjecture that binocular correlation and binocular disparity are processed by separate neural system, and that both modes of processing may be part of the functional repertoire of the neuron. (Research supported by USPHS, EY02966).

9.6 INTRINSICALLY PROJECTING PYRAMIDAL NEURONS OF MONKEY STRIATE CORTEX: AN EM-HRP STUDY. K.S. Rockland. E.K. Shriver Center, Waltham, MA. 02154

Periodic intrinsic connections have been reported in primate visual cortex within layers 2-3 and 4B (Rockland and Lund,'83). In layers 2-3, HRP injections show these connections to originate from pyramidal neurons, mainly small and medium in size (8.0 - 15.0um at soma base). These neurons have long horizontal axon collaterals that intracellular HRP injections (Gilbert and Wiesel, '83) show to branch at regular intervals. Still unknown from these light microscopic studies, however, is the type of synaptic contact made by these neurons. To address this issue, extracellular HRP injections were made in macaque striate cortex. After a 3 day postoperative survival, animals were perfused with a brief saline prewash, followed by 1.0% paraformaldehyde and 1.5% glutaraldehyde. Tissue blocks were vibratome sectioned at 50-60um, reacted with DAB, postosmicated, and plastic embedded. After light microscopic inspection to identify labelled areas, relevant portions were trimmed, reembedded for 1.0um sectioning, then reinspected and trimmed for electron microscopy (EM). Initial inspection has been restricted to layer 3. In this layer, EM reveals many HRP-filled axons, which can be clearly identified in longitudinal section as myelinated and of small caliber (commonly <0.75um in diameter). HRP reaction product is localized in large terminals (typically 1.0 x 1.5um), usually containing numerous mitochondria and a conspicuously high density of round vescicles. These terminals consistently form asymmetric synapses (about 0.25um in length) with dendritic spines. There are occasional examples of terminals synapsing with two adjacent spines. En passant synapses are also observed between large HRP-filled terminals (5.0um long in one ultrathin section) and two spines spaced 2.0um apart. These data are consistent with earlier lesion-degeneration studies on intrinsic cortical connections (Fisken et al.,'75), which reported a large population of degenerating terminals making asymmetric synapses with spinous profiles. The degeneration technique also labelled asymmetric synapses with dendritic shafts and stellate cell bodies, as well as a smaller proportion of symmetric synapses. Examples of these contacts have not yet been found in EM-HRP material. The present work indicates that intrinsically projecting pyramidal neurons in primate layer 3 form asymmetric, presumably excitatory synapses onto dendritic spines. Further experiments are necessary to determine whether these spines belong to other layer 3 pyramidal neurons, to sparsely spinous stellate neurons, or to other elements passing through layer 3.
Supported by EY04946

9.7 ULTRASTRUCTURAL CHARACTERIZATION OF LONG-RANGE CLUSTERED HORIZONTAL CONNECTIONS IN MONKEY STRIATE CORTEX. B.A. McGuire, C.D. Gilbert and T.N. Wiesel, The Rockefeller University, New York, NY 10021.

Most pyramidal cells in the striate cortex form long-range clustered horizontal connections (Gilbert and Wiesel, J. Neurosci., 3: 1116-1133, 1983). To learn more about the functional role of these connections in monkey visual cortex, we are studying them using electron microscopy of cells which have been injected intracellularly with horseradish peroxidase (HRP). Cortical neurons were impaled with a micropipette, their receptive fields mapped, and the HRP injected iontophoretically. After fixation by perfusion, the cortex was sectioned, reacted with diaminobenzidine, and processed for electron microscopy. The first cell we have examined ultrastructurally had a well-oriented complex receptive field located a few degrees from the fovea. It was a pyramidal cell with the soma located in layer IIIB of area 17. Light microscope reconstruction revealed that the axon arborized extensively within the superficial layers and also projected into the white matter. In surface view, the axonal field of this cell was long and narrow, projecting antero-posteriorly, perpendicular to the 17/18 border, and extending for 2 mm along its long axis. The axon collaterals branched in 4 distinct clusters within layers II-III, separated from one another at regularly spaced intervals. One cluster, located 300 μm lateral to the apical dendritic tree, was chosen for analysis and serially sectioned for electron microscopy.

The HRP-labeled collaterals were followed through consecutive sections and photographed with the electron microscope. Labeled terminals were found to make asymmetric synapses. From an initial sample of HRP-labeled synaptic contacts, 80% were axo-spinous, the remainder being axo-dendritic. No labeled contacts were found on cell bodies. Serial reconstruction of the postsynaptic dendrites suggests that almost all of the postsynaptic cells were spiny. Furthermore, our preliminary evidence is that a disproportionate number of the postsynaptic processes were apical dendrites belonging to pyramidal neurons. Taken together, our results indicate that the long-range lateral interactions are excitatory, and exist between clusters of pyramidal cells. These results are consistent with the results of Ts'o et al. (Abstr. Soc. Neurosci. 9: 476, 1983) who have found excitatory long-range interactions in striate cortex on the basis of cross-correlation analysis. We speculate that the apical dendrites of pyramidal cells may represent a specialization for receiving the long-range horizontal connections within the cortex.

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9.8 RELATIONSHIPS BETWEEN HORIZONTAL CONNECTIONS AND FUNCTIONAL ARCHITECTURE IN STRIATE CORTEX AS REVEALED BY CROSS-CORRELATION ANALYSIS. D.Y. Ts'o, C.D. Gilbert, and T.N. Wiesel. Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021

We have been investigating the functional relationships between cells participating in horizontal interactions in striate cortex which may be mediated by intrinsic horizontal connections that have been demonstrated anatomically. We have previously reported experiments in cat and monkey striate cortex using cross-correlation analysis of pairs of superficial layer cells recorded from two independently manipulated extracellular electrodes. These experiments revealed excitatory horizontal connections that spanned distances in the millimeter range. The observed interactions were found in pockets suggestive of the clustered nature of long range horizontal connections. The horizontal interactions were found between cells with matched receptive field properties such as orientation specificity. The dependence of the horizontal interactions on matching orientation specificity was strong evidence that these connections were intrinsic.

Further experiments in the cat have shown that these horizontal interactions extend over several millimeters and at the longest distances the cells had non-overlapping receptive fields. The cytochrome oxidase-rich patches (blobs) in the monkey visual cortex offer another opportunity to study the relationship between intrinsic connections and functional architecture. Experiments show a tendency for unoriented monocular cells in the blobs to be connected to unoriented monocular cells in other blobs and not to oriented non-blob cells. These blob-to-blob interactions are excitatory. Correlated firing between blob cells with the same eye preference is more prevalent than between blob cells with a different eye preference.

We have found that a large proportion of the observed correlated firing between cell pairs is due to common input from an intrinsic source. In some cases, the orientation tuning of the cells providing the common input could be established. Several experiments used local iontophoresis of a glutamate analogue (DLH) at each recording site to raise the firing rates of the cells under study instead of light stimuli. In these experiments DLH stimulation usually did not reveal a direct connection between the cells. The prevalence of common input is likely to be due to interconnections between large numbers of cells in columns of like functional specificity.
(Supported by grants EY05253, BNS9318794, and BNS8351738.)

9.9 VOLTAGE SENSITIVE DYES IN MACAQUE STRIATE CORTEX. G. G. Blasdel and G. Salama*. Departments of Ophthalmology, Physiology, and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Recent successes in the application of voltage sensitive dyes to mammalian cortex (Orbach, Cohen, and Grinvald, 1983) have encouraged us to explore the use of optical probes in monkey striate cortex. Macaque monkeys were anaesthetized and prepared in a manner suitable for physiological studies. We then imaged a small (1x1mm) patch of cortex on 4 photodiodes and stained the cortex with an absorbence dye (NK2367). While the cortex was illuminated with light at 720nm, currents from the photodiodes were amplified and fed into a computer where they were averaged as the monkey viewed 20-40 presentations of visual stimuli - blank screen, vertical, or horizontal gratings (0.5 cy/deg, moving 8 deg/sec). In order to control for movement artifacts, we randomly interleaved sham presentations, where the animal saw only a blank screen, and synchronized each sequence to the animals respiration and its EKG (as first suggested by Orbach et al., 1983). Visually driven responses consisted usually of a reduction (by 0.1%) in the light reflected from a specific region of stained cortex. They appeared believable because they: (1) coincided with the onset of visual stimulation (either Horizontal or Vertical) and returned to baseline with its cessation, (2) showed different responses at different sites, (3) were repeatable from one sequence to the next, (4) were of large amplitude - twice that of anything produced by respiration or heartbeat, and (5) because their strength depended upon the focal plane - the optimal plane lying some 100-200 micra beneath the cortical surface. Due to their relatively slow time course - 1-2 sec to develop or decay - these changes seem unlikely to derive directly from membranes yielding action potentials. They could develop, however, from the depolarization of glial cell membranes or from the invasion (with repeated depolarizations) of small dendritic processes.

Preliminary experiments, using a TV camera and frame averager (together capable of detecting 0.02% changes in reflected light), have revealed stripe-like patterns of activity in 1.5x2mm patches of cortex. These patterns develop in response to visual stimulation and are specific for orientation. Those resulting from stimulation with vertical are approximately 0.2 to 0.4 mm in width and, for the most part, separate from those resulting from stimulation with horizontal. The sets of patches seen with vertical and horizontal do not form simple complements of one another, however, since there are regions where the cortex appears to respond to both vertical and horizontal, as well as regions where it appears not to respond to either orientation. Supported by EY05403, AHA18253, and in part by EY05282.

9.10 REAL TIME OPTICAL MAPPING OF NEURONAL ACTIVITY IN THE MAMMALIAN VISUAL CORTEX IN VITRO AND IN VIVO. A.Grinvald, C.D.Gilbert, R.Hildesheim*, E. Lieke* and T.N.Wiesel. Laboratory of Neurobiology, Rockefeller Univ., New York, N.Y. 10021 and Dept. of Neurobiology, The Weizmann Institute, Rehovot, Israel.

The use of voltage-sensitive dyes and optical recording of neuronal activity (Cohen, et al; Grinvald, Ann. Rev. Neurosci., 1978, 1985) is a potentially powerful technique for the evaluation of local circuits using in vitro brain slices (Grinvald, et al., J. Physiol., 1982) and for studying the functional organization of exposed brain structures in vivo (Orbach, et al., J. Neurosci., 1983, 1985; Grinvald, et al., Nature, 1984). However, past attempts to use optical mapping for the investigation of the cat visual cortex in vivo and in vitro were not fruitful due to the lack of penetration by the dyes into the cortex and to the inadequate signal-to-noise ratio (S/N).

To overcome these difficulties we have evaluated the performance of 57 dyes, including 29 which were newly synthetized, with a hippocampal slice preparation. We were able to improve the S/N considerably by finding a better dye (giving a 3 to 5 fold improvement over the best dye available previously, RH-414), by making fluorescence rather than transmission measurements and by using a brighter light source. This yielded a total improvement in S/N by a factor of 10 to 20, thus facilitating the recording of a pattern of population acitivity in hippocampal slices with a S/N of 20 to 30 without signal averaging (the fractional change in fluorescence was 0.5%). Several dyes were then tested on slices of visual cortex from cats and monkeys, where the electrically evoked activity is much smaller than that in the hippocampal slice. We therefore averaged over 20 to 30 trials when using the dimmer tungsten light source. Optical mapping of local circuits in the visual cortex confirmed many features of the known microcircuitry of the visual cortex.

Some of the newly designed probes were then used in in vivo experiments. Following topical application for 1-1.5 hours on cat and monkey visual cortex, the full cortical thickness was stained. When we used a flashing grating on a monitor screen, we obtained large optical signals, with a fractional change in fluorescence of 0.07%. The S/N was 10 to 15 when averaged over 20 trials, and 6 to 8 such measurements could be repeated on the same cortical area. For other visual stimuli, however, the signals were smaller and the heartbeat associated movement artifact interfered with reliable interpretation of the different patterns that were observed for different visual stimuli.

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REGULATION OF PITUITARY FUNCTIONS I

10.1 THE PLASMA GROWTH HORMONE RHYTHM DRIVES THE RHYTHM OF EPISODIC SOMATOSTATIN RELEASE. M. Zorzaf A. Williams*, T. Hughes*, and L.C. Terry. University of Michigan and VA Hospitals, Ann Arbor, MI 48105.

In the rat, growth hormone (GH) is released episodically with a period of about 3 h between bursts. To account for the reduced responsiveness to iv injections of growth hormone-releasing factor (GRF) during the trough compared to the peak phase of GH release, Tannenbaum (Endocrinology, 115:1952, 1984) has proposed that hypothalamic somatostatin (SS) is also released episodically, about 180 degrees out of phase with the GH rhythm. This study was undertaken to determine if the GH rhythm induces the SS rhythm.

Chronically cannulated, unanesthetized, male rats were administered 500 ul antiserum to rat GRF (1-43) iv at 0930 h, which inhibited endogenous GH release to undetectable levels until at least 1600 h. GH release was stimulated by iv injection of 1 ug hpGRF (1-44) to which the antiserum to rat GRF showed no cross reactivity. Animals received two hpGRF injections either in phase (3 h apart) at 1245 and 1545 h or out of phase (4.5 h apart) at 1245 and 1715 h. No significant difference in pituitary GH responsiveness to hpGRF was found between the two injections whether delivered in phase or out of phase. Mean plasma GH (ng/ml) 5 min after the first and second injections was 1810 ± 288 and 1999 ± 438, respectively, in the in phase group (N=8) and 1610 ± 256 and 1730 ± 355 in the out of phase group (N=7). If SS continues to be released episodically at 3 h intervals even though the endogenous GH rhythm is suppressed, a significant difference in responsiveness to hpGRF injections delivered out of phase would have been anticipated. SS rhythmic release is apparently absent in GH suppressed rats.

A second experiment was designed to determine if an iv pulse of exogenous rat GH could induce SS release, again in rats treated with 500 ul antiserum to rat GRF at 0930 h. In rats given 1 ml normal sheep serum (NSS) at 1000 h, 20 ug rat GH in 250 ul saline was administered 1.75 h prior to a 1 ug hpGRF injection at 1245 h and 250 ul saline was administered 1.75 h before a 1 ug hpGRF injection at 1545 h. GH pretreatment significantly diminished the pituitary response to hpGRF at 1245 h. Mean plasma GH (ng/ml) 5 min after the first hpGRF injection was 500 ± 130 compared to 2180 ± 421 after the second (N=8). Administration of 1 ml antiserum to SS at 1000 h, instead of NSS, reversed the GH induced reduction of hpGRF responsiveness. In this group (N=6), mean plasma GH (ng/ml) 5 min after the first hpGRF injection was 1332 ± 362 and 1068 ± 220 after the second.

These results suggest that the GH rhythm drives the rhythm of episodic SS release. The hypothalamic somatostatinergic neurons constitute a GH dependent, passive oscillatory element in the control of the GH rhythm.

10.2 SOMATOSTATIN (SS) AUTOIMMUNIZATION INCREASED GROWTH RATE (GR) BUT REDUCED GROWTH HORMONE (GH) SECRETORY RESPONSIVENESS TO GROWTH HORMONE RELEASING FACTOR (GRF) IN LAMBS. M.A. Della-Fera, F.C. Buonomo*, C.A. Baile and M.J. Sabacky*. Nutr. Chem. Div. Monsanto, St. Louis, MO 63167

GH secretion is regulated primarily by two hypothalamic hormones: the inhibitory factor SS, and the releasing factor GRF. The balance between these two is thought to determine the frequency and amplitude of GH secretory episodes. Because of the inhibitory effect of SS on GH secretion, immunoneutralization should lead to increased GH secretion and thus increased GR. Lambs immunized with ovalbumin-SS developed high anti-SS titers and increased GH levels, but grew slower than controls (Varner et al, 1980). In contrast, lambs immunized with human a-globulin-SS (hg-SS) had increased GR compared to controls (Spencer & Garsen, 1983). We have tested two SS-antigens: 1) lambs (N=4/treatment) were immunized with thyroglobulin-SS (TG-SS) or TG (control); 2) lambs (N=7/treatment) were immunized with HG-SS or HG (control). In both cases GR, measured over a 16 wk period, was significantly greater in the SS-immunized sheep than in controls (TG-SS vs TG: .29 vs .24 Kg/day, $p<.01$; HG-SS vs HG: .31 vs .27 Kg/day, $p<.01$). Although there was no significant difference between TG-SS and TG sheep in plasma Somatomedin (SmC) levels, sheep immunized with HG-SS had increased plasma SmC levels compared to controls (0-16wk mean plasma SmC: 5.1 vs 4.2 U/ml, $p<.001$). To determine whether increased SS secretion was responsible for decreasing GH secretory responsiveness with multiple injections of GRF, SS-autoimmunized and control sheep were injected IV with GRF (.016 and .065 nmol/Kg doses in separate tests) hourly for three hours. In both TG-SS and HG-SS sheep plasma GH levels after each GRF injection (either dose) were significantly lower compared to controls. Thus, refractoriness to GRF may result from depletion of GH, changes in GRF receptor affinity or number and/or changes in post-receptor functions, but increased SS secretion does not appear to play a major role. Increased GR of SS-autoimmunized sheep, however, does suggest that SS-antibodies decreased the amount of SS reaching the somatotrophs, thereby resulting in greater GH secretion (as evidenced by increased SmC in one study), and increased GR.

10.3 SOMATOSTATIN IMMUNOCYTOCHEMISTRY IN GROWTH HORMONE-DEFICIENT SNELL DWARF AND "LITTLE" MICE. G.E. Hoffman and C.J. Phelps. Department of Anatomy, University of Rochester Medical Center, Rochester, NY 14642

Snell dwarf mice manifest a recessive genetic disorder which, when present in the homozygous condition, results in a pan-hypopituitarism; the condition includes failure to transcribe GH mRNA (Cheng et al., Endocrinology 113:1669, 1983). Mice possessing the unrelated "little" mutation exhibit an isolated GH deficiency, to 10% of normal levels (Ibid). GH has been shown to stimulate hypothalamic somatostatin (SS) levels, including restoration after SS depletion by hypophysectomy (Kanatsuka et al., Neuroendo. 29:186, 1979). Two recent reports have noted a significant decrease in radioimmunoassayed SS in median eminence, but not in pineal or stomach (Webb et al., Life Sci 36:1239, 1985), and in hypothalamus, but not other CNS sites (Fuhrmann et al., Brain Res. 328:161, 1985) among Snell dwarfs. The present study was undertaken to determine the anatomical distribution of SS in the brains of dwarf (dw/dw) and little (lit/lit) mice, and in their phenotypically normal counterparts, DW/? and LIT/?.

Brains of young adult (2-3 months old) male and female mice (Jackson Labs) were prepared by vascular perfusion with Zamboni's fixative; coronal sections were cut at 30 um and processed for immunocytochemistry using SS antiserum (Immunonuclear 1:1000), and the avidin-biotin technique. Specific staining was eliminated by substitution of non-immune serum, or by preabsorption of SS antiserum with 5-10 uM SS. In dw/dw mice, distribution of SS fibers and perikarya was comparable to the pattern observed in DW/? brains, except for immunoreactivity in hypothalamus. In dwarf median eminence (ME), there was a marked loss of SS terminals in the external zone; immunoreactive SS cells of the periventricular hypothalamus (PE) were extremely sparse and numbered less than 10% of normal. Despite the reduction in the PE-ME pathway, other hypothalamic SS cells and fiber projections appeared normal. In the brains of lit/lit mice, SS distribution was similar to LIT/? patterns in the PE-ME pathway, as well as in other regions.

These observations indicate that the SS abnormality in the hypothalamus of dwarf mice, detected by others biochemically, represents a specific defect in hypophysiotropic neurons. This defect maybe a primary manifestation of the Snell mutation, or be secondary to the pituitary disorder. The presence of normal SS patterns in the lit/lit mouse suggests that SS expression is independent of GH in that strain or that the small amounts of GH present in the little mice are sufficient to maintain SS expression.

Little mice were provided courtesy of the research facilities of Dr. W.G. Beamer. The study was supported by NIH grants HD18243 (CJP) and HD 18418 (GEH).

10.4 α-MELANOCYTE STIMULATING HORMONE EVOKES A STIMULATORY EFFECT OF β ENDORPHIN ON SOMATOSTATIN RELEASE. M.C. Aguila,* O. Khorram* and S.M. McCann. Dept. of Physiology, Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235

The influence of α-melanocyte stimulating hormone (α-MSH) and β-endorphin on the secretion of somatostatin (SRIF) from the median eminence (ME) was studied using an in vitro incubation system. Adult male rats were used as tissue donors. The ME's were first preincubated in 0.4 ml of Krebs-Ringer bicarbonate-glucose buffer with bacitracin, pH 7.4 at 37 C, in an atmosphere of 95% O_2, 5% CO_2 with constant shaking for 30 minutes. Medium was discarded and replaced by medium containing different doses of α-MSH, β-endorphin, or a fixed dose of α-MSH (10^{-7} M or 10^{-9} M) plus β-endorphin at various concentrations. By themselves α-MSH and β-endorphin were unable to affect basal SRIF release, but a 10^{-7} M concentration of α-MSH evoked a stimulatory effect of β-endorphin on somatostatin release. This effect was significant at 10^{-8} M and higher doses of β-endorphin. The permissive effect of α-MSH was observed at a dose as low as 10^{-9} M, but in this case the stimulatory effect of β-endorphin became evident only at higher doses tested (10^{-7} M). It is suggested that α-MSH and β-endorphin participate in the modulation of SRIF release. By themselves β-endorphin and α-MSH did not affect basal release of SRIF but in the presence of α-MSH, β-endorphin had a stimulatory effect on SRIF release. The mechanism for this interaction is unknown. The results are consistent with the possibility that β-endorphin neurons have stimulatory and inhibitory effects on SRIF release and that α-MSH by blocking the inhibitory components, discloses the stimulatory effect of β-endorphin on SRIF release. Experiments are being performed to test the validity of this hypothesis. (Supported by AM10073-19)

10.5 VENTRAL MEDIAL PREOPTIC NUCLEUS (vMPN) OR SUPRACHIASMATIC NUCLEUS (SCN) LESIONS COMBINED WITH ESTROGEN, PROGESTERONE TREATMENT; EFFECTS ON PROLACTIN (PRL) AND LUTEINIZING HORMONE (LH) IN THE CYCLING FEMALE RAT. M.A. Dykshoorn*, M.J. Kelly and O.K. Rønnekleiv. Department of Physiology, Oregon Health Science University, Portland, OR 97201 and Oregon Regional Primate Research Center, Beaverton, OR 97006.

The hypothalamic site of action of progesterone to induce an LH surge has been found to reside in the ventral medial preoptic nucleus (vMPN). Although SCN lesions abolish the proestrus and estrogen-induced PRL surge, the site of action of progesterone to induce a PRL surge has not been determined. In the present study bilateral electrolytic lesions were performed in the SCN (N=10) and/or the vMPN (N=6) of 4-day cycling female rats. The lesions were produced by passing 1-5 μA of anodal current for 3 min through a glass micropipette filled with Wood's metal, with a tip diameter of 60-75 μm and resistance of 2-10 MΩ. Vaginal smears were monitored before and 5-10 weeks after the lesions were made. Serial blood samples (0.4 ml) were obtained every 30-45 min for 6 1/2 hr on the afternoon of various stages post lesion. The plasma was retained for hormone determinations, the blood cells were resuspended in Plasmanate and reinjected. Lastly, partially (N=4) and complete (N=4) SCN-lesioned animals were injected subcutaneously with estrogen (25 μg) and then progesterone (2 mg) 48 h later. Control animals (N=4) received progesterone only on the morning of estrus.

Control lesioned animals cycled normally and exhibited low levels of LH and PRL during diestrus and estrus (3-50 ng/ml). PRL showed occasional spikes during the afternoon of estrus (100-250 ng/ml), and on proestrus PRL and LH surges occurred precisely on successive afternoons. Progesterone treatment on the morning of estrus induced PRL surges in all control animals. Discrete lesions in the SCN or the vMPN caused persistent estrus, interrupted occasionally with 1-2 days of diestrus-type smears. Animals with vMPN lesion or SCN lesions extending rostrally showed an estrus-type pattern of LH and PRL. Estrogen and progesterone treatment of vMPN or partial SCN-lesioned animals caused elevation of plasma PRL, whereas LH remained low. SCN-lesioned animals showed no significant change of plasma PRL or LH in response to estrogen and progesterone. Therefore, these findings indicate that the vMPN and the SCN both control the proestrus surge of PRL and that the SCN is necessary for the estrogen/ progesterone-induced surge of PRL.

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10.6 ACUTE EFFECTS OF PROPRANOLOL IN UNILATERAL DECORTICATED ANIMALS DURING LH AND FSH PULSATILE RELEASE. J.C. Bedran de Castro*, S.L. Petrovic*, S.M. McCann (SPON: S. Speciale). Dept. of Physiology, Univ. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Previous studies indicate the involvement of the cortico-rhinencephalic pathways in pulsatile LH and FSH release since unilateral removal of these structures shortened the cycle period for LH and had the opposite effects for FSH. At the same time there was a significant increase in β-adrenoreceptor binding density probably due to a decrease in firing rate of the noradrenergic neurons projecting to the remaining cerebral cortical structures. Recently a possible inhibitory influence on gonadotropin secretion emanating from the cortex via a β-adrenergic pathway was suggested. To assess the physiological significance of these observations during the pulsatile cycle, female Sprague-Dawley control (C) and hemidecorticated (HD) rats (180-200 g) maintained under controlled lighting conditions (14 h light/10 h dark) were used 21 days after ovariectomy. In freely moving rats blood samples (250 μl) were withdrawn every 10 min over a period of 3 1/2 h and LH and FSH were measured. After 1 1/2 h d,l propranolol (PROP) (3 mg/kg) was administered i.v. in C and HD. The analysis of hormonal profiles indicated changes in number of LH cycles and cycle period after PROP. The LH and FSH overall means before PROP were higher in HD ($p<0.05$) compared with C. After PROP a decrease in LH overall mean occurred at 60 min ($p<0.05$) and at 120 min ($p<0.05$) in the HD group. The lower overall LH mean suggests a β-adrenergic blockade induced by PROP in the unilateral decorticate animals. A cortico-rhinenecephalic β-adrenergic mechanism inducing modulations in LH release in ovariectomized rats may be due to the increase in β-adrenergic binding capacity in HD animals as previously demonstrated. The lack of acute modulation in FSH levels suggests once more a differential LH and FSH pulsatile modulation. (Supported by FAPESP and HD09988).

10.7 WHICH LHRH NEURONS PROJECT TO THE MEDIAN EMINENCE? A COMBINED RETROGRADE TRACING AND IMMUNOCYTOCHEMICAL ANALYSIS. Ann-Judith Silverman and Leo Renaud. Dept. Anatomy & Cell Biology, Columbia Univ., P&S, New York, N.Y. 10032 and Neuroscience Unit, Montreal General Hospital, McGill Univ., Montreal, Canada, H3G 1A4.

LHRH neurons in the rat CNS are not concentrated into a single nuclear group but are widely distributed in preoptic, hypothalamic, septal and olfactory regions. The major, if not sole, neurosecretory terminus where neurohormone is released is in the median eminence (ME). Since it is not known which specific LHRH neurons innervate this structure, we used a modification of the procedure of Wiegand and Price (J. Comp. Neurol. 1980 192:1) to answer this question. A 10% wheat germ agglutinin solution (WGA: EY Labs) was administered by pressure injection in nanoliter quantities via a micropipette (20-40 um tip) directly into the ME via a transpharyngeal approach in pentobarbital anesthesized male Sprague Dawley rats. 10-12 hrs later, animals were perfused (Zamboni's fixative) and the lectin localized immunocytochemically in 50-75 um vibratome sections (antibody: EY Labs) using an avidin-biotin-HRP procedure (Vecastain) with DAB as the chromagen. The reaction product was seen as discrete brown dots within neuronal cytoplasm corresponding to the vesicular structures within which WGA was transported. Sections were washed and then incubated in an antibody to LHRH (Benoit, LR-1) which was localized with an avidin-fluorescein procedure (Vecastain). Sections were analyzed to determine which LHRH neurons were retrogradely labeled, i.e., projected to the ME. Approximately 50% of the LHRH neurons found in the diagonal band of Broca, the medial and triangular septal nuclei, the medial and lateral preoptic area and the lateral hypothalamus just dorsal to the supraoptic nucleus also contained WGA. The doubly and singly labeled cells showed no obvious preferential distribution in any of these regions. Both smooth contoured and thorny or spiny LHRH cells were observed; both types were also doubly labeled. These data suggest that the neurosecretory cells that directly influence gonadotropin secretion are widely distributed in the rat CNS and that neighboring cells containing the same peptide may have different efferent projections.

Supported by USPHS HD10665 (AJS) and the Canadian MRC 5038 (LR).

10.8 CORRELATIVE STIMULATION OF SUPRAOPTIC NEURONS AND VASOPRESSIN RELEASE TO NORADRENERGIC STIMULATION IN VITRO W.E. Armstrong, M.J. Gallagher* and C.D.Sladek. Dept.of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163 and Depts. of Neurology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

When a neuroactive substance is implicated in the control of neurohypophysial hormone release, seldom does an investigator have the opportunity to directly measure the substance's action on both hormone release and on the electrical activity of the neurons synthesizing the hormone in question. Such an experiment can be accomplished in vitro using the isolated hypothalamo-neurohypophysial system, which retains intact a substantial number of supraoptic neurons and their projection to the neurohypophysis. In the present study, explants prepared from male albino rats were used to assess the action of noradrenergic agonists on the electrical activity of supraoptic neurons and vasopressin released into the perifusate.

Following their excision explants were perifused in a balanced salt solution at 2 ml/min. After a 2-3 hr recuperative period, extracellular recordings were made in the retrochiasmatic portion of the supraoptic nucleus, which contains a majority of neurons immunoreactive for vasopressin. Potentials identified antidromically from neural stalk stimulation were held for a 2 hr period during which drug applications were made and samples of the effluent collected every minute for radioimmunoassay.

Basal vasopressin release ranged from being undectable to approximately 5 pg/min. Transient injection of the α_1-agonist, phenylephrine (0.1 µm-1 mM) into the perifusate resulted in a dose-dependent release of vasopressin and a burst of action potentials in the recorded supraoptic neuron. Within an explant the mean within-burst firing rate for the first 30 sec of the burst was positively correlated with the peak rate of vasopressin release, which usually occured during the second minute of collection following drug application. In equimolar amounts, noradrenaline was associated with a greater rate of vasopressin release than was phenylephrine and also excited supraoptic neurons. Neither the β-agonist isoprenaline (100 mM) nor the α_2-agonist clonidine (100 mM) stimulated vasopressin release or supraoptic neurons.

The consistent ability of phenylephrine to release vasopressin is predictable considering that in additional electrophysiological experiments 88% of all retrochiasmatic supraoptic neurons were excited by this compound, including 48 of 56 slow/silent neurons and 16 of 17 phasic neurons. Since the great majority of these neurons synthesize vasopressin, the results indicate vasopressin release and excitation of vasopressin secreting neurons are directly associated during noradrenergic stimulation.

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10.9 ANTIDIURESIS PRODUCED BY α-ADRENERGIC AND DOPAMINERGIC STIMULATION OF THE RAT SUPRAOPTIC NUCLEUS. Sarah F. Leibowitz and Kenneth F. Garay.* The Rockefeller University, New York, N.Y. 10021.

To examine the role of central catecholamine (CA) systems in the control of urine formation, CA agonists were injected through a chronic cannula directly into the supraoptic nucleus (SON), and their effects on the urine output of freely-moving, hydrated rats was measured. l-Norepinephrine (NE) and l-epinephrine (EPI), in contrast to their d-isomers or various control solutions with adjusted pH, osmolarity or bitartrate concentrations, produced a clear state of antidiuresis, characterized by a strong suppression of urine flow (30-70%), an increase in urine osmolality and Na^+ and K^+ concentrations, but no change in the ratio of Na^+/K^+, nor in the clearance of creatinine. This antidiuretic effect was dose-dependent (0.05-100 nmoles), lasting 15-120 min and, in particularly responsive animals, could be observed at a dose at least as low as 1.0 pmole (170 pg free base). A 25 ng dose induced a similar response, comparable in magnitude and duration, to that induced by sc injection of 300 µU lysine vasopressin. A somewhat less robust antidiuretic effect was obtained with SON administration of dopamine (DA).

SON injections of the α-adrenergic antagonists, phentolamine, phenoxybenzamine and tolazoline, were effective in blocking the action of NE or EPI, whereas other receptor antagonists, including β-adrenergic, DA, cholinergic (muscarinic and nicotinic), and serotonergic blockers were,without effect. The DA antagonists, haloperidol and pimozide, in addition to the α-adrenergic blockers, antagonized DA-induced antidiuresis. At higher doses, both phentolamine and haloperidol, and also the CA synthesis inhibitor α-methyl-p-tyrosine, each acted in the opposite direction to the agonists, producing increased diuresis after SON injection.

Cannula mapping studies with NE (0.1-20 nmoles) showed that the hypothalamic paraventricular nucleus (PVN) can exhibit a similar, although somewhat less robust, antidiuretic response to α-noradrenergic stimulation. The sensitivity of this nucleus contrasted with that of the ventromedial nucleus, which responded only to a high dose (10 nmoles) of NE, and with that of the caudal lateral hypothalamus, the rostral perifornical hypothalamus just lateral to the PVN, the nucleus accumbens and caudate nucleus, which were generally unresponsive to NE.

From these results, it is concluded that NE (α) and DA, in the SON and PVN, act to induce a state of antidiuresis. Based on a variety of evidence, including measurements of cardiovascular changes, it is suggested that this antidiuretic effect may result from the release of vasopressin from magnocellular neurons in these hypothalamic nuclei.

(This research was supported by grant MH 22879 and by funds from the Whitehall Foundation.)

10.10 OPIOID PEPTIDES MEDIATE THE WATER-INDUCED REDUCTION IN PLASMA VASOPRESSIN AND OXYTOCIN CONCENTRATIONS OF HYPOVOLEMIC RATS. L.M. Rosella-Dampman*, R.D. Hartman*, and J.Y. Summy-Long (SPON: W.B. Severs), Dept. of Pharm., Penn St. Univ., Hershey, PA 17033.

Overhydration inhibits release of vasopressin (VP) and oxytocin (OT) from the hypothalamo-neurohypophysial system (HNS) stimulated by hypovolemia (Stricker and Verbalis, Soc. Neurosci. Abstr. 10:90, 1984). We proposed that endogenous opioid peptides (EOP) mediate inhibition of the HNS by H_2O during hypovolemia. The effect of naloxone (NAL), an opiate receptor antagonist, on plasma [VP] and [OT] was studied in normal and overhydrated male rats made hypovolemic by either hemorrhage (HEM) of 35% blood volume (B.V.; 65 ml/kg) or injection of polyethylene glycol (PEG; 20-M). B.V. was reduced by removing blood at a rate of 0.51 ml/min from catheters placed in the femoral artery 2 days earlier or by s.c. injection of 30% PEG (w/v, 35 ml/kg). When 10% of the B.V. was removed (HEM) or 6.75 hr after PEG, rats were intubated (no H_2O) or given H_2O (40 ml/kg, p.o.), which lowered plasma osmolality 8-18 mOsm/kg. Saline (SAL, 1 ml/kg) or NAL was injected 4 min before HEM (2 mg/kg, i.v.) was ended or 6.83 hr after PEG (5 mg/kg, s.c.). Rats were decapitated 5 min (HEM) or 10 min (PEG) later. Control animals were treated similarly but not HEM or were injected with SAL without PEG. Plasma VP and OT (pg/ml ± SE; n4-14) were quantified by RIA, and analyzed by ANOVA. HEM increased ($p < 0.05$) plasma [VP] (5±1 vs 334±92) and [OT] (83±10 to 329±72). Water reduced ($p < 0.05$) the rise in [VP] (127±27) and [OT] (146±27) after HEM, but not in controls (VP: 6 ± 1; OT: 78 ± 10). NAL elevated [OT] in HEM rats that were intubated (508±83) or overhydrated (469±86) without affecting [VP]. In control rats that were not HEM, plasma [OT] but not [VP] was increased by NAL after intubation (OT: 127±11) or overhydration (OT: 181±28). Since surgery (vs unoperated controls) in the HEM study altered basal levels of OT (83 ± 10 vs 6 ± 0.2 pg/ml), we examined NAL effects during hypovolemia produced by PEG, a less invasive protocol. PEG increased [VP] (2±0.2 vs 41±6) and [OT] (6±0.2 vs 77±17). Water suppressed ($p<0.05$) the PEG-induced rise in [VP] (13±1) and [OT] (22±4) without altering levels in controls (VP: 2 ± 0.3; OT: 6 ± 0.8). As during HEM, NAL augmented the rise in [OT] (395±75) but not [VP] (42±6) after PEG. However, in overhydrated animals, NAL partially reversed ($p<0.05$) the water-induced reduction in both [VP] (21±3) and [OT] (118±33) after PEG. Neither NAL nor H_2O affected [VP] or [OT] in controls. Similar results were obtained in the PEG study when data were expressed as pg hormone per mg plasma protein. Thus, H_2O attenuated release of hormones during hypovolemia rather than only diluting their concentrations. We conclude that EOP: a) inhibit release of OT after surgery and hypovolemia, and b) mediate, in part, the inhibitory effects of water on release of VP and OT during hypovolemia. (Supported by NIH HL 32826).

10.11 OXYTOCIN ANTISERUM BLOCKS STEROID-INDUCED PROLACTIN RELEASE AND ATTENUATES THE PROLACTIN RESPONSE TO SUCKLING. WK Samson, MD Lumpkin & SM McCann (SPON: A. Rupert). Physiol, Southwestern Med Sch, Dallas, Tex 75235.

We have reported previously that synthetic oxytocin (OT) possesses powerful and specific prolactin (PRL) releasing activity in vitro (Endo. 112:1711, 1983; Fed. Proc. 43:503, 1984) at doses similar to those found in hypophysial portal blood in vivo (Endo. 114:1216, 1984). Additionally, we have reported that OT surges in peripheral plasma preceed those of PRL following a suckling stimulus and after steroid treatment in the rat. Steroid treatment: Injection of estradiol benzoate (5µg, sc) in ovariectomized rats results two days later in a significant release of OT at 1200 and 1300h and PRL at 1300h. When 1ml normal rabbit serum was infused i.v. at 1000hr, the PRL peak at 1300h was still present (1100h:9.23±1.30; 1200h:17.64±5.84; 1300h:40.88±13.15; 1400h:23.20±12.60ngm PRL/ml plasma). However, infusion of 1ml antiserum specific to OT completely abolished the PRL surge (1100h:13.23±7.63; 1200h:10.28±3.91; 1300h:10.68±3.22; 1400h:13.53±3.36). These preliminary data suggest a possible physiological PRL releasing factor (PRF) role for endogenous OT released into hypophysial portal blood on the afternoon of proestrus (Sarker and Gibbs, Neuroendo. 39:481, 1984) just prior to the proestrous PRL surge. Lactation: Both OT and PRL are released in response to a suckling stimulus; however, if suckling is maintained, levels of PRL remain elevated, while those of OT return within 30 min to presuckling baseline (Soc. Neurosci. 136:12, 1983). When mothers were denied their pups for 4hr and 1ml normal rabbit serum (NRS) was infused (n=6) i.v. just prior to pup reinstatement, suckling induced PRL release occured as expected, however following infusion of 1ml antiserum to OT (n=9) the surge was significantly attenuated (prior to pup reinstatement:NRS 18.3±8.1, anti OT 21.0±2.9 ngm PRL/ml plasma; 45 min later:NRS 300.1±76.8, anti OT 113.8±40.4, $p<.05$; 60 min later:NRS 399.0±89.1, anti OT 115.2±25.6, $p<.005$). Infusion of a lower titer anti OT serum was without effect. Thus while some investigators feel that the initial PRL response to suckling is dependent upon dopamine withdrawal (Grosvenor and Mena, Neuroendo. Persp., Vol. 1, 1982) the initial release of PRL appears to be in part due to OT. The ability of OT antiserum to completely block this initial PRL surge is similar to results obtained by Abe et al. (Endo. 116:1383, 1985) using antiserum to VIP suggesting a multiplicity of peptidergic PRFs during this phase of PRL release. In summary, the ability of OT to stimulate PRL release in vitro coupled with our present data on passive immunoneutralization of endogenous OT strongly suggests a role for OT in the physiological control of PRL release.

10.12 HIGH GLUCOSE UTILIZATION IN THE PITUITARY NEURAL LOBE OF BRATTLEBORO RATS IS DECREASED BY DESMOPRESSIN. M. Kadekaro and P.M. Gross. Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20205, Div. of Neurosurgery, UTMB, Galveston, TX 77550* and Dept. Neurological Surgery, SUNY, Stony Brook, NY 11794. *Present addresses.

Our previous studies showed that high glucose utilization in the pituitary neural lobe of Brattleboro (DI) rats persisted after chronic treatment with arginine vasopressin (AVP), while other brain structures exhibited normal rates of glucose utilization (Brain Res. 275:189, 1983). This finding conflicted with the semi-quantitative studies of another group (Sutherland et al., Brain Res. 271:101, 1983) who found an apparent normal level of glucose utilization in the neural lobe of DI rats after acute treatment with desmopressin (dDAVP, 1-desamino-8-D-arginine vasopressin), a synthetic analogue of AVP with a more potent and prolonged antidiuretic effect. Using the quantitative autoradiographic deoxyglucose method we have reexamined the local rates of glucose utilization in the brain and neural lobe of DI rats given dDAVP. Four month-old DI rats were injected either with dDAVP (100µg/kg ip) once a day for two days (n=4) or with vehicle (n=4). Water intake was measured daily for five days before treatment with dDAVP and for two days afterwards. Local rates of glucose utilization were determined and compared to our previous results from rats receiving AVP for one week (reference above).

	Long-Evans (n=10)	DI (n=10)	DI+AVP (n=7)	DI (n=4)	DI+dDAVP (n=4)
Water intake (ml/100g/24hr)	11±1	84±5	22±4*	66±10	15±1***
Glucose utilization (µmols/100g/min)					
Subfornical organ	48±2	69±3	53±4*	60±3	45±2**
Paraventricular nuclei	59±3	58±2	57±3	58±1	47±3**
Supraoptic nuclei	58±2	61±3	55±2	52±2	51±3*
Neural lobe	41±2	83±5	84±6	69±5	45±6

Values are means+SEM; *p<.05, **p<.01, ***p<.001, compared to respective control

The results demonstrate that dDAVP has a potent inhibitory effect on water intake and on glucose utilization in the neural lobe and other brain structures. We suggest that the ineffectiveness of chronic AVP treatment in decreasing glucose utilization in the neural lobe of DI rats may be due to the reported phenomenon of physiological adaptation which leads to diminished response to the hormone.

SUBCORTICAL SOMATOSENSORY PATHWAYS I

11.1 CHARACTERIZATION OF CAT NASAL RECEPTORS PROJECTING TO TRIGEMINAL SUBNUCLEUS INTERPOLARIS. G. E. Lucier and R. Egizii*. Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1, Canada.

The ethmoidal nerve innervates the anterior portion of the nasal mucosa and constitutes the afferent limb of several upper respiratory tract protective reflexes. Previous anatomical studies (Lucier and Egizii, Proc. IUPS, 25:123.30, 1983) have shown that ethmoidal afferents project to all regions of the spinal trigeminal nucleus. The purpose of this study was to characterize the nature of the ethmoidal inputs onto cells in the subnucleus interpolaris.

Adult cats were anaesthetized with alpha chloralose. Two tracheal cannulae were inserted, one to provide access to the lungs for ventilation and a second to allow air and test odorants to be pulled through the upper airway. Arterial blood pressure and end tidal CO_2 were monitored. Bi-polar stimulating electrodes were placed on the ethmoidal, vagus, hypoglossal, glossopharyngeal and superior laryngeal as well as the ipsilateral, maxillary and mandibular canine tooth pulp. During recording, all animals were paralyzed with gallamine triethiodide and artificially ventilated. The activity of functionally identified single neurons in subnucleus interpolaris was recorded using tungsten microelectrodes.

The inputs to over 100 interpolaris neurons which had a discrete trigeminal receptive field were studied. Neurons which received short latency ($\leq$10 msec) input from the ethmoidal nerve also responded to non-noxious tactile stimuli either applied to the nasal region or facial receptive fields. A much smaller group of neurons were encountered which resembled neurons previously described in subnucleus caudalis which receive an ethmoidal projection. These neurons, in addition to having a long latency input from ethmoidal ($\geq$10 msec) also receive input of a nociceptive nature from other trigeminal divisions (i.e. tooth pulp stimulation, noxious radiant heat applied to the face). These cells could also be stimulated by non-electrical noxious ethmoidal input (i.e. ammonia vapour). Supported by the Canadian MRC.

11.2 STRUCTURE-FUNCTION-CONNECTIVITY RELATIONSHIPS FOR NEURONS IN SUBNUCLEUS INTERPOLARIS (SpVi). M.F. Jacquin, D. Woerner*, R.D. Mooney & R.W. Rhoades. Dept. of Anatomy, UMDNJ-School of Osteopathic Medicine & Rutgers Medical School, Piscataway, NJ 08854

Intracellular recording, antidromic stimulation, and HRP injection techniques were used to compare the morphological and physiological properties of 56 neurons in rat SpVi. In most experiments, stimulating electrodes were inserted in the trigeminal ganglion, cerebellum (CB), and subnucleus principalis (PrV) ipsilateral, and the ventral posteromedial thalamus (VPM) and deep layers of the superior colliculus (SC) contralateral, to the recording electrode. 20 cells projected to VPM and, of those tested, 91% also projected via axon collaterals to SC, 64% to PrV, 64% to adjacent parvicellular reticular formation, and 73% to SpVi itself. 6 of these cells projected to all of these targets, while none were driven from CB. VPM-projecting cells had medium or large sized multipolar perikarya (range of average diameters=20-48um) with widespread, transversely oriented dendrites. All but 2 were responsive to deflection of any 1 of a # of vibrissae (4-19). Significant correlations were noted between VPM conduction latency and axon diameter, and # of targets innervated and axon diameter. 9 other cells projected to CB with small or medium sized (16-31um) multipolar somata. None of these had multiple projections and all but 1 had a small receptive field (1 or 2 vibrissae). 2 cells projected to SC, but not VPM, and 1 of these projected to PrV as well. Each had multipolar perikarya (15 & 41um) and they responded to 4 and 6 vibrissae, respectively. 4 medium sized multipolar cells (20-24um) were backfired from PrV only and had dense compact local axon arbors; each responded to 1 vibrissa. For all of the above projection neurons, receptive field size was correlated with dendritic area in the transverse plane.

The 21 remaining cells did not have an identifiable long range projection and, in most cases, their axons terminated locally. 11 of these responded to 1 vibrissa and had small or medium sized (12-25um) round, fusiform or multipolar somata; all had similar, longitudinally oriented, compact dendritic and axon arbors. 5 responded only to guard hair deflection. These had small or medium sized (15-27um) fusiform or multipolar perikarya and widespread dendrites. 5 responded only to noxious pinch or pressure and their morphology ranged from small (18um) and round with compact dendrites, to medium sized (30um) and multipolar with widespread dendrites. Nociceptors were located along the medial border of SpVi. Other cells were topographically organized in a manner consistent with prior mapping studies in SpVi. We conclude that strong structure-function-connectivity relationships exist for individual neurons in SpVi. Supported by DE06528, EY03546, EY04170, the UMDNJ Foundation and the American Osteopathic Association.
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11.3 INTRINSIC AND EXTRINSIC CONNECTIONS OF THE SPINAL TRIGEMINAL NUCLEUS IN CATS: SUBNUCLEUS ORALIS. N.F. Capra and J.M. Bailey*. Dept. of Anatomy, U. Miss. Med. Ctr., Jackson, MS 39216.

The subnucleus oralis (Vo) of the spinal trigeminal nucleus receives primary afferent input from the face, temporomandibular joint, teeth, and periodontium. The diversity of its peripheral inputs suggests a complex role for Vo in orofacial mechanisms. Central connections which may serve to modulate activities in Vo are the subject of this report.

Discrete electrophoretic injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP; 2-4%) were made into physiologically identified regions of Vo. The animals were euthanized 12-24 hours later and perfused transcardially with saline followed by a mixed aldehyde fixative. Serial sections (40-60 μm) made through the brain stem, upper spinal cord, and portions of the cerebral cortex were processed for histochemical demonstration of HRP using TMB as a chromagen.

Eight cats selected for this study had discrete injection sites restricted to the rostral part of Vo. Labeled perikarya were identified throughout trigeminal sensory nuclei, dorsolateral reticular formation, nucleus cuneatus, and the orbital gyrus of the cerebral cortex. Of these, the largest number of neurons projecting to Vo originated in laminae IV and V of the ipsilateral medullary dorsal horn (MDH) and layer V of the contralateral orbital gyrus. Contributions from laminae I-III of the ipsilateral MDH, contralateral trigeminal nuclei, ventrolateral region of nucleus cuneatus, though sparse, were reliably observed. Anterogradely filled axons from Vo projected caudally to terminate on cells in trigeminal subnucleus interpolaris and the MDH. Rostrally directed axons either crossed exiting fibers of the facial nerve and terminated in the region of the trigeminal motor nucleus or decussated and ascended in the pontine tegmentum.

These results are consistent with electrophysiologic data suggesting that MDH neuron project rostrally and modulate the activity of neurons in Vo which receive primary afferent input from the tooth pulp (Hu et al., '84). These connections may provide an indirect avenue for the periaqueductal gray (PAG) and the nucleus raphe magnus (NRM) to exert inhibitory effects on primary afferent transmission from Vo. Both PAG and NRM project to MDH but not to Vo. Corticofugal fibers from the orbital gyrus, participate in modulation of orofacial reflexes and mastication. Efferent projections from Vo to trigeminal motor neurons complete the masticatory reflex pathways. Other extrinsic and intrinsic projections that originate in Vo may be involved in mechanisms which underlie the conscious appreciation of jaw position and oral stereognosis since many of the neurons in this region are activated by passive jaw movements and by pressure applied to the teeth (Capra and Anderson, J. Dent. Res., '85). Supported by DE06027

11.4 MECHANICAL RESPONSE PROPERTIES OF TRIGEMINAL THALAMIC NEURONS IN THE ALERT MONKEY. G.H. Duncan* and M.C. Bushnell. Fac. Medecine dentaire, Univ. de Montreal, Montreal, Que. CANADA H2V 3B5

Previous studies of trigeminal thalamic neurons have not yielded a consensus regarding the neurophysiological properties of the ventroposterior medial nucleus (VPM). Most investigators have observed an overwhelming majority of rapidly adapting low threshold (RA) neurons. Recently, however, some studies have documented smaller populations of slowly adapting (SA) low threshold, wide dynamic range (WDR), and nociceptive specific neurons. Most reports of VPM in monkeys are incorporated within larger surveys of the ventrobasal complex, so that frequently VPM is not systematically explored. In addition, most studies have been conducted in either anesthetized or paralyzed animals. The present study explores the properties of trigeminal cutaneous thalamic neurons in an alert monkey to determine receptive field and response properties in neurons unaltered by pharmacological agents.

One macaque monkey received juice reward for sitting quietly while an investigator probed the monkey's face with mechanical stimuli. Extracellular single-unit recordings were made from VPM neurons, and mechanical response properties were evaluated for each cell having an extraoral cutaneous receptive field.

Sixty-four VPM neurons with receptive fields on the face were characterized. Fifty-two (81%) of these neurons responded maximally to gentle touch of hair or skin. Twenty-nine (56%) of the low threshold cells adapted rapidly to sustained touch (RA), but 23 (44%) continued to respond throughout the stimulus presentation (SA). Most low threshold neurons had receptive fields on the contralateral side, but 7% showed fields confined to the ipsilateral face. Both RA and SA neurons were found throughout VPM. Six neurons (9%), classified as WDR, showed a graded response to increasingly intense stimuli, with a maximum discharge to noxious pinch. Receptive fields of WDR neurons were smaller than those previously observed in trigeminothalamic neurons of the medullary dorsal horn, and no larger than those of low threshold VPM cells in the current study. WDR cells were located primarily in the caudal third of VPM. Finally, the activity of six neurons (9%) was inhibited during innocuous mechanical stimulation. These cells had small contralateral receptive fields and were located in the caudal third of VPM.

In summary, response characteristics of VPM neurons show more diversity in the alert monkey than has been reported in paralyzed and/or anesthetized animals. In addition to the large proportion of RA neurons, there exists a similar population of SA neurons, and smaller populations of inhibitory and WDR cells. Thus, VPM contains neurons capable of coding both static and dynamic mechanical properties of noxious and innocuous cutaneous stimuli.

11.5 AROUSAL-RELATED RESPONSE MODULATION OF VP THALAMIC NEURONS TO SOMATIC AND SPINAL LEMNISCAL STIMULATION IN THE AWAKE MONKEY. T.J. Morrow and K.L. Casey, Neurology Research Laboratories, V.A. Medical Center, Ann Arbor, MI 48105.

RATIONALE: Our sensory systems are continually bombarded by inputs from which we select information as a basis for action, ignoring all the rest. It is reasonable to hypothesize that certain properties of a neuron's response to a specific sensory stimulus should covary with changes in behavioral state. Accordingly, the somatosensory responses of ventral posterior (VP) thalamic neurons would be expected to vary with the level of arousal in unanesthetized, behaving animals. Previous studies have failed to demonstrate this relationship, and have shown changes in only the spontaneous discharge of VP somatosensory neurons (Baker, 1971; Hayward, 1975).
METHODS: The responses of single neurons in the ventral posterior thalamus to a variety of controlled, calibrated, somatic stimuli and to electrical stimulation of the midbrain spinal lemniscus (SL) were recorded in four awake squirrel monkeys. Movements of the animal and EEG changes were continually monitored, and used to identify periods of drowsiness, quiet waking and waking with movement. Neural responses to stimuli delivered during these periods were separately analyzed by computer for changes which correlated with changes in the state of arousal.
RESULTS: Many VP thalamic neurons (29/51) exhibited changes in somatosensory responsiveness which correlated with shifts in arousal. Arousal-related changes were not selective for neurons responding to any specific stimulus type (ie. hair movement, touch, tap, etc.) and included modulation of 3 neurons in the wide dynamic range class (WDR). Most neurons discharged maximally during quiet waking. Responses were significantly reduced during drowsiness and either unchanged or reduced during periods of waking with movement. Similar arousal-related changes were seen in 13 of 30 neurons driven by SL stimulation. The spontaneous discharge of many somatically driven neurons also varied as a function of the state of arousal. All changes in stimulus evoked responses were, however, independent of the pre-stimulus background discharge frequency since reduced responses to somatic and spinal lemniscal stimulation were seen during increased or decreased background activity.
CONCLUSIONS: The somatosensory responses and the spontaneous discharge of VP thalamic neurons are significantly modulated during concurrent changes in the state of arousal. One modulating mechanism includes inhibition (possibly presynaptic) at the level of the VP thalamus.

11.6 CEREBELLAR RELATIONSHIPS WITH SOMATIC SENSORY PORTIONS OF THE PRETECTUM AND ZONA INCERTA IN THE CAT. M.S. Bull and K.J. Berkley. Dept. of Psychology, Florida St. Univ., Tallahassee, FL 32306.

Considerable evidence has accumulated showing that neurons in the dorsal column nuclei (DCN) project not only to the ventroposterolateral nucleus of the thalamus (VPL), but also to a number of other structures throughout the neuroaxis (e.g. Hand and Liu, 1966; Berkley and Hand, 1978). Recent evidence from several laboratories suggests that the group of DCN neurons projecting to VPL differs both anatomically and functionally from the groups projecting to these other targets (see Berkley, 1985).

One common feature of some of the extra-VPL-projecting groups of DCN neurons is that they project to the cerebellum and to precerebellar nuclei such as the inferior olive (IO) and PONS. In addition, another group of DCN neurons projects to the anterior pretectal nucleus (PTA) whose neurons in turn project to the DCN-recipient portions of the IO and PONS (Bull and Berkley, 1983).

In the course of studying these cerebellar-related pathways, it became apparent that the neurons in the DCN-recipient portion of the pretectum, (i.e. PTA), had reciprocal connections with the DCN-recipient portion of the zona incerta (ZI). It is difficult to speculate, however, what role the ZI might have in the context of a cerebellar-related system involving DCN and PTA. This difficulty may be due in part to ZI's diverse nature as a structure with putative motor as well as sensory (nociceptive) functions (Kaelber and Smith, 1979).

In an effort to understand what role, if any, the ZI inputs might play in a cerebellar-related system, the DCN inputs to ZI and PTA were compared with those from the cerebellum using a double orthograde labeling strategy. The results showed that the DCN and cerebellar terminations overlapped in PTA but did not share the same domain in ZI. (The DCN terminations were ventral, lateral and caudal to those from the cerebellum.)

That the DCN and cerebellar terminations converge in PTA, a structure which projects to the cerebellum through the PONS and IO, but do not converge in ZI, is consistent with the suggestion that the pretectum and its afferent DCN input is involved in somatic-motor feedback functions associated with the cerebellum. The separation of the DCN and cerebellar inputs in ZI, on the other hand, suggest that the afferent DCN input contributes to a different, perhaps more sensory (i.e. nociceptive) role for ZI.

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11.7 SYNAPSES UPON PHYSIOLOGICALLY CHARACTERIZED THALAMIC NEURONS WHICH RESPOND TO HAIR FOLLICLE STIMULATION: AN ELECTRON MICROSCOPE STUDY. P.T. Ohara, D.D. Ralston*, and H.J. Ralston, III. Department of Anatomy, University of California, San Francisco, CA 94143

The morphological features of the synaptic populations contacting single, physiologically characterized thalamic neurons which were iontophoretically labeled by the intracellular injection of HRP have been studied in the cat by the electron microscopic analysis of serial thin sections. The purpose of the study was to determine whether the populations of synapses contacting various regions of the cells, (soma, primary, secondary or tertiary dendrites) varied, and whether cells which responded to movements of hairs had different synaptic inputs than those upon thalamic neurons which responded to other nonnoxious or noxious peripheral stimuli. Cats were anesthetized with IM ketamine followed by intravenous pentobarbital, placed in a stereotaxic headholder and the cortex overlying VB removed by suction. A glass guide tube was placed on top of the thalamus, and a metal electrode used to search for receptive fields. Once the stereotaxic coordinates had been determined, a glass micropipette containing 4% HRP with a resistance of 100 MΩ was advanced into the thalamus and the receptive fields and response properties of neurons recorded. In this study, we report on 3 neurons with small receptive fields on the contralateral forepaw which responded to deflection of small clusters of hairs and which adapted rapidly to the stimuli. The cells were held intracellularly from 5-10 minutes, during which time 0.5 nA of positive current was injected. One to 4 hours later the animal was perfused with aldehydes and the thalamus serially sectioned at 50μm, reacted with DAB, and sections containing labeled neurons osmicated and embedded in epoxy resins. Drawings were made of labeled cells and the 50μm section cut serially for EM, with frequent drawings and photographs being made for the identification of various neuronal elements. In this way, most of the synaptic input to a single neuron can be examined. Examination of 425 consecutive sections of the first 40um of a dendrite, from cell body to the initial portion of a tertiary branch, has revealed 27 contacts made by large synaptic profiles with round vesicles (RL), 7 by presynaptic dendrites (PSD), 10 by flat-vesicle axonal profiles (F-1), 7 by F profiles of unknown origin, and 8 by profiles which could not be classified. No contacts by small profiles with round vesicles (RS) were seen on this protion of the dendritic tree. The serial sections permitted a determination of the dendritic character of several PSD's, as well as the synaptic input to these PSD's. In addition, PSD's could be found to follow a tortuous course, forming multiple contacts with the labeled dendritic shaft. These contacts were not involved in triadic synaptic relationships with RL profiles. An extensive description of the synaptic input to all regions of this physiological class of neurons will be presented. (Supported by NS-11614 from NIH).

11.8 THE CUNEATE NUCLEUS OF THE MONKEY: ANATOMICAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR SUBMODALITY SEGREGATION.

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The main cuneate nucleus (MCN) and the external cuneate nucleus (ECN) were investigated by means of transganglionic labeling with WGA-HRP, field potential mapping, and extracellular unit recordings in the anesthetized monkey. It was found that labeling following incubation of the deep radial nerve occurred in the pars triangularis of the MCN and in the ventromedial segment of the ECN. Field potentials evoked by electrical stimulation of low-threshold muscle afferents were likewise focused on these subdivisions of the ECN and MCN. By contrast, the pars rotunda of the MCN is projected upon by cutaneous afferents: loading of the superficial radial nerve or cutaneous infiltration of a single digit with WGA-HRP produced dense patches in this 'core' region of the MCN. After having established the territory of the 'proprioceptive' bulbar relay of the forelimb, single neurons were studied with respect to their sensitivity to small sinusoidal and ramp stretches of the extensor digitorum communis (EDC) muscle, to their convergence pattern, and to their projection to the thalamus and the cerebellum. In the MCN 27 neurons and in the ECN 8 neurons were found to project to the thalamus. In each nucleus two additional cells were found to project to both targets. With controlled longitudinal displacements of the EDC muscle, neurons in both ECN and MCN discharged phasically, often with partial entrainment in response to 100-200 Hz sinusoidal stretches. Many neurons were activated with minimal step amplitudes of 50 μm or less. The majority of neurons appeared to react specifically to one muscle. Our electrophysiological results corroborate previous suggestions that a subpopulation of ECN neurons contribute to a proprioceptive transmission system, via the thalamus, to the cerebral cortex (Boivie and Boman, 1981). The electrophysiological properties of the 'proprioceptive' bulbar neurons were very similar to those recorded in the thalamus and in cortical area 3a with the same experimental protocol. The transmission system thus functions with high synaptic efficacy, providing the cortex with precise musculotopic information about small changes of muscle length.

11.9 THE SOURCES OF PEPTIDERGIC INPUT TO THE LATERAL SPINAL NUCLEUS IN THE RAT. K.D. Cliffer, G. Urca,* R. Elde and G.J. Giesler, Jr. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455 and Dept. of Physiology and Pharmacology, Tel Aviv Univ., Ramat Aviv, Israel.

The lateral spinal nucleus (LSn) of rats is rich in fibers that are immunoreactive for met-enkephalin (ENK), substance P (SP), somatostatin (SOM), dynorphin (DYN) and FMRF-amide (FMRF-NH_2). In this study we examined the sources of these peptidergic fibers.

In rats with unilateral dorsal rhizotomies of at least three lumbar segments ten days prior to sacrifice, no difference was seen between sides in staining for any of the five peptides in the LSn, even though the staining for SP was greatly reduced in superficial laminae of the dorsal horn on the side of the rhizotomy. Thus, little, if any, of the peptidergic input to the LSn appears to come from primary afferents.

In spinal cords transected at low thoracic levels nine days before sacrifice, no loss of immunoreactivity was apparent for any of the peptides in the LSn below the transection. In addition, unilateral transections of the lateral funiculi resulted in no consistent difference between sides in the amount of immunoreactivity to any of the substances in the LSn. These results demonstrate that few, if any, of the peptide-containing fibers afferent to the LSn in lumbar cord originate in the brain or in upper levels of the spinal cord.

Since these experiments indicated that the peptidergic input to the LSn originates locally, we examined cells in the lumbar spinal cord of colchicine-treated rats for immunoreactivity for the five peptides. Immunoreactivity for ENK was found in cells in the marginal zone (MZ), substantia gelatinosa (SG), nucleus proprius (NP), lateral reticulated area (LRA) and near the central canal (CC). Cells were labeled by antibody to SP in MZ, NP, LRA and the dorsal lateral funiculus (DLf). Cells immunoreactive for SOM were found only in NP. Staining for DYN occured in cells in MZ, NP, SG and LRA. Cells had immunoreactivity for FMRF-NH_2 in MZ and NP. Following injections of fast blue into the LSn, retrogradely labeled cells were found ipsilaterally in all areas in which immunocytochemically stained cells had been seen. Contralaterally, cells were retrogradely labeled in SG and NP. While some label may have been picked up through axons of passage or from spread of the injection to the lateral dorsal horn, the cells afferent to the LSn should be among those labeled. These results, in conjunction with the results from transections and rhizotomies, indicate that virtually all peptidergic fibers afferent to the LSn originate locally in cells among those in the MZ, SG, NP, LRA, DLf or near the CC.

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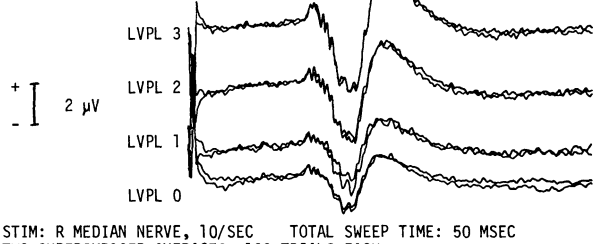
11.10 SOMATOSENSORY EVOKED POTENTIALS RECORDED FROM HUMAN THALAMUS C.D.Yingling, Y.Hosobuchi* and J.E.Adams*, Dept. of Neurological Surgery, University of California, San Francisco, CA 94143

Somatosensory evoked potentials (SEPs) have been recorded from the thalamic somatosensory nucleus (VPL) in over 30 patients permanently implanted with multi-contact electrodes (Medtronics) for relief of chronic deafferentation pain. Electrical stimulation of the median nerve at the wrist elicits a P17-N20-P24 complex with a limited spatial distribution (often seen at only 1 or 2 of the contacts, which are spaced 2 mm apart). In surface recordings of SEPs, P/N14 is thought to be generated by dorsal column nuclei or medial lemniscus, and N20 by the primary somatosensory cortex; it is thus likely that P17 represents the initial activation of the somatosensory thalamus. Recordings a few mm anterior to VPL during thalamotomy for movement disorders show a polarity reversal of P17 to N17. P24 is more variable and not recorded in all patients.

Of particular interest is the presence of fast oscillatory wavelets riding on the P17-N20 waves, with a frequency of 1 kHz or faster. These components are highly reproducible within each subject (see figure below) and may correspond to the scalp recorded high frequency waves reported by Eisen et al (EEGJ 59:388-395,1984) and others using 250 Hz high-pass digital or analog filtering.

The limited subcortical distribution of thalamic SEPs suggests that they are generated by relatively closed dipole fields, and are thus unlikely to contribute substantially to the major SEP components recorded from the scalp. On the other hand, this gives them clinical utility in guiding precise placement of thalamic electrodes in VPL. Finally, it is tempting to speculate that the fast oscillatory activity reflects a recurrent intrathalamic mechanism which may play a role in encoding the thalamocortical representation of sensory events.



STIM: R MEDIAN NERVE, 10/SEC TOTAL SWEEP TIME: 50 MSEC
TWO SUPERIMPOSED AVERAGES, 128 TRIALS EACH.
EACH RECORDING SITE IS 2 MM DEEPER THAN THE ONE ABOVE.

12.1 EXCITATORY EFFECTS OF NORADRENALINE ON BURSTING PACEMAKER NEURONS IN CULTURED SPINAL CORD CELLS. J.D. Vincent and P. Legendre . INSERM U.176 Rue Camille Saint-Saëns 33077 BORDEAUX-Cedex France.

Intracellular recordings were made from dissociated mouse spinal cord cells in primary culture (Legendre, P. et al., Brain Research 297 : 287, 1984). One type of neuron with a large cell body and several short thick neurites was found to fire rhythmic bursts of action potentials with a phase duration of approximately 1 sec when the membrane potential was depolarized to -55 mV. These cells remained generally silent at resting potential (-65 mV). Bursts did not arise from spontaneous synaptic input but appeared to result from endogenous ionic conductance properties of the membrane ressembling those observed in molluscan bursting pacemaker (BP) neurons. Pharmacological application of ionic conductance blockers and of modified ionic media suggested that Ca^{2+} is involved in burst generation and controls the burst duration by activating a K^+ conductance. Na^+ appeared principally involved in pacemaker potential generation and partially involved in spike generation.

Since Noradrenaline (NA) seems to be involved in the activation of spinal cord rhythmic generators, we looked at the effects of NA application on the electrophysiological properties of our cultured spinal cord BP neurons. Application of NA (Sigma 1 µM in the delivery pipette) onto the surface of BP neurons evoked, in a dosedependent manner, an increase in the input resistance (Ri) associated with a progressive depolarization of the membrane potential.

This response was potential dependent. The maximum change in Ri was observed at membrane potential values between -60 to -45 mV. No response was observed at membrane potential less than -80 mV. The extrapolated reversal potential (Rp) of the NA effect was close to -90 mV. The Rp value was not altered by increasing the intracellular Cl^- concentration whereas it was modified by increasing extracellular K^+ concentration. This excitatory effect was not blocked by extracellular application of tetraethylamonium but it was reversibly suppressed by Ba^{2+} and Cd^{2+} application. Ours results demonstrate that NA has an excitatory effect on pacemaker SC neurons in culture by decreasing a Ca^{2+} and potential dependent K^+ conductance. Alpha 1 receptors seem to be involved in this response since Phentolamine, an α 1 antagonist, prevent the effect of application whereas Clonidine, an α 2 agonist, did not mimick the NA response and since the β antagonist, L-Aprenolol, had no effect.

12.2 AN ISOLATED IN VITRO PREPARATION OF THE NEONATAL RAT BRAINSTEM AND SPINAL CORD. K. Walton and R. Llinás. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

We have developed a preparation suitable for the study of the development of supraspinal inputs to spinal motoneurons in rats during the first postnatal week. The preparation consists of the brainstem posterior to the inferior colliculus and the entire spinal cord (SC) with the dorsal roots (DR) and ventral (VR) roots attached; the cerebellum may also be included. Cranial nerves V, VIII, XI and XII are long enough to be stimulated individually via suction electrodes. The dissection was caried out under ether anesthesia. The preparation was pinned in a Sylgard-lined chamber and continually superfused with a modified Ringer's solution bubbled with O_2/CO_2 (95%/5%); oxgenation was supplemented with 0.004% H_2O_2. Recordings were made from the SC at the lumbar level using suction electrodes placed on the VR; glass micropipettes were used to record field potentials and intracellular responses. Stimulation was accomplished using suction electrodes on the DR and selected crainal nerves; a twisted pair of silver wires was used for stimulating the spinal column and brainstem areas. Typical spinal reflexes were recorded from the ipsilateral VR following DR stimulation and bilaterally following ventral column stimulation. The response consisted of a sharp monosynaptic component followed by a low amplitude polysynaptic component. Stimulation of the trigeminal nerve elicited responses in the lumbar ventral roots bilaterally. These comprised long latency, prolonged (about 50 ms) responses. Stimulation of the pyramidal tracts at the posterior margin of the pons, the nuclei gracilis and cuneatus, the posterior and the anterior funiculus at the cervical level also elicited ventral root potentials. Intracellular recordings indicated both excitatory and inhibitory responses to DR and ventral column stimulation. The preparation can be maintained in good condition for 8 hours at room temperature as indicated by VR potentials recorded following brainstem activation. These preliminary results indicate that this preparation should prove to be a valuable tool in studying the development of the descending synaptic inputs to spinal motoneurons and their interaction with DR inputs during the first week of postnatal life. Supported by USPHS grant NS-13742 from NINCDS.

12.3 AN INTRACELLULAR STUDY OF THE EFFECTS OF ACETYLCHOLINE ON MOTONEURONS IN A NEONATAL RAT SPINAL CORD SLICE PREPARATION. N. J. Dun and Z. G. Jiang* (SPON: R. S. Schmidt). Dept. of Pharmacol. Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings stable for many hours were made from motoneurons situated in thin (400-500µm) transverse slices in vitro of rat thoracolumbar spinal cord removed from neonatal rats aged 8-15 days old. Spinal cord slices were superfused with a Krebs solution saturated with 95% O_2 & 5% CO_2; the temperature of the solution was maintained at 34 ± 0.5°C. Motoneurons were identified by the appearance of an all-or-none action potential following stimulation of the ventral rootlets that remained attached to the spinal cord slices. Two types of motoneurons could be categorized on the basis of their electrical membrane properties. The first group of motoneurons, constituting the majority of motoneurons sampled here, had conduction velocities between 20-50 m/s, lower input resistance (5-40 MΩ) and shorter time constant (2-5 ms). The conduction velocity of the second group of motoneurons was below 10 m/s. These motoneurons exhibited higher input resistance (50-150 MΩ) and slower time constant (10-15 ms). These two groups of motoneurons observed here may correspond to the alpha and gamma motoneurons that have been extensively studied in the adult spinal cord. Acetylcholine (ACh) applied by either pressure ejection or by bath superfusion in known concentrations (10-100 µM) depolarized nearly all the motoneurons tested. In the majority of motoneurons the depolarization induced by ACh was slow in onset and lasted seconds to minutes. The slow ACh depolarization was accompanied by an increase in input resistance and the response was made smaller upon membrane hyperpolarization. Atropine (≤ 1 µM) completely and readily abolished the slow ACh depolarization. Tetrodotoxin (TTX, 0.1-1 µM) or low Ca (0.12 mM)/high Mg (12 mM) solution did not appreciably affect the slow depolarization induced by ACh. These findings collectively suggest that the slow depolarization evoked by ACh was probably due to a direct action of ACh on motoneuron muscarinic receptors. ACh evoked a biphasic depolarization in a small number of motoneurons tested; an initial brisk depolarization was followed by a slow depolarization. The fast and slow component was reversibly depressed by d-tubocurarine and atropine, indicating that the respective response may be due to an activation of nicotinic and muscarinic receptors. The effect of eserine (≤ 1 µM) on muscarinic depolarizations induced by ACh on motoneurons was biphasic; an initial enhancement was followed by a long lasting depression. The high incidence of ACh sensitive motoneurons observed here in conjunction of the report of the presence of nerve fibers exhibiting immunoreactivity to choline acetyltransferase near the motoneurons by others may suggest a transmitter/modulator role of ACh in the ventral horn. (Supported by NIH Grant NS18710).

12.4 MOTOR PATTERNS FOR RESPIRATION AND LOCOMOTION GENERATED BY AN IN VITRO BRAINSTEM-SPINAL CORD FROM NEONATAL RAT. J. C. Smith* and J. L. Feldman. Department of Physiology, Northwestern University, Chicago,IL. 60611

In vitro brainstem-spinal cord preparations of lower vertebrates are commonly used to investigate properties of central pattern generators (CPG's) for respiration and locomotion, but a suitable in vitro preparation for routine study of these CPG's in a mammalian system has not yet been developed. We have found spontaneous rhythmic respiratory motor activity, and locomotor patterns elicited by excitatory amino acids, in an in vitro preparation of the brainstem-spinal cord of the neonatal rat. The finding of spontaneous respiratory motor activity confirms the observations by Suzue (J. Physiol.,354:173,1984). The preparation consists of the brainstem and spinal cord up to the lumbar level from 0-4 day old rats placed in modified oxygenated Krebs solution (pH 7.4) at 27-30° C. Preparations including portions of the body wall (rib cage with intercostal musculature) with dorsal and ventral thoracic roots intact, preserving mechanosensory afferent input, have also been used. The respiratory pattern consists of a synchronous periodic discharge on the hypoglossal nerve, 4th and 5th cervical and thoracic ventral roots. The brainstem origin of the pattern was verified by transection experiments. Following spinomedullary transection, the periodic discharge persists on the hypoglossal nerve but is absent on ventral roots of the spinal cord. The spontaneous discharge frequency is increased by bath applied N-methyl D-aspartic acid (NMDA;1-10 uM), acetylcholine (ACH;0.01-1 mM) and reduced pH; preliminary observations indicate that the discharge frequency is reduced in a dose-dependent manner by GABA (0.01-1 mM) and atropine (1-10 uM). The respiratory burst pattern is altered by mechanical perturbation of the rib cage musculature indicating the presence of functional afferent circuitry. The locomotor patterns recorded on ventral roots are elicited by ACH (0.1-1 mM), NMDA (1-10uM) and D-glutamate (0.1-1 mM) in a manner similar to the induction of locomotor patterns in lower vertebrates (Grillner & Wallén, Ann. Rev. Neurosci.,8:233,1985; McClellan & Farel, Brain Res.,332:119,1985). These motor patterns range from alternation to synchrony in bursting between contralateral ventral roots in a given segment with burst frequencies varying with the excitatory neurotransmitter concentration. The results indicate that this preparation should be suitable for study of mammalian respiratory and locomotor CPG function. It may also provide data for a comparative analysis of CPG mechanisms among lower and higher vertebrates. The induction of locomotor patterns by similar amino acids in this and lower vertebrate preparations may suggest a phylogenetic conservation of mechanisms controlling the locomotor circuitry. Supported by NIH Grant HL-23820.

12.5 CONTROL OF ABDOMINAL MUSCLE ACTIVITY DURING VOMITING: ROLE OF VENTRAL RESPIRATORY GROUP EXPIRATORY NEURONS. A.D. Miller, I. Suzuki* and L.K. Tan. Lab. of Neurophysiology, The Rockefeller University, New York, NY 10021.

Vomiting is produced primarily by the synchronous contraction of the diaphragm and abdominal muscles (2,5). The brainstem mechanism controlling these muscles during vomiting is unknown. In an attempt to identify these neurons, we examined the firing patterns of ventral respiratory group (VRG) expiratory (E) neurons during fictive vomiting in decerebrate, paralyzed cats. VRG E neurons project to the region of abdominal motoneurons and are probably the main source of expiratory modulation of abdominal muscle activity (3,4). However, since VRG E neurons are inhibited during inspiration (6), it is uncertain what their firing patterns will be during co-contraction of the diaphragm and abdominal muscles.

Fictive vomiting was defined by co-activation of phrenic and upper lumbar abdominal nerves in response to sub-diaphragmatic vagal stimulation (1) or emetic drugs. We find that vagal stimulation (pulse trains) readily produces vomiting in non-paralyzed, decerebrate cats but does not elicit other expulsive acts in which the abdominal muscles and diaphragm are knownto be co-active, such as coughing, eructation, or defecation. Latencies from onset of vagal stimulation to onset of nerve co-activation average about 30 sec. Each episode of fictive vomiting consists of an average of 8 bursts of nerve co-activation. Abdominal nerve activity increases an average of about 10 times during fictive vomiting, as compared to respiration, while phrenic discharge increases about 3 fold.

Data have been obtained from 21 VRC E neurons,located 1.6-5.2mm caudal to the obex, that were antidromically identified as projecting to the lumbar cord. Eighteen cells were studied during 1-3 episodes of vagal-induced fictive vomiting while 4 cells were studied during drug-induced nerve co-activation. The activity of two-thirds of these neurons (14/21) was greatly reduced or abolished during nerve co-activation. These neurons typically fired between bursts of co-activation. The maximum firing rates of the 7 remaining neurons were either unchanged (N=3) or increased 2-4 times (N=4).

Thus, it would appear that abdominal muscle activity during vomiting is mainly controlled not by VRG E neurons but by some other as yet unknown descending input.

This study was supported by grants from NSF (BNS8317651), NASA (NAG2164, NSG2380), and NIH (NSO2619, RR07065).

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12.6 LONG LASTING HYPERPOLARIZATION OF SOLEUS ALPHA MOTONEURON ELICITED BY PONTINE STIMULATION IN DECEREBRATE STANDING CAT. T. Sakamoto*, Y. Ohta*, S. Mori (SPON: H. Asanuma),Dept. of Physiol.,Asahikawa Med. Col., Asahikawa, JAPAN 078-11.

Stimulation of the dorsal part of the caudal tegmental field (DTF) along the midline of the pons (P5 to P6, H -4.5 to H -5.5) suppresses the postural muscle tone of hindlimb in the decerebrate cat. Such a suppression continues for more than several minutes after termination of stimulation.

To understand the neuronal mechanisms underlying such a long lasting suppression, we attempted intracellular recording of soleus alpha motoneurons with glass micropipettes filled with 2M potassium citrate. While the animal was maintaining the standing posture, soleus motoneurons were identified by antidromically stimulating the nerve endings within soleus muscle. DTF stimulation (10-50 uA, 0.2msec, 50 cps) which lasted 5-10 sec always produced hyperpolarization in soleus motoneurons with amplitude ranging between 1 to 16 mV and these hyperpolarizations continued several minutes after termination of DTF stimulation.

To examine whether there were differences in the mechanisms for producing hyperpolarizations which were observed during and after DTF stimulation, following measurements were made. 1) Characteristics of Ia EPSPs which were evoked at stimulus strengths subthreshold for antidromic invasion of identified motoneurons before, during and 10 sec after DTF stimulation. 2) Delay of antidromic spike potentials which were evoked at a frequency of 1-2 Hz. 3) Differences in input resistance. These were determined by delivering depolarizing and hyperpolarizing current pulses through the impaling microelectrode and dividing the elicited voltage changes by the amount of injected current. 4) Reversal of hyperpolarization by injecting Cl^-ion through 3M potassium chloride microelectrode.

The results obtained were: During DTF stimulation, 1) there were reductions in amplitude and in the time of decay of the averaged EPSPs, 2) there was a suppression of antidromic impulse propagation,i.e., increased IS-SD delay or IS-SD block, 3) there was a reduction of the input resistance and 4) there was a reversal of the membrane hyperpolarization. None of such modifications were evident during the hyperpolarization which outlasted beyond the termination of DTF stimulation.

These results indicated that there were differences between different parts of long lasting hyperpolarizations, i.e., during and after DTF stimulation. It was concluded that postsynaptic inhibition played a major role during the DTF stimulation but disfacilitation or other mechanisms played a major role after termination of DTF stimulation.

12.7 A RETICULOSPINAL TRACT THAT PREVENTS SYNCHRONY OF DISCHARGE BETWEEN GAMMA MOTONEURONES IN THE CAT. P.H.Ellaway and N.J.Davey* (SPON: D.G. Stuart). Department of Physiology, University College London, London WC1E 6BT, England.

Neurones that share presynaptic stem fibres may show a tendency to synchrony in their discharges as a result of near simultaneous synaptic potentials. Background and reflex discharges of gamma motoneurones in the spinal cat show such synchrony of discharge (Ellaway & Murthy. Quart.J.Exp.Physiol., 70: 1985). In motor control the consequence of synchronized discharges of gamma efferents to muscle spindles would be a state of tremor. We show here that this undesirable tendency is prevented by activity in fibres of the descending dorsolateral funiculus.

The discharges of individual gamma motoneurones in the decerebrated cat are regular and show no tendency to synchrony. After complete spinal section, discharges of gamma motoneurones below the cut became irregular and a tendency to synchronized firing appeared. Synchrony was evident as a peak (half-width 3-5 ms) in the cross correlogram between the discharges of two efferents. Discrete lesions of the spinal cord were made, in the region T8-10, by section with a fine blade. The site and extent of the lesions were verified by histological preparation. The degree of synchrony between pairs of gastrocnemius gamma motoneurones, and the regularity of firing of the individual efferents, were assessed before and after each lesion. We found that discrete lesions of the dorsolateral funiculus needed to be made on both sides of the spinal cord to produce synchronous and irregular firing at the level observed after total spinal cord section. Large lesions of the cord that spared either the ipsilateral or contralateral, dorsolateral funiculus did not produce any marked change in regularity of firing or tendency to synchrony.

Reticulospinal neurones descend in the dorsolateral funiculus. They are known to control the flexion reflex by release of catecholamines (Anden, Jukes & Lundberg. Acta Physiol.Scand. 67: 387, 1966). In the spinal cat intravenous injection of 75mg/kg of L-Dopa, a precursor of catecholamines, caused a pronounced reduction in the degree of synchronized firing and an increase in regularity of gamma motoneurone discharge. This effect was antagonized by intravenous injection of chlorpromazine (1 mg/kg).

We conclude that the prevention of synchronized gamma efferent discharge is exercised through a bilateral reticulospinal tract that involves catecholaminergic transmission in the spinal cord.

Supported by NIH grant NS19215.

12.8 VESTIBULOSPINAL AND RETICULOSPINAL INTERACTIONS IN ACTIVATING AXIAL MUSCLE EMG IN THE RAT. S.L. Cottingham*, P.A. Femano, and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Lesion and electrical stimulation studies show that vestibulospinal and reticulospinal systems are necessary for lordosis, an hormonally-dependent female rat sex behavior (Modianos and Pfaff, 1976). Electrical stimulation of the medullary reticular formation (MRF) activates the axial muscles medial longissimus (ML) and lateral longissimus (LL) essential for the vertebral dorsiflexion of the lordosis reflex (Femano et al, 1984). The present study examines activation of ML and LL muscles by electrical stimulation of the lateral vestibular nucleus (LVN), and interactions between vestibulospinal and reticulospinal stimulation of these muscles.

Female rats were anesthetized with urethane, and two multistranded stainless steel EMG recording electrodes were placed in ML and LL at spinal level L4-L5. EMG units were amplified and displayed on an oscilloscope, and PST histograms were constructed. Electrical stimulation to MRF and LVN consisted of neg-pos biphasic pulses (0.2 msec/phase) at 200 Hz delivered in trains (30-100 pulses/train, one train/sec). Effective current range: 20-100 µA; 40-50 µA was typical. Data reported were from nucleus gigantocellularis in RF, and medial, central, and lateral placements in LVN. Electrical stimulation of LVN activated ML (threshold range 20-50 µA) and LL (thr 20-50 µA). Similarly, electrical stimulation of MRF activated ML (thr 20-40 µA) and LL (thr 20-50 µA). With repetitive delivery of trains to LVN or MRF, each successive train was more effective; that is, the tenth train elicited more units, at shorter latency than the first. LVN-evoked activity in the axial muscles can be facilitated by electrical stimulation of MRF. Facilitation was observed as a lowering of threshold to elicit muscle activity; for LVN stimulation already above threshold, an increase in number of units firing for a given LVN current was seen. MRF sites that produced facilitation of an LVN response were sites that evoked firing in axial muscles. LVN stimulation also facilitated MRF-evoked axial muscle activity. Trains using currents subthreshold for both MRF and LVN (the lower being 10-50% of threshold), delivered simultaneously to MRF and LVN, could lead to activity in axial muscles. Inhibitory MRF sites did not activate ML or LL, but ventroflexion of the animal was sometimes observed. Such inhibitory interactions were accompanied by rebound facilitation.

Ventral and medial motor control systems long have been suspected to be important for axial motor control (Lawrence and Kuypers, 1968). These EMG data in the rat show some ways in which lateral vestibulospinal and medullary reticulospinal neurons could cooperate in activating deep back muscles important for reproductive and other behaviors. (Supported by NIMH grant MH38273).

12.9 LOCUS COERULEUS FACILITATION OF LUMBAR MONOSYNAPTIC REFLEXES IN THE RAT. Julie Y.H. Chan, Simon J. Fung, Samuel H.H. Chan and Charles D. Barnes. Washington State University, College of Veterinary Med., Pullman, WA 99164-6520.

Stimulation of the locus coeruleus (LC) in the cat has been demonstrated in our laboratory to unequivocally produce facilitation of both extensor and flexor monosynaptic reflexes (MSRs) of the hindlimb. Since the majority of work on coerulospinal projections was performed on rodents, and there are known structural differences in the LC of cats and rats, the possibility arises for a specific difference in LC influences on spinal motoneurons. The present study was initiated to examine this possibility, by extending our previous investigations on cats to the rat.

MSRs were evoked in chloral hydrate-anesthetized male Sprague-Dawley rats by stimulating either the L5 dorsal root or the posterior tibial (TIB)/common peroneal (CP) nerves, respective representative of extensor/flexor muscle nerves. They were quantified by the amplitude of the compound action potential recorded from the L5 ventral root. Electrical activation of the LC, using a train of four 100- or 500-μs rectangular pulses at a train pulse frequency of 770 Hz, consistently promoted facilitation of these MSRs regardless of their origins. Such LC-evoked potentiations may vary in degree (37.5-147.4%), duration (70.6-72.9 ms) and latency (3.0-5.5 ms) among different animals. Further experiments, using the tracking technique, ascertained that our observations were specifically the result of activating LC neurons locally and not due to current spread to neighboring tissues.

While affecting minimally the control MSRs, intravenous administration of prazosin (20 μg/kg) significantly antagonized the enhancing effects of LC on MSRs. It is also interesting to note that the ventral root discharges elicited by LC stimulation were also depressed by this alpha$_1$-adrenoceptor blocker. These data confirmed the involvement of noradrenergic neurotransmission in the LC-promoted facilitation of MSRs.

Since the results obtained in the present study are in general agreement with previous observations from this laboratory on the cat, we conclude that the LC exerts similar actions on the spinal motoneurons in at least two animal species, cat and rat. We postulate that this pontine nucleus may directly or indirectly excite the motoneuron pool, allowing more neurons to be included in the discharge zone upon the arrival of afferent volleys, in a process that involves noradrenergic neurotransmission.

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12.10 STUDIES ON INPUT-OUTPUT ORGANIZATION OF MEDIAL PONTINE RETICULAR FORMATION. R.W. McCarley and K. Ito. Neuroscience Lab., Harvard Medical School and VAMC, Brockton, MA 02401.

Considerable data indicate the importance of mPRF for a number of behaviors, including saccadic eye movements, somatic motor activity and REM sleep, but there have been few physiological studies of mPRF organization and none explicitly addressing the question of whether some mPRF neurons are specialized to receive input ("input neurons") and others are specialized as "output neurons". We have recently examined this question with intracellular mPRF recordings and microstimulation (<85 uA) of reticular areas, using undrugged cats so as not to introduce anaesthetic confounds. Monosynaptic responses were defined as PSPs of <1.2 ms latency and antidromic (AD) responses by the usual intracellular criteria.

In 40 mPRF neurons antidromically activated from ipsilateral bulbar magnocellular tegmental field (FTM) we varied stimulation current to below AD threshold; in 38/40 neurons (95%) monosynaptic PSPs were seen, with 87% EPSPs and 13% IPSPs; these were the same proportions as seen in a larger sample (N=470) of mPRF neurons showing no AD activation (chi square test, p=0.5). Furthermore, the histograms of monosynaptic latencies for the AD and non-AD groups were not different (Kolmogoroff-Smirnoff test, $p > .4$) and the mean latency was identical (0.73 ms). Thus there was no evidence for any distinction on any of the input parameters between the population of "output neurons" (AD) and the total sample population, and thus no evidence for the presence of a specialized "input neuron". We simularly found no differences in the proportion of monosynaptic PSPs in mPRF neurons that were and were not AD activated by microstimulation of ipsilateral bulbar lateral and gigantocellular fields and contralateral gigantocellular PRF, although the small Ns (10) made detailed statistical comparisons impossible.

The apparent identity of input and output elements in mPRF suggests that a great deal of integration of input for response is done in individual mPRF neurons rather than having a "cascade" of information flow from one neuronal type to another as in cerebral cortex. The large size of mPRF soma and of dendritic fields and the absence of anatomical evidence for Golgi II neurons among mPRF neurons is also compatible with this concept. If there are no "interneurons" in mPRF, this raises the interesting question of the origin of relatively short latency IPSPs which follow EPSPs. On the ventral and lateral borders of mPRF we have recorded neurons with a burst discharge that occurs just after the time of EPSPs in mPRF neurons. While further data are necessary, these burst neurons are possible candidates for neurons inhibitory to mPRF.

12.11 **RETROGRADE LABELING OF SPINAL NEURONS FOLLOWING INJECTION OF HRP INTO THE FLEXOR DIGITORUM LONGUS MOTOR NUCLEUS IN THE CAT.** **D. Meyers*, J.W. Fleshman and B.J. Schmidt* (SPON: J.S. McIntosh). Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.**

It has been known for some time that EPSPs can be evoked in motoneurons following electrical stimulation of cutaneous nerves. Previous results from this laboratory (Fleshman et al., Exp. Brain Res. 54: 133, 1984 and Fleshman et al., this meeting) have shown that, following electrical stimulation of the superficial peroneal nerve, short latency EPSPs are generated in flexor digitorum longus (FDL) motoneurons. The segmental onset latency of these EPSPs is so short (1.3-2.0 ms) as to suggest the existence of a disynaptic pathway from the skin to the FDL motoneurons. If this pathway exists, the intercalated neuron should be located in the deeper laminae of the medial dorsal horn (the termination region of cutaneous afferents innervating the distal hindlimb). At present there is no evidence that this region of the grey matter contains neurons that are both presynaptic to motoneurons and monosynaptically driven by cutaneous afferents.

In the present work we are attempting to determine whether there are last-order premotor interneurons in the dorsal horn by making small pressure injections of lectin-conjugated HRP through a micropipette (tip size 5-10 μm) at a number of sites in the FDL motor nucleus. The pipette tip was positioned by recording the antidromic field potential elicited from the FDL nerve. Animals were perfused after a survival period of 24 hr. The cord was then sectioned and reacted with tetramethyl benzidene.

Of three experiments, one with effective injection sites about 1 mm in diameter is presented below. Retrogradely labeled neurons were found on both sides of the cord in the L6-L7 segments. Cells labeled contralateral to the injection site were restricted to lamina VIII. These cells may be the last order neurons in the crossed extension reflex pathway. As expected, most cells labeled ipsilateral to the injection site were located in laminae VII and IX with a few cells in lateral lamina VIII. There was, however, another group of labeled cells located in ventral lamina V and lamina VI. These data are consistent with the hypothesis of a disynaptic cutaneous pathway to FDL motoneurons. Further experiments are in progress using smaller injection volumes and techniques to minimize problems associated with uptake from damaged axons.

12.12 CYTOCHEMICAL LOCALIZATION OF CYTOCHROME OXIDASE IN THE SPINAL CORD AND DORSAL ROOT GANGLIA, WITH QUANTITATIVE ANALYSIS OF VENTRAL HORN CELLS IN THE MONKEY. M. Wong-Riley and D.A. Hoppe*. Dept. of Anatomy, Med. Coll. of Wisconsin, Milwaukee, WI 53226.

At the 1984 Neuroscience meeting, we (Wong-Riley and Kageyama) reported the histochemical pattern for cytochrome oxidase (C.O.) in the spinal cord and dorsal root ganglia of several mammalian species. The present report represents an extension of that study to the ultrastructural level in the macaque. The spinal cord exhibited a heterogeneous but consistent pattern of C.O. staining, which could be correlated positively with known levels of activity in various nuclear groups. We have selected the substantia gelatinosa, dorsal nucleus of Clarke, ventral horn and dorsal root ganglia for further EM analysis. In the lightly reactive substantia gelatinosa layer, there were relatively few mitochondria in both neurons and neuropil. Most of the neurons were small, spindle-shaped, and contained very few and lightly-reactive mitochondria. In the dorsal nucleus of Clarke, medium to large neurons contained numerous darkly reactive mitochondria intermixed with a rich array of other organelles. Some of the smaller neurons were much less reactive. In the ventral horn, many of the large motoneurons had lightly-reactive mitochondria, while small and medium-sized neurons often contained moderate to darkly-reactive mitochondria. Axon terminals in the ventral horn had varying degrees of C.O. reactivity. Medium-sized "F" terminals with flattened synaptic vesicles typically had darkly-reactive mitochondria, while small "S" terminals with spherical vesicles often had light to moderately-reactive ones. The largest "C" type terminals were richly supplied with lightly-reactive mitochondria. "M" terminals with Taxi bodies were rarely encountered. Quantitative analysis of 1770 ventral horn neurons from 3 macaque monkeys indicated that the distribution of cell sizes as well as optical densities (for C.O. activity) at every level of the cord fell on a continuum. There was a negative regression coefficient such that a greater proportion of large neurons tended to be less reactive than the small ones. However, neurons of all size categories had representations in the dark, moderate, and light ranges of C.O. reactivity. Dorsal root ganglion neurons likewise exhibited a heterogeneous distribution of C.O. reactivity in every size category. The darkly reactive ones had a large number of reactive mitochondria, while others with equivalent volume of cytoplasm had fewer mitochondria, most of which were only lightly reactive. Satellite cells were not reactive for C.O.

Thus, our analyses indicate that the oxidative enzymatic activity of neurons in the spinal cord and dorsal root ganglia cannot be correlated strictly with size, but it is likely to reflect their total functional demand which, in turn, is strongly governed by their cumulative levels of synaptic and spontaneous activities. (Supported by NIH NS18122.)

12.13 ANATOMICAL AND PHYSIOLOGICAL ASPECTS OF THE ELECTROGENIC SYSTEM IN THE SPINAL CORD OF GYMNOTUS CARAPO. E. Decima, J. A. Echague*, D. Lorenzo* and O. Trujillo-Cenoz*. Dept. of Anatomy, UCLA School of Medicine, Los Angeles, CA 90024, U.S.A., and Neurobiology, Inst. C. Estable, Montevideo, Uruguay.

The electrogenic system of the so-called "weakly electric fish" consists of a muscle-derived electrogenic organ (EO) and a complex neural command determining the characteristics of the EO discharge (EOD). The main components of the command network are located in the fish medulla and spinal cord. This report concerns the spinal cord portion of the system, particularly the electrogenic neurons. These represent the final output of the EO command system and they were unambiguously identified by means of the somatopetal transport of HRP previously injected in the EO. The HRP-labelled cells are located medially, close to and dorsal to the ependyma. They form a long slender column along the spinal cord. When silver-impregnated, each neuron appears as a monopolar pear-shaped cell (about 25 μm in diameter), whose axon runs perpendicular to the main axis of the fish. Under the EM the electromotor neurons show synaptic knobs apposed to the somatic plasma membrane. Most of these synaptic terminals exhibit the characteristics of both chemical and electrical synapses.

Electrophysiological recordings of extracellular activity (field potentials and unitary responses) were obtained from the spinal cord with glass micropipettes while the EOD was simultaneously monitored. In the first type of experiment a high spinal cord section was made and stimulating electrodes were implanted in the caudal stump. Their aim was to activate the descending tract. In a second design, the animal was immobilized with gallamine and artificially oxygenated. Under this condition the normal activation of spinal electrogenic cells was maintained. In both experiments, field potentials and unitary responses were recorded preceding the EOD by 0.6 to 0.8 ms.

It remains to be elucidated whether these electrical events correspond to the activity of the electrogenic neurons themselves or to their driving pre-synaptic elements (i.e., those axons from the medullary command centers).

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PRESYNAPTIC MECHANISMS I

13.1 MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC AUTORECEPTOR PROPERTIES OF (-)-APOMORPHINE: SEROTONERGIC INTERACTIONS. P.A. Broderick, Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York, 10461 and Department of Biology and Medical Technology, Bronx Community College, The City University of New York, Bronx, New York 10453.

(-)-Apomorphine's presently suggested role as prototypic dopamine autoreceptor agonist is intriguing. Small doses of (-)-apomorphine selectively stimulate presynaptic autoreceptors on dopaminergic nigrostriatal neurons and may serve as the mechanism for the suppression of firing rate and possibly release in dopamine neurons. This is in keeping with behavioral effects of (-)-apomorphine which show that administration of small doses of (-)-apomorphine decrease locomotor activity, similar to the effects of dopamine receptor blockers; nonetheless, larger doses of (-)-apomorphine produce an increase in locomotor activity (Strombom, U., Acta Physiol. Scand. suppl., 431:1-103, 1975). In vivo electrochemical procedures are particularly suited to further investigate autoreceptor hypotheses because they measure the ongoing dynamics of neurotransmitter release in the synaptic cleft. A previous paper using in vivo electrochemistry (Broderick, P.A., Annals of N.Y. Acad. Sci., 1985, in press) demonstrates evidence for a significant suppression ($p<0.05$) of dopamine release after intraperitoneal administration of small doses of (-)-apomorphine to male, Sprague-Dawley rats, stereotaxically implanted with stearate indicator electrodes in anterior striatum (for methodological details, cf. Broderick, P.A., Life Sci., 36(24):2269-2275, 1985). The data further show a reversal of this (-)-apomorphine effect at larger doses, possibly stimulating postsynaptic dopaminergic receptors and perhaps explaining unexpected, behavioral responses (Strombom, U., Naunyn-Schmiedeberg's Arch. Pharmacol., 292:167-176, 1976). The present paper further shows that the sensitivity of mesolimbic dopaminergic autoreceptors, as shown by dopamine release from tuberculum olfactorium, after systemic (-)-apomorphine administration, may be greater than that of striatum, in agreement with electrophysiological studies (Roth, R.H., Comm. Psychopharmacol., 3:429-445, 1979). Preliminary data showed that serotonin release from rat striatum after (-)-apomorphine appeared inconsistent; serotonin release from rat tuberculum olfactorium, after (-)-apomorphine, decreased 30-40% below basal levels. These data support previous data (Lee, E.H. and Geyer, M.A., Eur. J. Pharmacol., 94(3-4):297-303, 1983) which showed differential effects of dopaminergic autoreceptor agonist activity in striatal and mesolimbic serotonergic systems in rat brain.

13.2 ASSOCIATIVE PRESYNAPTIC INTERACTIONS IN THE CA_1 AREA OF RAT HIPPOCAMPUS. B. R. Sastry and J. W. Goh*. Neuroscience Research Laboratory, Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

A tetanic stimulation of stratum radiatum in the hippocampus results in a post-tetanic long-lasting potentiation (LLP) of the CA_1 neuronal population spike evoked by the same input. Some investigators reported that a coactivation of several input fibres is needed to induce LLP and suggested that the necessity for a coactivation of several input fibres and the associative nature of the induction of LLP could be explained if LLP is postsynaptic. In the present studies on rat hippocampal slices, we examined whether Schaffer collateral terminals interact with each other.

Individual CA_3 neurones were antidromically activated with an electrode in the CA_{1b} area to determine the threshold for the generation of an action potential in the terminal region of the fibre. The threshold was usually decreased (by about 15% at 50 ms delay) for about 300 ms following the activation (with a separate electrode in the CA_1 area) of other nearby fibres in stratum radiatum (5 conditioning pulses at 100 Hz, n = 14). The decrease in the threshold was less intense if the conditioning was given in Ca^{++}-free (substituted with Mn^{++}) medium (n = 8). Exposure of slices for 1 min to a medium containing 4.5 mM K^+ (control medium had 3.1 mM K^+) also decreased the threshold (by 11-36%, 5/6). When the number of conditioning pulses was increased to 10 (at 100 Hz), the threshold was increased (by 88-294% at 50 ms delay, 3/7; by 83-278% at 300 ms delay, 5/7). Exposure for 1 min of slices to a medium containing 6 mM K^+ resulted in an increase in the threshold (by 22-215%, 5/7) while an exposure to a 12 mM K^+ containing medium caused even a larger increase in the threshold (by 55-300%, 7/7). Subsequent to the exposure of the slices to 12 mM K^+, the threshold was maintained at higher than control for a long period of time (increase in threshold 20 min post 12 mM K^+: 25-76%, 5/7) as was seen during LLP of the population spike.

These results indicate that presynaptic terminals in the hippocampal CA_1 area interact with each other to alter their excitability. The interaction may be a consequence of a build-up of extracellular K^+ or secondary to the release of transmitters. In view of these results, future studies should examine if such interactions play a role in associative induction of LLP.

(Supported by the Medical Research Council of Canada.)

13.3 PROPERTIES OF EXCITATORY POSTSYNAPTIC POTENTIALS MEDIATED BY LARGE MYELINATED CLUB ENDINGS ON THE GOLDFISH MAUTHNER CELL.
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The major excitatory inputs to the goldfish Mauthner (M-)cell are the large myelinated club endings (LMCE) which arise from the saccular nerve and terminate on the lateral dendrite. Ultrastructural studies demonstrated that all the endings established morphologically mixed synapses on the M-cell (Nakajima,Y., J.Comp.Neurol. 156:375,1974). In contrast, our electrophysiological investigations, utilizing simultaneous pre- and postsynaptic intracellular recordings, show that impulses in most of the saccular fibers sampled (>80%) produce only electrotonic coupling potentials in the M-cell, i.e. they are chemically 'silent'. In the remaining cases, chemical excitatory postsynaptic potentials (EPSPs) are recorded as well. Presynaptic HRP injections demonstrate that the individual afferents send one process to the M-cell which terminate as LMCEs. Thus EPSP fluctuations could be studied under conditions where branch point failure does not occur and presynaptic spike variations can be monitored directly.

The average unitary EPSP amplitude was 137±77μV (n=15) and was significantly less than the electrotonic coupling potential (826±410μV) produced by impulses in the same fibers. When individual saccular fibers were activated repetitively at intervals of 1.5 to 3 msec., the EPSPs showed pronounced facilitation; the second and third EPSPs averaged 91% (n=6) and 102% (n=5) larger than the first, respectively. The coefficient of variation of the second EPSP was always smaller than that of the first, which is expected if transmitter release is quantal and probabilistic. Furthermore, fluctuations in the amplitudes of the first and second EPSPs were uncorrelated. This independence strongly suggests a presynaptic origin for the facilitation and is consistent with the notion that it occurred at any active site regardless of whether or not it had undergone exocytosis.

Repetitive stimulation of the 'silent' junctions failed to evoke any detectable EPSPs following the second or third spikes. Similarly, while 4-aminopyridine increased EPSP amplitude and duration, it had no detectable effect on the silent junctions. The contrast between facilitation of functioning chemical synapses and the inability to evoke EPSPs at silent synapses suggests that in the latter case either: (1) the postsynaptic receptors, if present, did not respond to the released transmitter, or (2) there is an overriding mechanism in presynaptic terminals which block release at all active sites and is insensitive to the facilitatory mechanisms described here. Supported by NIH Grant #NS-15335.

13.4 QUANTAL ANALYSES DURING PRESYNAPTIC INHIBITION AND CORRELATED MORPHOLOGY OF CRAB MOTOR AXON SYNAPSES. F.W. Tse* and H.L. Atwood, Dept. of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8

The crab limb stretcher muscle is innervated by one excitor (E) axon. In Pachygrapsus crassipes, this single axon provides individual muscle fibers with synapses that show diverse synaptic performance. At low frequencies of stimulation, low-output and high-output synapses are apparent. Low-output synapses (1 mV EPSPs at 1 Hz stimulation) show strong facilitation at higher frequencies of stimulataion and high-output synapses (20 mV EPSPs at 1 Hz stimulation) show depression or poor facilitation. The E axon receives diverse strength of presynaptic inhibition (PI) from a specific inhibitor (SI) axon that is known to form axo-axonal synapses with the E axon. The distribution of synaptic terminals and the branching patterns of both axons can be observed with fluoresence microscopy using rhodamine 123 to stain the nerve endings. Such staining has no significant effect on synaptic transmission or on the muscle fibers' resting membrane potential. Focal extracellular ("loose patch") recordings of synaptic currents from morphologically identified neuromuscular synaptic sites show that PI is stronger at sites with high-output synapses. Quantal output of the E axon at identified sites was analysed statistically with a computer programme. Binomial statistical parameters (n and p) were calculated before and during PI. Without PI, the output of the E axon can be described by binomial statistics which assume that a focal electrode records from a fixed number (n) of equivalent synapses, each with a uniform and stationary probability (p) of releasing one quantum of transmitter. With PI, the reduction in probability of output at a group of synapses is seldom uniform and sometimes non-stationary. Usually, the probability of quantal release at some synapses in a site is reduced to almost zero, while that of adjacent synapses in the same site is still appreciable. In some cases, PI is strong and stationary; the output of the E axon can be described by binomial statistics with a reduced number (n) of detectable synapses. Fluorescence microscopy shows that high-output synapses with strong PI occur on compact, end-plate like clusters of boutons, while low-output synapses with weaker PI occur on more diffuse axonal branches with sequential boutons. In electron microscopy, active synapses can be distinguished from inactive ones by an uptake of extracellular markers (e.g. HRP) after stimulation. This method is being applied to verify whether there are inhibited synapses adjacent to active ones during presynaptic inhibition.
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13.5 DIFFERENTIAL MODULATION OF ENDPLATE POTENTIALS IN SLOW AND FAST MAMMALIAN MUSCLES.
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The modulation of endplate potentials during double pulse stimulation and repetitive stimulus trains was studied in the fast twitch extensor digitorum longus (EDL) versus the slow-twitch soleus (Sol) muscles of the rat. Nerve-muscle preparations were isolated from 8 rats and the muscular contraction was abolished by decreasing the calcium level of the perfusate. The muscle nerve was stimulated by a suction electrode and the synaptic potentials were recorded using surface-extracellular and conventional intracellular recording methods. Twin-pulse facilitation (F) was measured from 32-sweep computer averages. Tetanic potentiation (TP) and posttetanic potentiation (PTP) were evaluated from computer averages of endplate potentials at the end and immediately following stimulus trains of 200 pulses. The data revealed that F, TP and PTP were substantially higher in EDL versus Sol synapses (see the table below).

Interpulse interval		F 100(V2/V1-1)	P(max) (% p o t e n t i a t i o n)	PTP
25ms	EDL	*30.3±2.0 (5)	*251.1±17.7 (11)	*44.0±5.7 (11)
	Sol	25.6±2.0 (5)	169.4±12.7 (14)	22.6±10.7(14)
100ms	EDL	13.6±2.0 (4)		
	Sol	11.7±3.0 (4)		
200ms	EDL	*10.5±1.0 (5)	*41.3±6.5 (9)	*12.5±2.9 (9)
	Sol	3.1±1.0 (5)	16.4±2.2 (10)	7.2±3.7 (10)

These data are based on surface recordings from populations of synapses. The number of recording sessions (each from a different recording site) are in parentheses. Sig- nificant differences between the respective means of EDL and Sol (two tail t-test P<0.001) are denoted by asterics (*).

The results thus demonstrate that the synaptic potentials generated in neuromuscular synapses of slow twitch mammalian muscles are modulated differently from those generated in fast twitch muscles. Further studies regarding the basis for these differences are now in progress.

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13.6 SEROTONIN PRODUCES A DECREASED CONDUCTANCE EPSP AND BROADENING OF THE ACTION POTENTIAL IN GROWTH CONES OF APLYSIA SENSORY NEURONS. F. Belardetti*, S. Schacher, E. R. Kandel, and S. A. Siegelbaum. Howard Hughes Medical Institute and Center for Neurobiology & Behavior, Dept. of Pharmacology, Columbia Univ., College of P & S, and N. Y. State Psychiat. Instit., New York, NY 10032.

Serotonin (5-HT) facilitates transmitter release from sensory neuron terminals in Aplysia abdominal ganglia, and produces a slow EPSP and broadening of the action potential (AP) due to closure of a specific class of K^+ channels (S channels). In the past, exploration of the relation of the S channel modulation to presynaptic facilitation was restricted to studies of the cell body membrane. To explore whether K^+ channel closure also occurs in presynaptic terminals, we have used the whole cell patch clamp and studied the action of 5-HT on both intact and isolated growth cones (g.c.), the precursors to the mature synaptic terminal of Aplysia sensory neurons in culture.

Using patch pipettes filled with an "intracellular" solution (containing KCl, EGTA, MgATP, GTP, and HEPES at pH 7.3) we recorded resting potentials of -50 to -60 mV. Recordings from cell bodies (n=8) show that a brief puff of 5-HT (100 uM serotonin creatinine sulfate) produces a slow depolarization of 4-10 mV, a 15-20% increase in input resistance, and a 14-30% increase in the action potential duration. Recordings from intact growth cones (n=4) show that before application of 5-HT, the growth cones are either inexcitable or fire only a small AP. After application of 5-HT either on the growth cones or the cell bodies, the growth cones show a slow depolarization of 2-7 mV and a 3-14% increase in input resistance. In addition, all growth cones now display action potentials, and in those that had action potentials prior to 5-HT, there is an increase in duration (25%).

To determine whether these effects of 5-HT result from a modulation of ionic conductances within the growth cone itself, we have begun to record from growth cones that had been mechanically isolated from their axons with a glass needle. Most of the growth cones remained morphologically and electrically intact and depolarizing current pulses elicit an action potential. In preliminary experiments, we have found that 5-HT (n=2) induces a 22% broadening in action potential duration, a slow 2-5 mV depolarization of the resting potential, and a 12-24% increase in input resistance. Application of a control solution (n=2) (creatinine sulfate) had no effect. These findings indicate that 5-HT induces a slow EPSP in sensory neuron growth cones that is similar ot the EPSP produced in the cell body, and suggest that an increase in excitability and in the broadening of the AP at the terminals contributes to presynaptic facilitation.

13.7 PRESYNAPTIC FACILITATION IN APLYSIA SENSORY NEURONS: A PROCESS INDEPENDENT OF K^+ CURRENT MODULATION BECOMES IMPORTANT WHEN TRANSMITTER RELEASE IS DEPRESSED. B. Hochner*, S. Schacher, M. Klein*, and E. R. Kandel. H. Hughes Medical Institute and Center for Neurobiology and Behavior, Columbia P & S.

Sensitization in Aplysia involves cAMP-mediated presynaptic facilitation of transmitter release from sensory neurons. This process is accompanied by reduction in a K^+ current (S-current). Closure of these channels can produce spike broadening which increases Ca^{++} influx and enhances transmitter release. Two recent findings raised the possibility that another process might also contribute to facilitation: 1) 5-HT increases the Ca^{++} transient (measured with arsenazo III) caused by long depolarizing commands under voltage clamp (Boyle et al., 1984); 2) Blockade of 5HT-induced facilitation using K^+ channel blockers reduces not only the S current but also the increase in Ca^{++} transient (Hochner et al., 1984).

To reveal additional processes in facilitation we prevented the voltage changes produced by 5-HT-induced closure of the S-channel using voltage clamp. To achieve optimal control of the terminals, we isolated sensory neurons and their followers and plated them in close proximity in dissociated cell culture. The synaptic connections formed are similar to those in vivo and in some cases we could control transmitter release from the cell body in normal sea water using a two-electrode voltage clamp. The dependence of PSP amplitude on voltage step duration is steep. Release starts with a duration of 1.5 msec and asymptotes at 10 to 20 msec. In the range of 1.5 msec to 3 msec, a 0.5 msec increase in duration causes a 2 to 3 fold increase in transmitter release, suggesting that in the normal range of release spike broadening plays an important role in facilitation. This input-output relationship is not mantained, however, when this synapse undergoes homosynaptic depression with repeated stimulation as in vivo. In the depressed state, the increase in release caused by increasing step duration is greatly reduced. This suggests that now the amount of transmitter available for release is limiting; this is also supported by the fact that the PSPs produced by short pulses are more refractory to depression. When 5-HT is applied to depressed synapses, there is only little or no facilitation of the PSPs produced by short duration pulses, but large facilitation for PSPs elicited with longer duration pulses. In addition, 5-HT restores the steepness of the input-output relation. TEA does not mimic this effect.

These data suggest that 5-HT induces presynaptic facilitation by two mechanisms: 1) By closure of S-channels. This predominates for short duration pulses (comparable in duration to the action potentials) and for normal (nondepressed) levels of release. Here release is strongly dependent on pulse duration. 2) By mobilization of transmitter or other steps in the release mechanism. This predominates when the synapse is in a depressed state and might be related to 5-HT modulation of Ca^{++} handling. In the intermediate range of release, duration and mobilization both regulate transmitter release, and here, 5-HT-induced facilitation presumably involves synergistic interaction of the two processes.

13.8 RELEASE OF TRANSMITTER FROM APLYSIA SYNAPTOSOMES. G.J. Chin, E. Shapiro & J.H. Schwartz. Howard Hughes Med. Inst., Columbia Univ. Coll. of Physicians & Surgeons, New York, NY 10032.

We have developed a preparation of isolated nerve endings from Aplysia to examine the biochemistry and physiology of transmitter release. We find that this synaptosomal preparation releases neurotransmitter in a Ca^{2+}-dependent manner and displays a Co^{2+}-inhibitable Ca^{2+} influx. To prepare synaptosomes, central ganglia from 8-10 Aplysia (trimmed of all extraneous connective tissue, but not desheathed) were homogenized in buffered 0.8 or 1.1M sucrose using a ground-glass tissue grinder. Connective tissue debris was removed manually, and the remaining suspension (10 mg protein in 1.0 -1.5 ml) was centrifuged for 10 min at 1000 x g on a two-step density gradient (0.8M/1.1M sucrose). We isolated three fractions: P1, the pellet below the 1.1M sucrose layer; P2, the material suspended in the 1.1M layer and the 0.8M/1.1M interface; and P3, the 0.8M layer and floating material. The two lighter fractions were diluted with several vol of Ca^{2+}-free seawater and centrifuged for 10 min at 10,000 x g to obtain membrane pellets P2 (2 mg protein) and P3 (1 mg protein).

For electron microscopy, the pellets were fixed and sectioned and then compared stereometrically (n=4). Profiles containing synaptic vesicles enclosed by external membrane were called synaptosomes. The % area occupied by synaptosomes in the unfractionated homogenate was 7.1, P1 contained 4.2% synaptosomes, P2 14.4% synaptosomes, and P3 22.1%. The average diameter of an Aplysia synaptosome is 1 um, with many profiles as large as 3 um.

To measure Ca^{2+} uptake, the membranes were resuspended and incubated for 30 sec at room temperature in normal or high K^+ solutions containing ^{45}Ca. Uptake assayed on glass fiber filters was greatest in the P3 fraction; it was stimulated by high K^+, and inhibited by 5 mM Co^{2+}. Transmitter release was assayed similarly. Membrane fractions were labeled with ^3H-choline for 30 min at 15°C, then washed and suspended in Ca^{2+}-free seawater at 4°C. Aliquots were exposed for 30 sec to normal or high K^+ seawater at room temperature, then rapidly filtered. Release of radioactivity from the P3 membrane was stimulated by high K^+ only in the presence of external Ca^{2+}.

We are currently extending these investigations in two directions: First, we are prelabeling identified Aplysia neurons with radioactive transmitter by intracellular injection before homogenization to study modulation of release from identified, isolated presynaptic terminals. Second, we have begun to characterize these fractions biochemically by examining protein phosphorylation.

13.9 EXPRESSION, PACKAGING, AND REGULATED RELEASE OF HUMAN GROWTH HORMONE IN PC-12 CELLS. E.S. Schweitzer and R.B. Kelly. Univ. Cal. Sch. of Med., San Francisco, CA 94143.

We have transfected the gene coding for human growth hormone into the rat pheochomocytoma-derived cell line, PC-12, in order to study the processing and secretion of a foreign peptide by a cell with neuronal characteristics. The genomic DNA coding for hGH was introduced in a plasmid containing the selectable marker for neomycin resistance, permiting the selection of stably transformed cell lines. The hGH gene is expressed in these cells, and immunoreactive growth hormone that co-migrates with authentic hGH is secreted into the medium. In addition to the major form of the hormone (MW=22,000), a smaller form of Mr=20,000 is secreted. This peptide is also found in normal human (but not rat) pituitary, and is thought to be the result of alternative RNA splicing.

Human growth hormone is stored within the cells in a compartment that appears to correspond to the endogenous catecholaminergic vesicles. These vesicles can be specifically immunoprecipitated by either of two vesicle-specific monoclonal antibodies. On sub-cellular fractionation, hGH co-purifies with the norepinephrine-containing vesicles. Treatment of the cells with NGF causes a 2-3 fold increase in the storage of hGH.

Stimulation of the transfected PC-12 cells either by elevated external K or 5 mM carbachol causes a large, rapid increase in the release of hGH. This release peaks in less than 2 min; approximately 40% of the total stored hGH is released in 10 min. The kinetics of the hGH release parallel those of norepinephrine, supporting the suggestion that they are packaged into the same vesicles. Differentiation of the cells with NGF causes an increase in the stimulated secretion of hGH. In these cells, stimulated release is 25-fold higher than the basal release.

The packaging and release of hGH from synaptic vesicles involves segregation of secretory proteins, since not all proteins seem to be packaged into these same vesicles. For example, laminin, a soluble protein that is a component of extracelluar matrix, is secreted by PC-12 cells independently of stimulation. Carbachol causes no increase in the rate of its release. PC-12 cells therefore possess at least two pathways for protein secretion, and are capable of selectively packaging a foreign hormone into one of the two paths.

13.10 ECTO-PROTEIN KINASE ACTIVITY AT THE SURFACE OF INTACT NEURAL CELLS. Y.H. Ehrlich, T.B. Davis*, E. Kornecki and R.H. Lenox. Neurosci. Res. Unit, Dept. of Psychiatry, Univ. of VT, Burlington, VT 05405

Extracellular adenosine triphosphate (ATP) exerts potent effects on the activity of excitable cells, neurons and muscle. Furthermore it is known that ATP is released in association with neurotransmitters at synapses and neuromuscular junctions. The molecular mechanisms underlying the modulation of neuronal function by extracellular ATP may involve the phosphorylation of surface proteins by ecto-protein kinase activity. Conclusive evidence for the existance of an ecto-kinase and the identification of its specific protein substrates requires that studies be carried out with a homogenous population of intact, viable cells. In the present study, this was done by incubating intact, viable cells of neuronal origin, the neuroblastoma x glioma hybrid clone NG108-15, with extracellular radiolabeled ATP. ^{32}P-incorporation into specific protein components was measured by autoradiography of SDS-solubilized proteins separated in slab polyacrylamide gels. To exclude the possibility that the measured activity arises from serum proteins or resides in membrane fragments formed when some cells break during harvest, NG108-15 cells were grown and differentiated for 6-8 days in a chemically defined (serum-free) medium supplemented with insulin, transferrin and linoleic acid. Cells were grown and assayed while attached to individual wells of 96 well plates. Prior to the assay, the cells were rinsed with a modified Krebs-Ringer buffer (containing 145mM NaCl, 6mM KCl, 0.8mM $MgCl_2$ and 1.8mM $CaCl_2$) and the reactions were carried out in this physiological medium. Intact cells utilized extracellular ATP to phosphorylate surface proteins within 30 seconds. The labeling of intracellular proteins with equivalent amounts of inorganic ^{32}Pi proceeded with a much slower time-course, and produced a different pattern of protein phosphorylation. Evidence for the ecto-enzymatic nature of the measured activity includes the ability of intact NG108-15 cells to phosphorylate an exogenous protein (casein), and the elimination of the activity by mild treatment of intact cells with 0.01% trypsin. The major protein substrates of ecto-kinase activity in NG108-15 cells have apparent M.W.'s of 190K, 120K, 97K and 44K. When NG108-15 cells are treated with 1mM dibutyryl cyclic AMP for 4-6 days they differentiate morphologically, biochemically and physiologically, and acquire many properties characteristic of mature neurons. In such differentiated NG108-15 cells the phosphorylation of endogenous proteins by extracellular ATP was increased compared to nontreated cells, suggesting that ecto-protein kinase activity is associated with the neuronal properties of these cells. A possible function for this activity was indicated by our finding that extracellular ATP induced an increase in the uptake of Ca^{++}-ions by intact NG108-15 cells.

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13.11 ATP RELEASED POSTSYNAPTICALLY CONTRIBUTES TO PRESYNAPTIC NEURONAL ATP POOL AND MODULATES NEURONAL CALCIUM UPTAKE. C.A. Lindgren and D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

Stimulation of the excitor axon to the opener muscle in the crayfish walking leg causes bath levels of ATP, ADP, AMP and adenosine to increase significantly. Addition of the postsynaptic antagonist, γ-methyl-glutamate, prevented the increase in ATP and reduced the levels of ADP, AMP, and adenosine below their limit of detection (1 pmol). This suggests that activation of the muscle causes it to release ATP and possibly also its products of hydrolysis.

The released ATP may contribute to the neuronal intracellular ATP pool. Addition of ATP to the bath solution increased ATP levels in the nerve. Moreover, addition of 2-deoxy-D-glucose and [γ-^{32}P]-ATP to the bath resulted in the formation of [^{32}P]-2-deoxy-D-glucose-6-P by the nerve. This implies that the extracellular ATP must have entered the neuron chemically intact because these results cannot be explained by extracellular hydrolysis of the ^{32}P-ATP to ADP and ^{32}P-orthophosphate. We conclude that ATP released by the muscle is capable of contributing to the neuronal ATP pool.

The released ATP may also modulate transmitter release from excitor nerve terminals. In cells with normal intracellular ATP levels, exogenous ATP was found to inhibit evoked transmitter release by 43%; neither ADP, AMP nor adenosine had any effect. Omission of glucose from the bathing solution caused nerve ATP levels to decrease by 40% within 80 minutes and an additional 10% over the next two hours. Under these conditions, 5 mM exogenous ATP increased release by 26%. This latter effect did not result from changes in intracellular ATP levels induced by the extracellular ATP since low intracellular ATP levels per se do not significantly alter average quantal content, synaptic delay nor interquantal latency.

These effects may be related to changes in the cell's Ca^{2+} permeability. Stimulation-induced ^{45}Ca uptake was reduced by exogenous ATP. However, 5 mM ATP increased resting ^{45}Ca uptake. These results are interpreted as follows. Exogenous ATP normally inhibits evoked transmitter release by reducing stimulation-induced Ca^{2+} influx into the synaptic terminal. In the absence of glucose, however, 5 mM ATP increases transmitter release because under these conditions the nerve has insufficient intracellular ATP levels to buffer the increased resting Ca^{2+} influx. Supported by NIH grant NS13600.

13.12 CYCLIC AMP REGULATES CALCIUM UPTAKE IN CORTICAL SYNAPTOSOMES OF THE RAT. J. A. Wagner, I.J. Reynolds and S.H. Snyder. Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Electrophysiological evidence suggests that an adenylate cyclase system in heart cells regulates calcium conductance through voltage-sensitive calcium channels. No such evidence exists for calcium channels in the brain, but modulatory agents such as enkephalins and adenosine, which influence adenylate cyclase, regulate release of neurotransmitters through their effects on calcium flux in nerve terminals.

Calcium flux into nerve terminals was measured as $^{45}Ca^{++}$ uptake into rat brain synaptosomes. Synaptosomal fractions were prepared according to Hajos (Brain Res. 93:485-489, 1975). Synaptosomal fractions were preincubated with or without drugs at 30°C for 30 min and incubated with $^{45}Ca^{++}$ for 45 sec. Undepolarized (3 mM KCl) and depolarized (57.5 mM KCl iso-osmotically substituted for NaCl) $^{45}Ca^{++}$uptake was measured. Uptake was stopped by rapid filtration and assessed as radioactivity trapped on filters.

Agents that enhance the adenylate cyclase systems, dibutyryl cyclic AMP, forskolin, and fluoride, enhance depolarized but not undepolarized synaptosomal $^{45}Ca^{++}$ uptake in a dose-dependent fashion. At 100 μM dibutyryl cyclic AMP maximally enhances $^{45}Ca^{++}$ uptake by 33°/°. Forskolin increases uptake maximally at 10 μM by 33°/°. Fluoride enhances uptake by 100°/° at 20 mM. These effects are all pharmacologically specific to adenylate cyclase because inactive analogs of these agents do not affect $^{45}Ca^{++}$ uptake. In addition, agonists at adenosine A_1, $GABA_B$ and opiate receptors, which block neurotransmitter release and inhibit adenylate cyclase, block depolarization-induced $^{45}Ca^{++}$ uptake. These findings suggest that adenylate cyclase is a major regulator of calcium uptake into nerve terminals in the brain and thereby modulates neurotransmitter release. (J.A.W. was supported by an NSF graduate fellowship.)

SENSORY SYSTEMS: AUDITORY PATHWAYS I

14.1 Representation of Envelope Information in Cochlear Microphonic Responses to Sinusoidal Amplitude Modulation

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Amplitude modulated sinusoids produce responses in VIII nerve fibers synchronized to the carrier (C), upper and lower sideband (U and L) and envelope (E) frequencies (Javel, E. J. Acoust. Soc. Amer. 68:133-146, 1980). For this to occur, the signal components (C, U and L) must fall within the unit's response area. The neuron's response to the modulation envelope is presumed to occur as a result of the relative response amplitudes and phases of these components. The amplitude of the VIII nerve response to the envelope varies with modulation frequency (Fm). The function describing this variation (the modulation transfer function or MTF) is low pass with a cutoff frequency which increases as the characteristic frequency of the fiber increases, reflecting a decrease in discharge modulation as the width of C, U and L exceed the width of the fiber response area (Palmer, A. R. Arch. Otorhinolaryngol. 236:197-202, 1982).

However, there are instances in which MTFs may not be directly predictable from single fiber filter functions. At high intensities, low CF fibers show synchronized responses to envelopes at their CFs, although the C, U and L frequencies do not fall within their response areas (Javel, 1980). The responses therefore arise from distortion products propagated to low frequency regions.

In this study, round window cochlear microphonic (CM) responses from guinea pigs were obtained to amplitude modulated sinusoids of different frequencies. Modulation frequency was varied from 25-800 Hz. Fourier Transforms (FFTs) of the CM waveforms revealed major frequency components at E and C with minor components at U and L. The magnitude of E, now expressed as a ratio of E/C, varied as a function of Fm. The MTF was bandpass with a maximum around 100 Hz.

These findings suggest that a distortion component at Fm is generated within the cochlea in response to sinusoidal amplitude modulation. Our data supports the hypothesis that this could partly determine those aspects as the response modulation observed in VIII nerve fibers which are not attributable to filter bandwidth. This work was supported by grant NS #21769.

14.2 Single-Unit Responses at Cochlear Nuclei to Group Delay in Noise

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The cochlea analyzes sounds with multiple frequency components into narrowband frequency channels. The outputs of these channels have amplitude envelopes which slowly vary in time. We modified the phase spectrum of a wideband noise to produce a second noise with envelopes delayed relative to those in the first by an amount proportional to frequency. A comparison of unit responses in the cochlear nuclei to the two noises show temporal delays which are predictable from a unit's characteristic frequency and the envelope or group delay appropriate to that frequency. In addition to providing a rapid technique for estimating characteristic frequency using complex sounds, this experiment illustrates that the envelope waveform resulting from narrowband filtering is a major determinant of the temporal pattern of responding for units in the cochlear nuclei.

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14.3 CAT COCHLEA DENDRITE RESPONSES FOR DIFFERENT Rm VALUES AND SYNAPTIC PULSE TRAINS. J.L. Winslow*, Faculty of Dentistry, U. of Toronto, Toronto, Ont. M5G1G6

The responses of cat cochlea inner and outer afferent dendrites to synaptic input were investigated by solving an electrical compartment model based on their geometry. There is still no clear identification of the role played by outer afferent dendrites in the peripheral auditory system. The response potential V(x,t) at location x, time t, was described by a system of ordinary differential equations, which is the compartmental version of the cable equation for dendrites. The synapses were driven by the nonlinear boundary condition $D_x V(x,t)|_{x=0} = -(\rho/\pi r^2) * G(t) * [V(0,t) - Vr - Ena]$, where Vr is resting potential, Ena is sodium equilibrium potential, ρ is axoplasm resistivity, r is radius, and input is post-synaptic conductance G(t). For inner dendrites of diameter 1 muM, length 25 muM, followed by a constricture of diameter 0.3 muM, length 20 uM, and axon of diameter 2 muM, a system of ordinary differential equations was obtained and solved using fast numerical stiff integration techniques. The responses were as expected with membrane capacitance 1.3 muF and membrane resistance 4000 ohm*sqcm, giving potential pulses that followed synaptic conductance pulses. The dendrite showed temporal summation for pulse trains of 1 KHz. Using the same model with only the different geometry for outer dendrites (shaped like a long handled comb with 10 to 100 synapses over 1 mm), response were similarly calculated. These responses exhibit spatial summation. However, their response potential at the spike generating site is too small to generate spikes at the start of the myelin. When the membrane resistivity is increased to 20 K ohm*sgcm, the space constant and hence response at the AP generating site increases, but still not sufficient to trigger spikes. When input for the synapses was taken to be conductance pulse trains, the responses showed temporal summation.

14.4 S^{35}-CYSTEINE UPTAKE LABELS NEURONS AND SYNAPTIC TERMINALS IN THE GERBIL COCHLEAR NUCLEUS. I.R. Schwartz, Division of Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024

Autoradiographic studies of localization of label following H^3-GABA uptake in synaptic terminals (Schwartz, I.R. 1985, in Auditory Biochemistry, D. Drescher, ed.) and histochemical studies of glutamic acid decarboxylase (GAD) immunoreactivity localization in synaptic terminals and cell bodies (Adams, J.C., Neurosci. Absts. 10:393, 1984; Moore, J.K. & Moore, R.Y., Neurosci. Absts. 10:843, 1984) provide data which suggests the presence of several populations of GABAergic neurons and synaptic terminals in the cochlear nucleus (CN) and superior olivary complex (SOC). However, Oertel et al (Neurosci. 6:2701-2714, 1981) have shown that GAD from rat liver and brain is indistinguishable from the protein of cystein sulfinic acid decarboxylase (CSD). Further, Iwata et al (J. Neurochem. 38:1268-1274, 1982) have demonstrated transmitter-like properties of uptake and release of cysteine sulfinic acid (CSA) by a rat brain preparation. To investigate the possibility that some of the data suggesting a transmitter role for GABA in the CN and SOC might be related instead to CSA, or some other closely related compound, we used light microscopic autoradiography to examine the distribution of label localization following incubation of fresh gerbil brain slices with S^{35}-cysteine (CYS).

At the light microscopic level CYS incubations produced labeling of neuronal somata, in contrast to the absence of neuronal somatic labeling observed in the cat after incubation with D- or L-aspartic acid, glutamic acid, glycine, GABA, taurine or alanine. In the gerbil CN labeled small neurons were found sparsely distributed in the molecular layer of the dorsal and anterior ventral CN (DCN & AVCN). Neither fusiform cell bodies in the DCN, nor spherical or globular cell bodies in the AVCN were labeled, although a few small labeled cells were found within the AVCN. Granule cells appeared generally unlabeled. The heaviest labeling was observed in terminals and small processes in the outer molecular layer of both DCN and VCN, the regions where both radioactive label following GABA uptake and GAD immunoreactivity are greatest. No labeled neurons were observed in the SOC.

These observations emphasize the need for additional data, especially on the "identity of action criteria", before it can be concluded whether GABA, CYS or some other compound is a transmitter of the labeled or immunoreactive neural elements.

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14.5 AGE-DEPENDENT EFFECTS OF COCHLEA REMOVAL ON THE COCHLEAR NUCLEI OF MICE. D.B. Webster. Kresge Hearing Research Laboratory of the South, Departments of Otorhinolaryngology and Anatomy, Louisiana State Univ. Med. Center, New Orleans, LA 70119.

The left cochlea was surgically removed in four groups of mice with seven mice in each group. Group 1 mice had the cochlea removed at 12 days after birth (DAB) and were sacrificed at 90 DAB; group 2 mice had their cochlea removed at 24 DAB and were sacrificed at 90 DAB; group 3 mice had their cochlea removed at 45 DAB and were sacrificed at 90 DAB; and group 4 mice had their cochlea removed at 45 DAB and were sacrificed at 123 DAB. All ears and brains were prepared for serial section, light microscopic analyses. All right ears were normal and the cochlea, including spiral ganglion, was totally destroyed in all left ears. In the ventral cochlear nuclei volumes and numbers of neurons were determined for the anterior ventral cochlear nuclei, intermediate ventral cochlear nuclei, and posterior ventral cochlear nuclei. The volumes of the dorsal cochlear nuclei were measured. The cross-sectional areas of spherical cells, octopus cells, and globular cells were measured in the ventral cochlear nuclei.

There were no differences ($p > 0.05$) in dorsal cochlear nuclear volumes between left and right sides in any of the 4 groups. In the ventral cochlear nuclei, the volume of each subdivision and the cross-sectional area of each cell type were significantly smaller ($p < 0.01$) in the left (operated) side than on the right in all four groups. The number of neurons in the ventral cochlear nuclei was significantly less ($p < 0.01$) on the left side than the right in all four groups. Analyses by subdivisions showed that all significant cell death occurred in the anterior ventral cochlear nuclei. Age-dependence of these results were found only for the volume of ventral cochlear nucleus, the cross-sectional area of octopus cells, and the number of neurons in the anterior ventral cochlear nucleus. For these three measurements, the effects were significantly ($p < 0.01$) more profound in the mice with earlier cochlear removals. Cell death in the anterior ventral cochlear nucleus continued between 90 and 123 days after birth: i.e. there were fewer neurons in the anterior ventral cochlear nucleus group 4 mice than in group 3 mice.

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14.6 SELECTIVE RETROGRADE LABELING OF LATERAL OLIVOCOCHLEAR NEURONS IN BRAINSTEM BASED ON PREFERENTIAL UPTAKE OF 3H-D-ASPARTIC ACID IN THE COCHLEA. A.F. Ryan* and I.R. Schwartz (SPON: D. Strelioff). Division of Otolaryngology, VA Medical Center & UCSD School of Medicine, La Jolla, CA 92093 and Division of Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Cochlear perfusion with probe concentrations of 3H-D-aspartic acid (D-ASP) results in preferential labeling of 60% of the efferent terminals under the inner hair cells (IHCs), based on EM autoradiographic reconstruction, while efferents beneath the outer hair cells (OHCs) are not labeled. Perfusion with 3H-GABA results in labeling of all efferents under the OHCs, and 40% of the efferent terminals under the IHCs. Cochlear afferents are not labeled with either amino acid (Schwartz and Ryan, Hearing Res. 9:185, 1983; unpubl. obs.). To identify the central neurons whose projections give rise to D-ASP-labeled fibers and terminals, gerbils were perfused perilymphatically with D-ASP in artificial perilymph and allowed to survive for 24 or 48 hours. The brains were then prepared for light microscopic autoradiography.

At 24 hours after D-ASP perfusion, densely-labeled cells and neural processes were observed in the ipsilateral lateral superior olivary nucleus (LSO). The cells were primarily fusiform in shape and were found throughout the LSO, although with a tendency to be located at the margins of the nucleus and in the medial and middle limbs. Labeled cells and processes were observed in the contralateral LSO, but they represented only 5-10% of the number observed ipsilaterally. At 48 hours after D-ASP perfusion, a smaller number of similarly distributed cells and axons in the LSO were labeled.

At both 24 and 48 hours after D-ASP perfusion, densely-labeled, scattered fibers could be traced dorsally from the ipsilateral LSO. After passing on the medial side of the trigeminal nucleus, the fibers turned laterally and aggregated to form bundles. Labeled fibers from the contralateral LSO passed under the floor of the fourth ventricle to join the ipsilateral fibers at this point. The fibers could be traced along the ventromedial edge of the vestibular portion of the VIIIth nerve root to the nerve stump. Adjacent to the cochlear nucleus (CN), densely-labeled collateral fibers left the bundles and crossed the VIIIth nerve root to enter the CN. Labeled fibers and apparent terminals were prominent throughout the central region of the ventral CN.

The D-ASP-labeled cells and fibers clearly belong to the lateral olivocochlear system identified by Warr and Guinan (Brain Res. 173: 152, 1979) in the cat. Neither retrograde transport of D-ASP by medial olivocochlear neurons, nor anterograde transport by afferent VIIIth nerve fibers, were observed. Retrograde transport of D-ASP thus allows the cells, axons and collaterals of the lateral olivocochlear system to be studied in isolation.

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14.7 REPRESENTATION OF A SINGLE LOW FREQUENCY TONE BY THE AVERAGE DRIVEN RATE OF AUDITORY-NERVE FIBERS. W.P. Shofner and M.B. Sachs. Dept. of Biomed. Eng., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

At low sound pressure levels, the profile of discharge rate vs. best frequency (BF) of the population of auditory-nerve fibers in response to a single pure tone shows a peak at the frequency of the tone. The peak is maintained over a wide range of sound pressure levels for high frequency tones (Evans, in Neuronal Mechanisms of Hearing, pp. 69-85, 1981), but not for low frequency tones (Kim and Molnar, J. Neurophysiol., 42:16, 1979). One interpretation of this result is that a place mechanism is used for encoding high frequencies, while low frequencies are encoded by temporal information such as phase-locking. On the other hand, studies using more complex stimuli such as vowels (Sachs and Young, JASA, 66:470, 1979) and tones in noise (Costalupes and Helstrom, Soc. Neurosci. Abs., 10:392, 1984) have shown that a rate-place representation of the acoustic stimulus is preserved over a wide range of levels by the low spontaneous rate (SR) auditory-nerve fibers. Because of the different functional and morphological characteristics of low SR fibers, we have re-examined the rate representation of a single low frequency tone in the population of auditory-nerve fibers.

Single auditory-nerve fibers were recorded from anesthetized cats, and average discharge rate during a 400 msec 1.5 KHz tone burst was determined at 4 sound pressure levels. The profiles of rate as a function of BF for high SR fibers (SR $>$ 19 spikes/sec), medium SR fibers (SR= 1 to 19 spikes/sec) and low SR fibers (SR $<$ 1 spike/sec) were plotted separately.

At the lowest sound pressure levels studied, all 3 populations of auditory-nerve fibers show a peak in the average rate profile around 1.5 KHz. At intermediate sound levels, this peak is lost in the rate profile of the high SR population, but is present in the rate profile of the low and medium populations. At the highest levels studied (87 dB SPL), the driven rates in both high and medium SR populations are saturated across a wide range of BFs. However, even at high sound pressure levels there is a peak around 1.5 KHz in the average rate profile for the low SR auditory-nerve fibers. The peak in the rate profile of the low SR population at high levels is undoubtedly the result of their higher thresholds (Liberman, JASA, 63:442, 1978) and wider dynamic ranges (Sachs and Abbas, JASA, 56:1835, 1974; Schalk and Sachs, JASA, 67:903, 1980; Evans and Palmer, Exp. Brain Res. 40:115, 1980). These results show that a rate-place representation of a single low frequency tone exists in the auditory-nerve over a wide range of sound pressure levels.
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14.8 CHANGES IN EXTERNAL EAR POSITION SHIFT THE SPATIAL TUNING OF AUDITORY UNITS IN THE CAT'S SUPERIOR COLLICULUS. J.C. Middlebrooks and E.I. Knudsen. Dept. of Neurobiology, Stanford University, Stanford, CA 94305

A neural map of auditory space has been demonstrated previously in the cat's superior colliculus in a preparation in which the highly mobile external ears are fixed in place. However, one might predict that movements of the ears would modify that map, since the acoustical properties of the ears largely determine the auditory cues from which the nervous system must derive the locations of sound sources. We have confirmed this prediction, at least for anesthetized cats, by measuring the influence of external ear position on the auditory responses of units in the superior colliculus. We recorded the responses of single units to noise burst stimuli presented in a free sound field. All units were selective for the horizontal and vertical location of the sound source. We represent the spatial tuning of a unit by its "best area", which is the area within which a given stimulus activated the unit to within 75% of its maximum firing level. Best areas were measured first with both external ears in a forward, symmetrical position, then with one ear turned approximately 50^{o} to the side. We refer to the ears as contra- or ipsilateral with respect to the side of the recording site.

The spatial tuning of units was influenced by changes in the position of either external ear. Whenever we turned the contralateral ear to the side, the best areas of units shifted contralaterally. The best areas of most units shifted ipsilaterally when we turned the ipsilateral ear to the side; this effect was greatest for best areas that were centered away from the area of greatest sensitivity of the contralateral ear. For a standard change in position of the contra- or ipsilateral ear, the variability in the directions and magnitudes of best area shifts was substantially greater between units than was the variability due to errors in positioning the ears.

The influence of external ear position on auditory spatial tuning has several consequences for the map of auditory space in the superior colliculus of the anesthetized cat. First, the shifts in best areas associated with changes in ear position imply that the portion of auditory space that is represented in the colliculus by best areas also can change. Second, the variability in best area shifts observed among different units implies that changes in the position of one ear can change the internal order of the auditory map on both sides of the brain. Finally, the portion of the auditory map that is least affected by a change in the position of one ear is the portion that represents the region of greatest sensitivity of the ear that is not moved. This suggests that the representation of locations in front of each external ear is relatively unaffected by changes in the position of the other ear.

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14.9 PATTERNS OF IMMUNOSTAINING WITH ANTISERA TO PEPTIDES IN THE AUDITORY BRAINSTEM OF CAT. J.C. Adams[1] and E. Mugnaini[2]. [1]Depts. of Otolaryngology and Anatomy, Medical University of South Carolina, Charleston, SC 29425, [2]Dept. of Biobehavioral Sciences, University of Connecticut, Storrs, CT 06268

Antisera against a variety of neuropeptides produce similar staining patterns of fibers and terminals in the auditory brainstem. In the inferior colliculus, particularly extracentral portions, antisera to cholecystokinin-8 (CCK-8), substance P, neurostensin, and met-enkephalin show a dense network of immunoreactive fibers and boutons. Following local injections of colchicine all four antisera show immunoreactive cell bodies in the colliculus as well. Far more cells and fibers are shown by antisera to CCK-8 and substance P and these antisera show cells and fibers within the central portions of the colliculus. All immunoreactive fibers may not be of intrinsic origin, however, because all antisera also show cells in the adjacent central grey, within and near the brachium of the inferior colliculus, and in the posterior medial geniculate body. Previous work has shown that cells in these areas are labelled following HRP injections of the inferior colliculus. More caudally, there are also similarities of staining patterns in the superior olive and the cochlear nucleus. The ventral nucleus of the trapezoid body and the cochlear nucleus contain fibers that are immunoreactive to all antisera. It is known that cells in the inferior colliculus send projections to these nuclei so it is possible that the subcollicular terminals shown by the immunostaining have origins in the inferior colliculus. This interpretation is complicated by the presense of cells in the ventral nucleus of the trapezoid body that are stained with antisera to met-enkephalin and to neurotensin. Also, in the cochlear nucleus there are cells that are CCK-8 and enkephalin immunoreactive. It seems likely that some of the subcollicular immunoreactive terminals arise from these subcollicular cell bodies. The present preliminary data serve as guide for experiments to determine the origins of these various fiber systems.

14.10 THE ULTRASTRUCTURE OF THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS OF THE RAT. C. E. Ribak and R. C. Roberts*. Dept. of Anatomy, Univ. of Calif., Irvine, CA 92717.

A few descriptions of the ultrastructure of the inferior colliculus have been made but none have described this structure in rats. Our analysis of the inferior colliculus in a normal strain of rat would serve as a baseline for future studies of the genetically epilepsy prone rat that displays a 100-200% increase in the number of GABAergic neurons specifically in this brain region. Therefore, Sprague-Dawley rats were perfused with either saline followed by 4% paraformaldehyde, 1% gluteraldehyde and 0.0002% CaCl in phosphate buffer or ringers solution followed by 1% paraformaldehyde and 1.25% gluteraldehyde in phosphate buffer followed by 3% gluteraldehyde in phosphate buffer. Tissue from the ventral lateral portion of the central nucleus was dehydrated, embedded in plastic, thin sectioned and examined under the electron microscope.

Three sizes of neurons were observed in the central nucleus. All somata contained the usual organelles but had varied amounts of perikaryal cytoplasm depending on somal size. Many small neurons (10-15 um in diameter) were spherical in shape and usually contained infolded nuclei. Occasionally two nucleoli were observed in the same nucleus. Only a few symmetric axosomatic synapses were found for this size neuron. Medium sized neuronal somata (15-20 um in diameter) were often ovoid in shape with highly infolded nuclei. Synapses were sparsely located on their somata. Large neurons were observed only occasionally, and their somata were elongated or round in shape. Their nuclei were occasionally infolded, but not to the extent of the medium sized neurons. In contrast to the medium and small cell types, many synapses occurred on the somata of the large neurons.

Many myelinated axons as well as unmyelinated axons were observed in the central nucleus some of which were observed to be in continuity with axon terminals that had three basic types of characteristics: 1) dark matrix and densely packed with round vesicles, 2)lucid matrix with flattened or pleomorphic vesicles and 3)lucid matrix with round vesicles. In addition to neuronal elements, the inferior colliculus contained many glial elements including astrocytes and oligodendrocytes.

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14.11 RECIPROCITY OF AUDITORY CORTICOTHALAMIC AND THALAMOCORTICAL PROJECTIONS: STUDY WITH AUTORADIOGRAPHIC AND HORSERADISH PEROXIDASE METHODS IN RAT MEDIAL GENICULATE BODY. D.T. Larue* and J.A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We analyzed the overlap of corticothalamic axon terminal fields and of thalamocortical cells of origin in the rat medial geniculate body (MGB). Our goal was to assess whether the distribution of auditory cortical axons projecting upon the ipsilateral MGB is coextensive with retrogradely labeled thalamocortical somata. Anesthetized adult Sprague-Dawley rats received unilateral injections of mixtures of [^{3}H]leucine (100 µCi/µl; 0.01-.2 µl total volume) and horseradish peroxidase (HRP; 20-30%). After 24-96 hours, the animals were reanesthetized and perfused transcardially with saline and mixed aldehydes, and the brains were blocked stereotaxically and then frozen-sectioned in a series of one 30 µm thick autoradiograph and two 60 µm thick TMB-reacted sections. Other experiments in which leucine or HRP alone were injected were also available. Using brightfield and darkfield microscopy, the numbers of silver grains and the disposition of labeled somata were plotted onto adjacent, independently studied sections and the separate results superimposed. The subdivisions of the MGB were defined in Nissl and Golgi stained preparations and were related to previous studies in the cat (Winer, J.A., Adv. Anat. Embryol. Cell Biol., 1985, 86:1-98).

Our primary finding was that zones of silver grains and HRP-labeled cells were not in complete continuity, although there was substantial overlap between them. Often, patches of silver grains 3-20 times above background (as assessed in the contralateral MGB) occurred, while the adjoining HRP section had few or no labeled cells; conversely, labeled cells were present in zones without corresponding levels of anterograde labeling. Such patterns of discontinuity occur in the nuclei of both the ventral and dorsal divisions of the MGB. The silver grains in both divisions were concentrated primarily in the neuropil. However, in experiments in which most somata in the adjoining, HRP-reacted section were labeled, the number of silver grains in the ventral division was consistently many times greater than in the dorsal division. We conclude that, besides zones of anterograde-retrograde continuity and discontinuity in the MGB, there are also regional variations in the distribution of corticogeniculate terminal fields which are independent of reciprocity. This research was supported by USPHS grant RO1 NS16832.

14.12 CONNECTIONS OF AUDITORY CORTEX IN SQUIRRELS. L. E. Luethke,* L. Krubitzer, and J. H. Kaas (Spon.: R. B. Langdon). Department of Hearing and Speech Sciences and Deparatment of Psychology, Vanderbilt University, Nashville, Tennessee 37240.

The connections of auditory cortex were investigated in grey squirrels, which have a larger brain and architectonically more distinct cortical subdivisions than commonly used laboratory rodents. Microelectrode multiunit recordings were used to determine the best frequencies for recording sites in auditory cortex. In each case, the borders of AI were estimated from the recordings, and lesions were placed to mark known physiological borders for later correlation with cortical architecture. Following mapping, single injections of approximately 0.06 microliters of 1% wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) were placed in AI of adult squirrels. Cortex was separated from the brainstem, flattened, and cut parallel to the surface. The brainstem was cut separately in the frontal plane. Alternate sections were reacted with tetramethylbenzidine or stained for fibers or cells. Reciprocal connections were found between AI and at least two adjoining subdivisions of auditory cortex, one immediately ventral and one rostroventral to AI. Other ipsilateral connections were with a newly discovered somatosensory representation, the parietal ventral area (PV), which is immediately ventral to the second somatosensory area, contains a systematic representation of the body surface, and is also responsive to auditory stimuli. Injections placed in AI, but extending slightly posterior to the caudal border of AI, resulted in additional connections with more caudal cortex in the temporal lobe. Callosal connections of AI were with AI and adjoining auditory fields. Subcortical connections of AI were with the principal division of the medial geniculate nucleus and the external nucleus of the inferior colliculus. These results confirm subcortical connections of auditory cortex in other mammals and reveal the convergence of somatosensory and auditory information within a specific cortical field, PV, along the lateral margin of the cerebral hemisphere.

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CARDIOVASCULAR REGULATION I: GANGLIONIC AND SPINAL MECHANISMS

15.1 **VENOUS DISTENSION LEADS TO EXCITATION OF NEURONS IN THE GUINEA-PIG INFERIOR MESENTERIC GANGLION.** K.D. Keef* and D.L. Kreulen. Department of Pharmacology, Health Sciences Center, University of Arizona, Tucson, Az. 85724.

The mesenteric sheath of the distal colon containing the inferior mesenteric artery and vein and the inferior mesenteric ganglion (IMG) was dissected free and pinned in an experimental chamber. Intracellular recordings were made in ganglionic neurons and nerve fibers were stimulated with bipolar electrodes. The colon was removed and the vein cannulated with a glass micropipette attached to a gravity-driven pressure head. To distend the vein the pressure was rapidly raised from 0 mmHg to 40 mmHg, maintained for 2 minutes, then lowered back to 0 mmHg. In 37% of cells (145 cells, 23 preparations) continuous excitatory post-synaptic potentials (EPSPs) were observed in the absence of distension. The frequency of this activity ranged from 1 to 100 EPSPs per minute with a mean maximum amplitude of 4.1 ± 2.5 mV. The activity was blocked by TTX (3×10^{-7} M) and hexamethonium (1×10^{-4} M). Three different types of responses to venous distension were observed: 1) In 5 of 46 cells exhibiting continuous activity venous distension was associated with an increase in the frequency of EPSPs. 2) In 17 of 48 cells venous distension gave rise to depolarization and an increase in membrane resistance. The mean depolarization for these cells was 3 ± 2 mV and the mean increase in membrane resistance was 20 ± 8%. Depolarization began within 15 seconds of distension and usually persisted for the duration of distension. When pressure was returned to 0 mmHg the cells repolarized. 3) When presynaptic nerve fibers were stimulated at intensities that produced subthreshold EPSPs (9 to 12 mV), venous distension was associated with conversion of EPSPs to action potentials in 16 of 49 cells. Conversion to action potential generation was associated with depolarization in 63% of cells. In conclusion we have demonstrated that venous distension increases the excitability of a population of neurons in the guinea-pig IMG. Since each type of response can occur independent of the others, the possibility exists that venous distension may involve several different neural pathways to the IMG. (Support: HL27781)

15.2 AN ELECTROPHYSIOLOGICAL DESCRIPTION OF RENAL AFFERENT FIBERS. Mark M. Knuepfer and Lawrence P. Schramm. Department of Biomedical Engineering, The Johns Hopkins University School of Medicine 21205.

Afferent renal nerves have been implicated in control of arterial pressure. Neuroanatomical and histological studies have described a predominance of unmyelinated fibers and a small population of myelinated fibers which project to the lower thoracic and upper lumbar spinal cord. This study was designed to characterize renal afferent fibers and their spinal projections using electrophysiological techniques.

Sprague-Dawley rats were anesthetized with chloralose and cannulated for arterial pressure measurement and venous drug administration. Rats were paralyzed, artificially respired and placed in a stereotaxic frame. The lower thoracic and upper lumbar spinal cord was exposed and covered with mineral oil. Each exposed dorsal root (DR) was cut at its entry into the spinal cord, and the peripheral end was electrically stimulated through a bipolar hook electrode. The left kidney and renal nerve were exposed by a lateral laparotomy and kept under mineral oil. A branch of the renal nerve was placed on a bipolar stainless steel hook electrode and cut or crushed distal to the electrode. Rats were treated with atropine (0.3 mg/kg) and hexamethonium (20 mg/kg) to prevent collateral activation in sympathetic ganglia and to reduce sympathetic postganglionic activity. Antidromic evoked responses were elicited in the renal nerve by stimulation of DR. Averaging of 10-100 responses was used to determine the number of fibers activated with fixed latency.

Of 284 antidromically activated fibers recorded in 22 renal nerve branches, 80% had conduction velocities under 2 m/s and 5% had conduction velocities over 10 m/s (range = 12-32 m/s). Thresholds for activation of these fibers, ranging from 1.5 to 50 µA, were inversely related to conduction velocities. Single fibers followed twin pulses at 1.1 to 22 ms interpulse intervals. Although DR from T8 to L3 were stimulated, antidromic responses in renal nerves were observed only during stimulation of DR T9 to L1. Seventy-five percent of responses occurred during stimulation of DR T11 to T13. Histological examination of renal nerves after recording verified the existence of the larger myelinated fibers.

These data corroborate anatomical evidence for a predominantly unmyelinated population of renal afferents. In addition, we have demonstrated that myelinated and unmyelinated fibers are evenly distributed over these roots. We have electrophysiologically identified a group of fibers of intermediate conduction velocity for which we have not observed anatomical correlates.

Supported by NIH Grant HL16315.

15.3 **RESPONSES OF THORACOLUMBAR SPINORETICULAR NEURONS TO RENAL MECHANORECEPTOR STIMULATION.** **W. Steve Ammons. Dept. of Physiology, University of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190.**

Renal afferent fibers are activated by mechanical stimuli applied to the kidney, particularly renal venous or ureteral occlusion. These mechanoreceptor stimuli may also elicit cardiovascular and renorenal reflexes. However, the central substrates for such reflexes are unknown. The goal of the present study was to test the hypothesis that neurons in the thoracolumbar spinal cord that project to the reticular formation respond to activation of renal mechanoreceptors. Experiments were performed on 17 α-chloralose anesthetized cats. Spinoreticular neurons were located by antidromic activation of neurons from the medial medullary reticular formation in or near nucleus reticularus gigantocellularis (15 cells) or the ventrolateral reticular formation near the lateral reticular nucleus (17 cells). After location of a spinoreticular neuron its response to electrical stimulation of the renal nerves was characterized and the type of renal afferent input (Aδ or C) determined. Responses to somatic stimuli were also examined. Twelve cells were tested for responses to renal vein occlusion. Eight responded with an increase in activity from 4 ± 2 to 15 ± 5 spikes/s. Renal vein occlusion also caused an increase in blood pressure (10 ± 4 mmHg); however, cell activity was not secondary to blood pressure changes as injection of phenylephrine caused pressor responses but no change in cell activity. Twenty-two cells were tested for responses to ureteral occlusion and 15 increased their activity from 6 ± 3 to a peak of 19 ± 6 spikes/s. Ureteral occlusion caused presser responses that averaged 6 ± 2 mmHg. Responses to either stimulus were rapid in onset (1-2 s) and often were maintained through the period of the occlusion. Seven responses to ureteral occlusion were of the slowly adapting type while 3 completely adapted. Almost all cells with responses to occlusion of the ureter or renal vein received both Aδ - and C-fiber input whereas many of the non-responding cells had Aδ -renal input only. All cells responded to somatic stimuli. Somatic receptive fields were usually in the left flank region. These experiments show that spinoreticular neurons receive renal mechanoreceptor input. These cells may participate in reflexes initiated by renal mechanoreceptors. Such reflexes could be part of the affective component of renal pain. (Supported by NIH Grant HL 30985).

15.4 **CONVERGENT INPUTS ONTO SYMPATHETIC PREGANGLIONIC NEURONS IN THE THORACIC SPINAL CORD OF THE CAT.** **Roger Thies and Robert Bourlier*. Dept. of Physiology & Biophysics, Univ. of Okla. Health Sci. Ctr., Oklahoma City, OK 73190.**

This study examined the responses of sympathetic preganglionic neurons (SPNs) on the left and right sides of the spinal cord to electrical stimulation of various nerves. We recorded with tungsten extracellular microelectrodes the activity of 16 SPNs in the intermediolateral cell column of the T2 and T3 segments of nine chloralose-anesthetized cats, relaxed with i.v. pancuronium (7 ug/kg/min). Neurons were identified as SPNs by antidromic stimulation of white rami that could be blocked by collision in the axon from orthodromic activation.

SPNs were found at depths of 1910 ± 210 (S.D.) from the surface, with ten on the left side and six on the right. Eight SPNs at T2 had axons with conduction velocities of 3.4 ± 0.4 (S.E.) m/sec, while eight SPNs at T3 had axons with conduction velocities of 6.2 ± 0.6 m/sec, a significant difference. Mininum afferent conduction velocity (MACV) for activation of T2 SPNs by stimulation of the T3 root was 7.6 ± 0.7 m/sec, and for activation of T3 SPNs by stimulation of the T2 root was 5.1 ± 0.4 m/sec. SPNs could be activated from stellate ganglia with a MACV of 8.2 ± 1.2 m/sec from the same side of the cord, and of 8.7 ± 1.3 m/sec from the opposite side of the cord.

Thoracic SPNs did not respond to electrical stimulation of either radial nerve, but they did fire to stimulation of the left or right third intercostal nerve. In addition, 90 V. stimuli to thoracic vagal afferents 50 msec before stimuli of 1.1x threshold across the stellate ganglion completely inhibited the response of SPNs to 6-9 of 10 stimuli on the left side, and to 4-8 of 10 stimuli on the right side in two of four SPNs tested. This suggests a descending inhibitory input onto SPNs from brain stem centers that relay vagal afferent information.

These studies show that SPNs in the thoracic spinal cord of the cat have visceral and somatic inputs from both sides of the body. In addition, the descending inhibitory input from vagal afferent excitation might suppress sympathetic input at the spinal level, similarly to the input to thoracic spinothalamic (1) and spinoreticular (2) neurons.

1. Ammons, W.S., Blair, R.W. and Foreman, R.D. Vagal afferent inhibition of spinothalamic cell responses to sympathetic cell responses to sympathetic afferents and bradykinin in the monkey. Circ. Res. 53:603-612, 1983.
2. Thies, R. and Foreman, R.D. Inhibition and excitation of thoracic spinoreticular neurons by electrical stimulation of vagal afferent nerves. Exp. Neurol. 82:1-16, 1983.
(Supported by Oklahoma Affiliate of American Heart Association).

15.5 MORPHOLOGY OF SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT SPINAL CORD REVEALED BY INTRACELLULAR STAINING WITH HORSERADISH PEROXIDASE. C.J. Forehand. Dept. Anat. and Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Understanding the central neural control of autonomic functions requires a knowledge of the morphology of the preganglionic neurons; for example, the dendritic arborizations of preganglionic neurons should indicate which central pathways have access to these cells. To this end, individual preganglionic neurons in the thoracic spinal cord of the rat have been examined by intracellular injection of horseradish peroxidase (HRP).

Rats (1-2 weeks postnatal) were anesthetized with pentobarbitol, cooled on ice, and decapitated. The vertebral column was removed and placed in ice-cold saline. The spinal cord was dissected free, hemi-sected longitudinally, and placed with the lateral surface uppermost in a recording chamber continuously superfused with oxygenated saline at room temperature. Individual preganglionic neurons in the vicinity of the intermediolateral cell column (IML) were impaled with HRP-filled microelectrodes while stimulating the appropriate ventral root. Cells exhibiting an antidromic action potential in response to ventral root stimulation were filled with HRP by iontophoresis. The HRP reaction product was visualized in transverse sections of the cords.

Most of the injected cells were located within the IML; others lay in the lateral funiculus (LF) adjacent to IML or in the intercalated nucleus (IC) medial to IML. The average cell body diameter was 22 um and total dendritic length averaged 2021 um. Dendritic arborizations were quite different depending on cell soma location. Dendrites of IML cells traversed the full mediolateral extent of the ipsilateral cord; typically, 2-3 dendrites extended from the cell body to the pia and 2-3 others extended from the cell body to the vicinity of the central canal. These cells also had dendrites that extended 300-500 um in the rostrocaudal dimension within the IML. Preganglionic neurons that lay in the LF or IC had strikingly different morphologies in that they lacked transversely oriented dendrites that spanned the mediolateral extent of the cord. Rather, these cells had dendritic arbors that extended into the IML and into the dorsal and ventral horns, traversing a rostro-caudal distance of about 400 um.

The different dendritic arborizations of preganglionic neurons in these spinal nuclei implies that the connectivity of preganglionic neurons varies according to cell body location. A further implication is that these groups may represent functionally different classes of preganglionic cells.
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15.6 SOMATIC AND DENDRITIC MORPHOLOGY OF INTRACELLULARLY LABELLED SYMPATHETIC PREGANGLIONIC NEURONS. J.B.Cabot, N.Bogan* and L.J.Reid*. Dept. of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794.

Recently there has been an impressive accumulation of anatomical data documenting both the subnuclear organization within the vertebrate spinal cord of sympathetic preganglionic neurons(SPN's), as well as the spinal and supraspinal localization of cells providing afferents to the SPN neuropil. Despite the seminal nature of such findings, there still remains a critical need for certain types of anatomical data on single SPN's. For example, information correlating (a) location, (b) somatic structure, (c) proximal dendritic size and number, and (d) distal dendritic shape, orientation, and arborization are not currently available. Within this context, the following observations are presented.

Experiments were performed in pigeons (Columba livia). Extracellular recordings from 51 antidromically activated and collided SPN's were obtained. Eleven of these cells were successfully labelled intracellularly with HRP. The mean latency for antidromic activation of the 11 SPN's was 6.6 ± 3.7 msec (±SD); median latency was 5 msec. Statistically there is not a significant difference between the antidromic latencies of the population of unlabelled SPN's and those intracellularly filled.

Nine of the 11 HRP-labelled SPN's were located within the principal SPN cell column. Two cells were located laterally in the nucleus intercalatus(IC). Four different somatic shapes were observed. Three were fusiform with major x minor axis diameters ranging from 35-43 x 16-28 um. Three were pear-shaped with major and minor diameters being approximately equal (22-31 x 21-28 um). Three were elongated and multipolar. One of these was quite large (61x33 um) and the other two were medium sized (30x22 um). The remaining SPN was small (21x12 um) and stellate-shaped.

The dendritic trees of 9 labelled SPN's exhibited simple planar organizations restricted to the longitudinal axis of spinal cord. The other 2 SPN's, both located in IC, had their principal dendritic processes oriented dorsoventrally. For one of these, a terminal arbor extended to the dorsolateral funiculus, ending adjacent to the lateral margin of the dorsal horn.

The first-order, and proximal portions of the major secondary dendrites were smooth. The number of primary dendrites/SPN varied from 4-8 and ranged widely in diameter (<1.0 to 5.3 um). Ten cells had at least one dendrite extending laterally into the intermediate spinal laminae. Moreover, 7 had processes reaching out into the lateral funiculus. The terminal portions of secondary and higher-order processes were "beaded or varicose" in appearance. Dendritic spines in the classical sense were not observed. (Supported by HL24103 to JBC).

15.7 LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS ON AXON COLLATERALS OF SYMPATHETIC PREGANGLIONIC NEURONS. N. Bogan* and J. Cabot (SPON: S. Yazulla). Department of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, New York 11794.

There is a longstanding debate as to whether sympathetic preganglionic neurons(SPN's) give rise to axons that have intraspinal collateral branches. All available light and electron microscopic data indicate that SPN axons do not branch, whereas some physiological observations support their presence. This report provides definitive evidence showing the existence of SPN axon collaterals.

Experiments were performed in pigeons (Columba livia). SPN's in T1 were identified electrophysiologically using antidromic activation and collision techniques. Eleven cells were intracellularly labelled with HRP. The axons of 5 SPN's could be traced to the ventral rootlets. The main axon of two cells branched intraspinally and none of these collaterals were recurrent onto the SPN of origin. While these two cells were similar in that the latencies of antidromic activation were among the shortest observed (2 and 5 msec, estimated conduction velocity: 4.7 and 1.9 m/sec), they differed significantly in spinal location, somatic structure, and dendritic organization. The cell with the slower conducting axon was pear-shaped, located in the principal SPN cell column (column of Terni), and had dendrites oriented horizontally; the other SPN was elongated and multipolar, localized in the nucleus intercalatus (IC), and had dendrites oriented dorsoventrally.

The parent axon of the SPN in IC branched twice. The first branch originated and terminated laterally within spinal lamina VII; the other arose at the intermediate spinal gray/funicular border and continued on into the white matter. Both collaterals (a) were significantly smaller in diameter than the parent axon; (b) travelled and ramified in the longitudinal spinal axis; and (c) exhibited bulbous terminal endings.

The SPN within the column of Terni gave rise to a single axon collateral 280 um from the soma. The collateral projected medially, and terminated in a restricted region at the lateral edge of the column of Terni. This branch, its endings, and the parent axon have been examined electron microscopically. A serial section analysis has revealed that the main axon was myelinated and of a caliber consistent with the estimated conduction velocity. The collateral tapered rapidly in diameter, became unmyelinated and branched. Each branched process was extremely small in diameter and periodically expanded to form varicose regions which were the exclusive sites of synaptic contact. These boutons contained small round electron-lucent vesicles and made only asymmetric, axo-dendritic synapses. (Supported by HL24103. The authors thank S.Van Horn for her aid).

15.8 REFLEX RESPONSES OF SINGLE SPLENIC NEURONS ELICITED BY STIMULATION OF INTESTINAL RECEPTORS AND BARORECEPTORS. R. Meckler and L. Weaver. Dept. Physiol., Mich. State Univ., E. Lansing, MI 48824.

Stimulation of visceral afferent nerves can produce unequal magnitudes of excitation or inhibition in multifiber recordings of splenic and renal nerve activity. Splenic nerve excitation is greater than renal nerve excitation in response to stimulation of splenic or intestinal receptors. Activation of arterial and cardiopulmonary pressoreceptors often results in less inhibition of splenic than renal nerve discharge. Is a greater response of one nerve caused by general excitation or inhibition of all neurons or by particular responses of only some neurons? This study was done to assess responses of individual splenic neurons to stimulation of intestinal receptors and baroreceptors. Experiments were done in chloralose-anesthetized cats that were artificially ventilated. Femoral arteries and veins were cannulated for monitoring arterial pressure and for infusions of drugs and solutions. The intestine was exteriorized through a laparotomy and placed in a small plastic bag filled with physiological saline. Splenic nerve bundles were identified close to the spleen, carefully dissected from surrounding tissue, and severed. Small fibers were teased from the central end and were placed on bipolar platinum electrodes for recording electrical activity. The nerves were immersed in a pool of warmed mineral oil. Pressoreceptors were stimulated by intravenous injections of 1 to 5 µg norepinephrine. Pressoreceptors were "unloaded" by small hemorrhages of blood that was subsequently returned. Intestinal receptors were stimulated by superfusion of the small intestine with 1 to 25 ml of physiological saline containing 1 µg/ml bradykinin triacetate. Between periods of drug application, the serosa was thoroughly rinsed with warmed normal saline. Discharge rates of spontaneously active neurons ranged from 0.6 to 7.5 spikes/s under resting conditions (average = 3.2 ± 0.5 spikes/s). Most neurons were baroreceptor sensitive: activity of 50% of the cells tested tended to be excited (from 3.1 ± 0.4 to 4.0 ± 0.4 spikes/s) by decreasing arterial pressure from 132 ± 3 to 110 ± 3 mmHg, activity of 70% of the neurons was excited (from 3.1 ± 0.5 to 5.0 ± 0.5 spikes/s) by decreasing arterial pressure from 132 ± 3 to 95 ± 3 mmHg, and activity of 82% of the cells was inhibited (from 3.2 ± 0.4 to 1.6 ± 0.4 spikes/s) by increasing arterial pressure from 127 ± 4 to 166 ± 4 mmHg. Serosal application of bradykinin caused excitatory responses (from 3.1 ± 0.7 to 6.7 ± 0.7 spikes/s) in 86% of the cells tested despite the concomitant blood pressure increases that ranged from 2 to 67 mmHg. Recruitment of previously inactive neurons was rarely observed during the excitatory responses. It appears that most of these efferent cells react similarly, although responses of splenic neurons are not homogeneous to stimulation of visceral afferent nerves. Support: NIH, HL21436.

15.9 SPLENIC AFFERENT NERVE RESPONSES TO STIMULATION OF SPLENIC RECEPTORS. J. Tobey and L. Weaver. Dept. of Physiol., Mich. St. Univ., E. Lansing, MI 48824.

Non-noxious stimulation of splenic receptors produces patterns of neural responses that differ from those produced by noxious stimuli. Non-noxious stimuli, such as congestion, produce equal magnitudes of splenic and renal excitation, whereas noxious stimuli, such as capsaicin, produce greater splenic than renal nerve excitation. Previous experiments employing two different levels of splenic congestion indicate different patterns of neural responses can be produced. Increase in splenic venous pressure (SVP) to 40 mmHg produces equivalent splenic and renal neural responses (18% increase and 11% increase, respectively). In contrast, increase in SVP to 50 mmHg produces greater splenic (51% increase) than renal (23% increase) excitation. The present study investigated responses of splenic afferent nerves that initiate the reflex responses. Two possible hypotheses are: 1) Unequal reflex responses are mediated by a different afferent population than the equal reflex responses, or 2) Intensity of afferent stimulation determines the pattern of reflex response. Experiments were done in anesthetized, vagotomized, sinoaortic denervated cats with vascularly isolated spleens. Splenic nerve bundles were identified next to the splenic artery and severed. Small fibers were teased from the distal cut end and prepared for recording afferent electrical activity. Nerve bundles with spontaneous activity of one or two units were tested. Splenic efferent nerve activity was monitored simultaneously to evaluate the reflex responses. Receptors of the vascularly-isolated spleen were stimulated by capsaicin (CAPS) or congestion with isotonic saline. Discharge rates of spontaneously active units ranged from 0.2 to 10.7 spikes/s (average = 2.7 ± 1.0 spikes/s). Two patterns of afferent response to stimulation of splenic receptors were found. One group of units responded to congestion only. Increase in SVP to 40 mmHg increased unit activity from 3.4 ± 2.3 spikes/s to 9.6 ± 5.3 spikes/s. The other group of units responded to non-noxious and noxious stimuli. Congestion increased activity in these units, from 2.9 ± 1.5 spikes/s to 4.8 ± 1.6 spikes/s. 5 µg CAPS increased unit activity from 2.0 ± 0.8 spikes/s to 8.4 ± 2.8 spikes/s. These results suggest the pattern of reflex responses is determined both by the intensity of afferent stimulation and by different afferent populations. Unequal reflex responses of splenic and renal nerves appear to be due to more intense activation of receptors responsive to mechanical and chemical stimulation, while equal reflex responses appear to be produced by less intense activation of these receptors and mechano-receptors. Support: NIH, HL07006, HL21436.

15.10 SPINAL TRANSECTION ELEVATES RENAL NERVE SYMPATHETIC ACTIVITY IN ANESTHETIZED RATS. L.P. Schramm, R.H. Livingstone, and M.M. Knuepfer. Department of Biomedical Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Tonic sympathetic activity is generally thought to be driven by supraspinal systems. However, we have observed substantial sympathetic activity in the renal nerves of anesthetized rats after spinal transection. The present studies are determining the effect of spinal cord transection on the magnitude and sources of renal sympathetic activity.

Male Sprague-Dawley rats weighing 250 to 490 g were anesthetized with ether followed by 100 mg/kg alpha-chloralose, and they were kept deeply anesthetized throughout experiments with supplemental doses of chloralose. Arterial blood pressure was measured through a carotid arterial cannula. Drugs were delivered via a femoral venous cannula. Rats were paralyzed with gallamine triethiodide, artificially respired, and mounted in a stereotaxic apparatus. The cervical spinal cord was exposed through a dorsal laminectomy and covered with mineral oil. A left renal nerve bundle was approached through a laparotomy and placed on bipolar stainless steel electrodes. Sympathetic activity was amplified, rectified, integrated, and stored on magnetic disc or tape.

Spinal transection between C1 and C2 slightly reduced mean arterial pressure from 90 +/- 19 to 83 +/- 9 mmHg. However, renal sympathetic activity rose, on the average to 230 +/- 70% of control ($P < 0.001$, $N = 14$, paired t-test). We hypothesized that supraspinal drive for renal sympathetic activity was replaced, within 5-15 minutes after spinal transection, by drive from spinal primary afferents. Two observations were consistent with this hypothesis. First, analgesic doses of morphine (4-40 mg/kg) dramatically reduced renal sympathetic activity after spinal cord transection. Naloxone restored the activity. Second, electrical stimulation (20 Hz, 25-250 µA, 0.3 ms pulse duration) in the cervical dorsolateral funiculus, after spinal cord transection, reduced or abolished the spontaneous and sensory-evoked firing of dorsal horn neurons, and it simultaneously reduced renal sympathetic activity by approximately 60%. The system responsible for the evoked sympathoinhibition did not descend from the brainstem since renal sympathetic activity could still be inhibited by stimulation caudal to chronic lesions of the dorsolateral funiculus made several weeks prior to the acute experiments.

We conclude that spinal afferents may be disinhibited by spinal cord transection in the rat. The resulting afferent drive, which may be particularly great in our surgically prepared animals, is more than sufficient to overcome the loss of supraspinal drive to the sympathetic preganglionic neurons, and it elicits elevated renal sympathetic activity.

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15.11 LONG-TERM POTENTIATION (LTP) IN DESCENDING SPINAL SYMPATHETIC PATHWAYS. Ralph L. Myers* and Donald N. Franz. Department of Pharmacology, Univ. of Utah School of Medicine, Salt Lake City, Utah, 84132.

LTP is readily produced in the bulbospinal sympathetic pathways of cats. Brief tetanic stimulation of the ipsilateral cervical dorsolateral funiculus in unanesthetized spinal cats produces a 20-30 minute enhancement of subsequent transmission to sympathetic preganglionic neurons (SPGNs). Stimulation within the range of physiological frequencies can produce 50-300% increases in intraspinal transmission (measured from thoracic white rami at 0.1 Hz) which decays along a single-exponential time course with a time constant of 9.5 minutes. This study investigated the ability of polysynaptic sympathetic reflex pathways and contralateral descending excitatory sympathetic pathways to produce LTP of transmission to SPGNs.

Tetanic stimulation (50 Hz for 10 sec) of intercostal nerve T4 in intact alpha-chloralose anesthetized cats did not induce LTP in the spinal-bulbospinal reflex pathway, nor did spinal sympathetic reflexes alone exhibit LTP or PTP. A modest LTP was induced in the polysynaptic spinal reflex pathway of spinal cats by tetanization (50 Hz/10 sec) of the intraspinal (descending) pathways, suggesting that some component of this LTP may be postsynaptic on the SPGNs.

Tetanization of ipsilateral pathways induced LTP of transmission through both ipsilateral and contralateral pathways, and vice versa. The maximun LTP in a given pathway was nearly identical whether the contralateral or ipsilateral pathways were tetanized, even though singly evoked contralateral evoked responses were much smaller than ipsilaterally evoked responses.

That LTP can be induced in contralateral and ipsilateral pathways by stimulating either pathway, suggests a convergence of these pathways at some level. The interaction may be presynaptic, postsynaptic, or both, and may involve mediation by an interneuron. The properties of LTP in spinal sympathetic pathways suggest a role for LTP in central regulation of cardiovascular function and, by virtue of its long duration, a possible role in the pathogenesis of primary hypertension. (Supported by HL-24085, GM-07579, and Utah Heart Association.)

15.12 ROLE OF SPINAL VASOPRESSINERGIC MECHANISMS IN THE CARDIOVASCULAR EFFECTS PRODUCED BY STIMULATION OF THE PARAVENTRICULAR NUCLEUS. J.P. Porter* and M.J. Brody. Dept. of Pharmacology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Vasopressin-containing neurons extend monosynaptically from the paraventricular nucleus (PVN) to the spinal cord. In the present investigation we sought to determine if vasopressin released from these spinal nerve terminals could alter sympathetic outflow to the peripheral vasculature.

In initial experiments, we compared the hemodynamic responses produced by stimulating the PVN in Long Evans rats (LE) and Brattleboro rats (BRAT) which lack hypothalamic and spinal vasopressin. Rats were anesthetized with urethane and instrumented with pulsed Doppler flow probes on the mesenteric and renal arteries and the lower abdominal aorta. Posterior parvocellular PVN was then stimulated with 25-200 µA before and after acute sinoaortic deaferentation (SAD). Stimulation of PVN in baroreceptor-intact BRAT produced responses which were not significantly different from LE controls. The response was characterized by an increase in arterial pressure and vasoconstriction in the mesenteric and renal vascular beds and vasodilation in the hindquarters. Following acute SAD, the pressor and vasoconstrictor responses were significantly enhanced in both groups except for mesenteric vasoconstriction which was not augmented in BRAT. In both groups the hindquarter vasodilation was reversed to a vasoconstriction.

In separate experiments, we sought to determine if vasopressin could specifically activate V1 receptors within the subarachnoid space of the spinal cord to produce hemodynamic effects similar to those produced by stimulating PVN. In conscious Sprague-Dawley rats with indwelling intrathecal catheters 1-17 pmoles of vasopressin (AVP) was injected into the area of the thoracic spinal cord. Within 1 min after AVP, arterial pressure increased 47 ± 8 mmHg accompanied by bradycardia and vasoconstriction in all three vascular beds. This effect appeared to be produced by an action of AVP within the spinal cord since prior blockade of peripheral V1 receptors did not prevent the increase in pressure. Intrathecal injections of oxytocin (30-1500 pmoles) had no effect. Pretreatment intrathecally with 0.5 nmoles of a V1 vasopressin receptor antagonist completely prevented the hemodynamic effects of subsequent intrathecal injection of AVP. However, intrathecal administration of the antagonist had no effect on the increase in pressure produced by stimulating PVN.

Taken together, these data suggest that AVP can act specifically on V1 receptors within the spinal subarachnoid space to produce pressor and vasoconstrictor responses. However, these receptors do not appear to be functionally involved in mediating the hemodynamic effects produced by stimulation of the PVN.

FEEDING AND DRINKING: CCK

16.1 EFFECT OF BOMBESIN, CHOLECYSTOKININ, CALCITONIN AND GLUCAGON ON CONSUMMATORY BEHAVIORS IN WOLF PUPS. J.E. Morley, A.S. Levine, T. Tandeski, H. Hertel, U.S. Seal. Neuroendocrine Research Laboratory and Geriatric Res Education and Clinical Center, VA Medical Center, Minneapolis, MN, 55417 and Sepulveda, CA, 91343.

We have previously examined the effect of a variety of satiety factors on food intake in the dog and the effect of opioid blockade on food intake in the wolf. In the present study we evaluated the effect of various satiety factors on 1 hour food intake in 24 hour deprived wolf pups (n = 5) weighing between 15 - 28 kg. Baseline food intake was measured following administration of 4 different vehicles used to dissolve the peptides. Intramuscular administration of bombesin (BB) (5 and 1 µg/kg) and cholecystokinin (CCK) (5 µg/kg) depressed food intake ($p < 0.05$) in 5/5 pups (Table). Calcitonin (CT) (5 and 1 U/kg) decreased food intake in 3/5 pups and 4/5 pups, respectively. Glucagon (G) (0.5 mg/kg) only decreased food intake in 2/5 pups.

	Food Intake (g)	
Vehicles	622 ± 75	
BB (5 µg/kg)	319 ± 84*	$p < 0.05$
BB (1 µg/kg)	489 ± 95*	$p < 0.05$
CCK (5 µg/kg)	418 ± 61*	$p < 0.05$
CT (5 U/kg)	531 ± 95	
CT (1 U/kg)	589 ± 82	
G (0.5 mg/kg)	575 ± 98	

Eating, drinking, moving and grooming behaviors were observed each minute and scored as the number of behavioral bouts which occurred in a 1 hour period. Although BB and CCK suppressed food intake, the number of eating bouts in one minute was not decreased (vehicle: 22 ± 3; BB - 5 µg/kg: 18 ± 5; BB - 1 µg/kg: 21 ± 6; CCK - 5 µg/kg: 20 ± 3 bouts/h). In contrast, CT at the 5 U/kg dose (14 ± 3 bouts/h) and 1 U/kg dose (15 ± 2 bouts/h) resulted in a significant decrease in observed eating behavior. Drinking behavior was decreased following administration of BB (1 µg/kg: 1.4 ± 0.4 bouts/h) and CCK (5 µg/kg: 1.8 ± 0.4 bouts/h) compared to the vehicle controls (3.4 bouts/h). Resting, moving and grooming behaviors were unaffected by any of the drug treatments. Of interest is the fact that the effect of these peptides on food intake does not necessarily correspond to the number of bouts of eating in a one hour period. These data indicate that food intake in wolf pups is decreased following intramuscular administration of CCK and BB, but not following CT or G. In other species, including the dog, CT is generally a potent anorectic agent. Thus, we have observed again that various species respond differently to the effects of peptides on consummatory behaviors.

16.2 HYPERPHAGIA AND OBESITY INDUCED BY NEUROPEPTIDE Y INJECTED CHRONICALLY INTO THE PARAVENTRICULAR HYPOTHALAMUS OF THE RAT. B.G. Stanley, S.E. Kyrkouli*, S. Lampert* and S.F. Leibowitz. The Rockefeller Univ., New York, NY 10021.

Neuropeptide Y (NPY), a 36 amino acid peptide which coexists with norepinephrine and is found in great abundance in the brain, has recently been shown to stimulate feeding behavior following intracerebroventricular or hypothalamic injection in satiated rats. NPY is the most powerful chemical stimulant of feeding behavior tested to date, with a single injection into the paraventricular nucleus of the hypothalamus (PVN) eliciting, in 4 hr, the equivalent of what rats normally eat in 24 hr. NPY acts within min after injection and is behaviorally specific.

The present experiment tested the effect of chronic PVN injection of NPY on 24 hr food intake and body weight gain of adult female Sprague-Dawley rats with bilateral 26-gauge cannulas aimed at PVN. After 5 days of preinjection baseline measurements, testing was initiated by injecting NPY (1 ug/0.3 ul saline, n=14) or vehicle (0.3 µl saline, n=6) bilaterally into the PVN every 8 hr (8:00, 16:00 and 24:00 hr) for 6 days. Body weight and food intake (67% Purina rat chow, 33% vegetable oil) were measured every 24 hr, and food intake was also measured 3 hr after each of the injections. NPY chronically injected into the PVN dramatically increased daily food intake and body weight gain. Food intake increased from a control baseline of 17 g/day, to 25 g the first day, 28 g the second day and not less than 36 g/day on days 3-6. With animals weighting 318 g when the injections were initiated, body weight gain increased from a control average of 2 g/day to 15 g/day during the 6 days of testing. The weight gain of individual subjects ranged from 8 to 24 g/day during this period. NPY was equally effective in increasing food intake in the light and in the dark phases.

This peptide-induced hyperphagia and weight gain demonstrates that chronic stimulation by exogenous NPY injected into the PVN can override mechanisms of satiety, as well as short- and long-term mechanisms of body weight regulation. It remains to be determined whether endogenous NPY mediates normal feeding, and whether it is involved in the hyperphagia and obesity produced by other experimental manipulations.

(Supported by NIH grant MH 22879).

16.3 CAPSAICIN PRETREATMENT ATTENUATES CCK-INDUCED SUPPRESSION OF SUCROSE INTAKE DURING SHAM FEEDING AND REAL FEEDING. D. Yox and R.C. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843.

Cholecystokinin (CCK) is a peptide which is localized in gut endocrine cells and in some neurons. Administration of exogenous CCK or its biologically active octapeptide (CCK-8) reduces consumption of solid and liquid foods. Recently, we demonstrated that pretreatment of adult rats with capsaicin, a neurotoxin that destroys small-diameter primary sensory neurons, markedly attenuates CCK-induced suppression of food intake (Ritter and Ladenheim, 1985). Consequently, we hypothesized that capsaicin destroys a population of neurons that directly mediates CCK-induced satiety. It is also possible, however, that capsaicin destroys neurons that mediate changes in gastric emptying produced by CCK, or impairs some interaction between CCK and receptors for gastric distension, thereby indirectly attenuating CCK's satietogenic effects. If this latter hypothesis were correct, then CCK-induced satiety in capsaicin-pretreated rats should not be attenuated when ingesta is allowed to drain from their stomachs via open gastric fistulae (sham feeding). Therefore, we have examined the effect of CCK injection on sham feeding and real feeding of 15% sucrose in capsaicin- and vehicle-pretreated rats. CCK-8-induced suppression of real feeding was significantly attenuated in capsaicin-pretreated rats. Vehicle-pretreated control rats suppressed their intake by 66.7±15.6%, 79.3±5.8%, and 86.1±4.1% in response to CCK-8 doses of 1, 2, and 8µg/kg respectively, whereas capsaicin-pretreated rats suppressed their intakes by 38.1±12.0%, 26.0±9.4% and 58.8±7.5% in response to the same doses of CCK-8. During sham feeding, suppression of sucrose intake by capsaicin-pretreated rats was significantly attenuated relative to suppression produced in vehicle-pretreated rats. In sham feeding vehicle-pretreated rats, CCK-8 (1, 2, or 4µg/kg) suppressed intake by 47.4±7.4%, 71.5±8.8% and 70.8±3.2% respectively. Capsaicin-pretreated rats, however, exhibited suppressions of only 1.7±8.9%, 29.4±9.2% and 21.6±17.0% respectively in response to the same three doses of CCK-8. These results confirm the previous findings of Gibbs et al. (1973) that CCK-induced satiety in the rat does not require accumulation of ingesta in the stomach. Furthermore, our results demonstrate that attenuation of CCK-induced satiety by capsaicin is due to the toxin's direct action on CCK-sensitive afferents and not to secondary impairment of gastric mechanoreception or gastric emptying.

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16.4 ATTENUATION OF CCK-INDUCED SATIETY BY SYSTEMIC, INTRAVENTRICULAR OR PERIVAGAL CAPSAICIN ADMINISTRATION. E.H. South and R.C. Ritter. WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843, and Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Capsaicin is a neurotoxin which destroys small-diameter primary sensory neurons. Previous work from our laboratory has demonstrated that CCK-induced satiety is attenuated in rats pretreated with intraperitoneal injections of capsaicin (Ladenheim and Ritter, 1984). Localization of the capsaicin-sensitive neurons which participate in CCK-induced suppression of food intake has not yet been accomplished. However, Smith et al. have demonstrated that CCK-induced satiety is abolished by section of the gastric vagal branches. Furthermore, capsaicin causes degeneration of vagal primary sensory terminals in the commissural and medial subnuclei of the nucleus of the solitary tract (Dinh and S. Ritter, 1985), where most gastric vagal sensory fibers terminate (Leslie et al., 1982). Therefore, the available data are consistent with the hypothesis that capsaicin destroys a population of vagal sensory neurons which participate in the mediation of CCK-induced satiety. In an initial test of this hypothesis we have examined CCK-induced satiety in rats treated with capsaicin via several different routes of administration. In confirmation of Ladenheim and Ritter, we found that intraperitoneal (IP) capsaicin (3 x 50mg/kg) attenuates CCK-induced satiety. Percent suppression of intake by IP vehicle treated rats was 80±2%, while in IP capsaicin treated rats percent suppression by CCK was only 27±4%. Capsaicin (225µg/3 doses) treatment via the fourth cerebral ventricle virtually abolished CCK-induced satiety, while administration of the same capsaicin dose IP did not significantly attenuate CCK-induced satiety. In rats treated with capsaicin applied directly to the vagal trunks, CCK suppressed food intake by 44±12%, whereas in vehicle treated rats CCK suppressed intake by 73±10%. Capsaicin applied intrapylorically failed to attenuate CCK-induced satiety. In contrast to CCK-induced satiety, suppression of food intake by bombesin was not significantly attenuated by fourth ventricular or perivagal capsaicin application. However, IP capsaicin caused a small but significant attenuation of bombesin-induced suppression of food intake. These data suggest that capsaicin attenuates CCK-induced satiety by damaging vagal sensory neurons. The data further suggest that bombesin's effects on feeding are mediated in part by capsaicin-sensitive neurons which are not of vagal origin.

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16.5 DORSAL HINDBRAIN PARTICIPATION IN CHOLECYSTOKININ-INDUCED SATIETY. G.L. Edwards, E.E. Ladenheim and R.C. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164, and WOI Regional Program in Veterinary Medical Education, University of Idaho , Moscow, ID 83843.

The satietogenic effect of exogenous CCK appears to depend upon the integrity of the gastric vagus (Smith et al., 1981). However, the area postrema (AP) has also been proposed as a central CCK receptor, necessary for suppression of feeding by CCK (Van der Kooy, 1984). Our previous work indicates that the AP is not essential for expression of CCK's satietogenic effect (Edwards and Ritter, 1981). Nevertheless, the gastric vagus projects densely to both the medial subnucleus of the NST, just rostral to the AP, as well as to the commissural subnucleus of the NST immediately subjacent to the AP. Therefore, it is probable that these areas are involved in the mediation of CCK-induced satiety as has been previously suggested by Crawley and Kiss (1984). In order to resolve the current uncertainty regarding the participation of dorsal hindbrain structures in CCK-induced satiety, we have compared the satietogenic action of CCK-8 in rats with lesions aimed to destroy the following structures: 1) the AP and adjacent commissural subnucleus of the NST [AP-lesion], 2) the medial subnucleus of the NST [mNST-lesion], and 3) all three structures mentioned above [AP/mNST-lesion]. We have found that lesions aimed to destroy only the AP and adjacent commissural nucleus do not attenuate CCK-induced satiety. Likewise, lesions directed to destroy only the medial subnucleus of the NST do not attenuate CCK-induced satiety. However, lesions which were aimed to destroy the AP, the commissural subnucleus, and the medial subnucleus of the NST strongly attenuated CCK-induced satiety. The completeness of AP-lesions was verified antemortem utilizing a conditioned food aversion (CFA) to paraquat. Paraquat-induced CFA has been shown to be mediated by the AP (Dey et al., 1984). Subcutaneous injection of paraquat (48 µmoles/kg) did produce CFA in all rats with an intact AP. Rats with complete lesions did not develop paraquat-induced CFA. We conclude 1) that the AP is not necessary for expression of CCK-induced satiety, and 2) both the commissural and medial subnuclei of the NST probably contain vagal terminals which participate in CCK-induced satiety.

Table I. PERCENT SUPPRESSION OF FOOD INTAKE BY CCK-8 IN BRAIN-LESIONED RATS

	CCK-8 Dose (µg/kg)			
	1	2	4	8
Controls	45.4±8.6	56.6± 7.5	70.3±5.8	76.3± 9.4
AP-lesions	36.1±9.2	53.7±10.1	56.4±5.5	73.0± 6.6
mNST-lesions	38.4±7.3*	52.3± 9.2*	76.3±4.8*	77.8± 9.2
AP/mNST-lesions	7.3±9.0	12.7± 5.0	38.6±9.1	62.8±10.6

* significantly different from controls

This work was supported by NIH Grant RO1 NS20561 to R.C. Ritter.

16.6 INFUSION OF CCK-8 BETWEEN MEALS INTO FREE-FEEDING RATS FAILS TO PROLONG THE INTERMEAL INTERVAL. D.B. West, L. Prescod*, and J. Triscari*. Vassar College, Poughkeepsie, NY 12601, and Hoffmann-La Roche Inc., Nutley, NJ 07110.

We have previously demonstrated that meal-contingent infusion of the octapeptide of cholecystokinin (CCK-8) into free-feeding rats persistently suppressed meal size, but total food intake recovered due to an increased feeding frequency (Am. J. Physiol. 246: R776-787, 1984). The failure to prolong the intermeal interval (IMI) in this previous study may be due to the relatively short half-life of the peptide in the circulation.

We have further evaluated the role of CCK-8 in regulating the IMI by infusing the peptide into 4 adult, male, Sprague Dawley rats during the intermeal interval. The rats were first habituated to computer-monitored enclosures where 45 mg food pellets were provided ad libitum. After 1 week of habituation, intraperitoneal catheters were implanted and the rats were infused with saline at the end of each free-feeding meal and every 5 minutes between meals. When stable growth rates were observed, 5 days of control data were collected. The rats were then infused for 6 days with a CCK-8 solution at a dose of 1.87 µg/meal per rat at the end of each free-feeding meal. The rats also received a CCK-8 infusion every 5 minutes between meals. The dose of the intermeal infusion was increased gradually over the 6 days of drug infusion. On the last day of CCK-8 infusion, the rats received a 20 second infusion containing 0.63 µg of CCK-8 every 5 minutes during the IMI.

At no time during the 6 days of drug infusion was the total daily food intake suppressed relative to control days when no drug was infused. Average control daily intake was 28.0 ± 1.5 g while mean intake during CCK-8 infusion was 25.8 ± 1.0 g. The CCK-8 infusion at the end of each meal persistently suppressed meal size by an average of 27.3% over the 6 day period. Meal number also increased from an average of 11.9 ± 0.9 meals/day during the control period to an average of 15.2 ± 1.4 meals/day over the 6 drug days. Average IMI decreased with CCK-8 administration from 81.1 ± 3.5 minutes to 64.4 ± 3.4 minutes. Intermeal infusion of CCK-8 clearly did not prolong the IMI in this study. These findings suggest that CCK-8 may be involved in the termination of a meal and the initiation of postprandial behaviors; but it does not regulate the intermeal interval.

16.7 COMPARATIVE EFFECTS OF CAERULEIN ON FOOD INTAKE AND PANCREATIC SECRETION IN DOGS. R. D. Reidelberger*, T. J. Kalogeris*, and T. E. Solomon* (SPON: R. C. McClure). Depts. of Medicine and Physiology, Univ. of Missouri Med. Sch. and Truman V.A. Hosp., Columbia, MO 65201.

Cholecystokinin (CCK) inhibits food intake in several species when large doses are administered peripherally. Whether physiologic doses of CCK produce satiety is not established. Since the primary role of CCK is thought to be regulation of exocrine pancreatic secretion, we compared dose-response effects of the CCK analog caerulein on food intake and exocrine pancreatic secretion in dogs adapted to one meal per day. Nine fasted mongrel dogs with gastric and pancreatic fistulas received intravenous scalar doses of caerulein (0, 6.25, 12.5, 25, 50, 100, 200, 400 pmol/kg-h, each for 30 min); pancreatic secretion was measured at 10 min intervals. Maximal stimulation of pancreatic enzyme secretion occurred at 50 pmol/kg-h. A similar maximal pancreatic secretory response occurred when five dogs received a single caerulein dose of 100 pmol/kg-h for 4 h. In feeding studies, eight pure-bred beagle dogs received intravenous infusions of caerulein (0, 200, 400, or 800 pmol/kg-h, one dose per day) for 15 min before presentation of canned dog food and during the 45 min feeding period. Food intake was inhibited significantly only at 400 and 800 pmol/kg-h (49 % and 98 % inhibition, respectively). These results do not support a role for CCK as a satiety hormone, since the minimal effective dose for inhibition of food intake was 4 to 8 times larger than the maximal dose for stimulation of pancreatic enzyme secretion.

16.8 PANCREATIC GLUCAGON IN COMBINATION WITH CHOLECYSTOKININ INHIBITS SHAM FEEDING IN THE RAT. J. LeSauter*, J. Arle*, T. Cornacchia* & N. Geary. Dept. of Psychology, Columbia University, New York, NY 10027.

Pancreatic glucagon (PG) elicits a behaviorally specific inhibition of meal size when injected before meals. PG does not, however, inhibit intake in rats sham feeding with open gastric cannulas (Geary & Smith, Peptides 3: 163, 1982). This suggests that some gastric or postgastric food stimulus that is absent during sham feeding is necessary for PG's satiety effect. Therefore, we tested whether PG regains its potency to inhibit sham feeding when it is administered in combination with injections of bombesin (BBS) or cholecystokinin (CCK), two peptides that may mediate the satiety effect of food in the stomach and small intestine (Gibbs & Smith, Peptides 3: 553, 1982). Ten rats were equiped with chronic gastric cannulas and maintained on Purina chow. Sham feeding of evaporated milk was tested in non-deprived rats after i.p. injection of PG, two doses of BBS, or two doses of CCK either singly or in combination. None of the single doses, except the higher CCK dose, inhibited sham feeding in comparison to control injections. Combinations of PG + BBS also failed to inhibit sham feeding. Combinations of PG + CCK, however, significantly potentiated the effect of CCK alone. The lower BBS + CCK dose failed to inhibit sham feeding, but in combination with PG, BBS + CCK did inhibit sham feeding. Mean ± SEM 30 min sham intakes were:

	Peptide (mcg/kg)					
	CON	CCK.15	CCK.30	BBS.25	BBS.50	CCK.15+BBS.25
without PG	50.5 ±3.6	47.5 ±3.5	37.8* ±4.9	46.1 ±3.7	48.7 ±4.3	43.8 ±5.1
with PG (200)	53.4 ±3.8	30.8##** ±5.1	21.0##** ±4.7	48.9 ±3.7	46.6 ±3.2	27.0##** ±4.1

*p < .05 vs. control, Dunnett's test; **p < .01.
##p < .01 vs. without PG, Tukey's test.

These data indicate that PG synergizes with CCK to inhibit sham feeding. Further, PG can inhibit sham feeding in combination with doses of CCK or CCK + BBS that alone have no effect on sham feeding. This demonstration of potentiation extends Hinton & Geary's (FPA Proc: 56: 30, 1985) report of similar synergistic effects of satiety peptides on real feeding.

16.9 NEUROMEDIN C INHIBITS FOOD INTAKE IN RATS. J.A. DiPoala* and J. Gibbs (SPON: C. Shamoian). Dept. of Psychiatry, Cornell Univ. Med. Coll., and Eating Disorders Institute and E.W. Bourne Behav. Res. Lab., The New York Hosp., White Plains, NY 10605.

Bombesin (BBS) and BBS-like peptides produce large, dose-related and specific inhibitions of food intake. A new BBS-like decapeptide, neuromedin C (NMC), has been isolated from rodent brain and porcine spinal cord and shown to have BBS-like smooth muscle bioactivity (Roth et al, 1983; Minamino et al, 1984). We tested NMC to determine if it, like BBS, inhibited food intake.

METHODS: Adult male Sprague-Dawley rats (n=8) on a 12:12h light:dark cycle (lights on at 0700) were maintained on food pellets and tap water. At 1000, pellets were removed and a predeprivation 30-min access to liquid food (BioServ, 40%) was provided. Rats were deprived of all food from 1030-1330. At 1330, they were injected intraperitoneally with 0.15M NaCl (vehicle control), or 2,4,8, or 16 $\mu g\text{-}kg^{-1}$ NMC or BBS. Liquid food was presented immediately and consumption was measured during the 30-min test meal.

RESULTS: Neuromedin C and BBS inhibited feeding:

	Dose ($\mu g\text{-}kg^{-1}$)				
Peptide	0	2	4	8	16
BBS	19.1±1.1	10.6±1.3** (-41)	9.5±0.9** (-48)	9.0±0.9** (-51)	
NMC	19.7±0.8	19.0±2.0 (-1)	13.9±0.9* (-28)	14.4±2.1* (-30)	9.8±1.2** (-49)

Values are mean food intakes in ml±SEM. Values in parentheses are mean % change from control. *p<0.05, **p<0.01; Tukey's test following ANOVA.

Both peptides produced large inhibitions of food intake. On a weight basis, BBS was more potent than NMC: threshold doses were 2 $\mu g\text{-}kg^{-1}$ (BBS) and 4 $\mu g\text{-}kg^{-1}$ (NMC); 50% inhibition was seen at 8 $\mu g\text{-}kg^{-1}$ (BBS) and 16 $\mu g\text{-}kg^{-1}$ (NMC). On a molar basis, NMC was approximately 40% as potent as BBS; this is similar to the relative potencies of these peptides for contraction of smooth muscle. All rats showed behaviors typical of normal satiety and no abnormal behaviors were noted.

CONCLUSION: Neuromedin C inhibits food intake in a potent, dose-related fashion. This inhibition may represent a satiety action of exogenous NMC.

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16.10 BOMBESIN INCREASES POSTPRANDIAL INTERMEAL INTERVAL. S. Mindell*, J.A. DiPoala*, S. Wiener*, J. Gibbs, and G.P. Smith. Dept. of Psychiatry, Cornell Univ. Med. Coll., and Eating Disorders Institute and E.W. Bourne Behav. Res. Lab., The New York Hospital, White Plains 10605.

Bombesin (BBS) decreases meal size (MS) and increases the length of the postprandial intermeal interval (IMI) when it is injected intraperitoneally before a test meal (Wiener et al, 1984). We determined the effect of BBS on postprandial IMI by administering it at the end of the meal.

METHOD: Adult male Sprague-Dawley rats (n=16) were maintained on a condensed milk (50% v/v) diet. After an 18h overnight food deprivation, a high carbohydrate liquid diet (BioServ, 40% v/v) was provided for a daily 140 min test. At the end of the initial meal, a single injection was given of a 0.15M NaCl vehicle control or of tetradecapeptide BBS (2,4, or 8 $\mu g\text{-}kg^{-1}$). For comparison, the synthetic C-terminal octapeptide of cholecystokinin (CCK-8, 8 $\mu g\text{-}kg^{-1}$) was also administered. IMI length was determined by observing rats once each min throughout the test.

RESULTS: (1) All doses of BBS and CCK-8 produced large, highly significant increases in IMI length. (2) The effect of BBS was dose related: 8 $\mu g\text{-}kg^{-1}$ BBS increased IMI 60%; this was significantly more than the effect of 2 $\mu g\text{-}kg^{-1}$ (48% increase) or of 4 $\mu g\text{-}kg^{-1}$ (47% increase). (3) BBS was more potent than CCK-8: 8 $\mu g\text{-}kg^{-1}$ of BBS increased IMI 60%, while 8 $\mu g\text{-}kg^{-1}$ of CCK-8 increased IMI 42%.

CONCLUSION: Exogenous BBS prolongs the postprandial IMI. This effect is dose-related. BBS is apparently more potent for increasing IMI than CCK-8, while previous work (Gibbs et al, 1979) showed that BBS was less potent than CCK-8 for decreasing meal size.

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16.11 SUPPRESSION OF FOOD INTAKE IN RATS BY MICROINJECTION OF CHOLECYSTOKININ (CCK) TO THE PARAVENTRICULAR NUCLEUS (PVN). P.L. Faris* and J.W. Olney, Dept Psychiatry, Washington U. St. Louis, MO 63110.

The presence of CCK fiber terminals in the ventromedial hypothalamic nucleus (VMN) (Zaborsky et al, Br Res 303,225,'84) and fiber varicosities in the PVN indicates that these areas are sites of endogenous CCK action. Since both regions are classically implicated in food intake control, the VMN and PVN are candidate regions where CCK may act to produce satiety. We have examined the effect on food intake of CCK microinjection to these areas. Adult male rats were implanted stereotaxically with chronic indwelling unilateral guide cannulae terminating 1.5 mm dorsal to either the PVN or the VMN. Following recovery, each rat (n=7 for both PVN and VMN) received saline, 4.4 pmoles of desulfated CCK-8 (DES-CCK), 4.4 pmoles CCK and 22 pmoles CCK on alternate days in a counterbalanced fashion. The injection cannulae extended the guides by 1 mm. Following injection, dry food pellets were removed and replaced with a pre-weighed cup of wet food mash. Food cups and spillage were weighed 30,60 and 120 min following the injection. Cannulae placements were histologically verified.

Microinjections of both doses of CCK to the PVN significantly decreased food intake. The suppression appears to be both dose and time dependent. The low dose (4.4 pmoles) reduced food intake by 27% during the first 30 min. Food intake was reduced by 47% at 30 min and by 66% during the second half hour interval as a result of treatment with 22 pmoles CCK. DES-CCK did not significantly affect food intake (Table I).

Direct injections of CCK to the VMN did not have a significant effect on food intake (Table II). Thus, it is likely that the suppression of food intake by CCK administered to the PVN is a regionally specific effect, implicating the PVN as a central site for CCK-mediated control of food intake.

Table I. Mean Food Intake (grams ± SEM) Following CCK to the PVN

	Saline	4.4 pmoles DES-CCK	4.4 pmoles CCK	22 pmoles CCK
30 min	8.8 (±1.1)	7.6 (±1.2)	6.5^{A} (±1.7)	4.7^{B} (±1.2)
60 min	2.6 (±0.8)	2.5 (±0.7)	1.7 (±0.7)	0.9^{A} (±0.5)
120 min	2.3 (±1.3)	3.0 (±0.9)	3.6 (±0.8)	2.7 (±1.0)

^{A} $p < 0.05$; ^{B} $p < 0.01$

Table II. Mean Food Intake (grams ± SEM) Following CCK to the VMN

	Saline	4.4 pmoles DES-CCK	4.4 pmoles CCK	22 pmoles CCK
30 min	7.5 (±2.7)	5.6 (±2.0)	5.5 (±1.6)	8.7 (±2.8)
60 min	1.6 (±1.1)	3.1 (±1.1)	2.5 (±1.1)	2.2 (±1.0)
120 min	3.4 (±1.0)	4.0 (±1.0)	1.7 (±0.6)	0.6 (±0.1)

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16.12 CCK-8 INTERACTS WITH PREFEEDING TO IMPAIR RUNWAY PERFORMANCE. J. E. Cox. Dept. Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

We previously reported that doses of cholecystokinin octapeptide up to 4 μg/kg did not produce decrements in runway performance of food deprived rats (Cox et al., Behav. Brain Res., 14: 41, 1984. These results suggested that this substance does not reduce feeding motivation prior to contact with food, consistent with the hypothesis that CCK injections reduce food intake by acting late in the meal to prematurely trigger satiety. To further examine this hypothesis, runway performance was assessed after CCK-8 administration in combination with prefeeding. After 21 hr food deprivation, male Sprague-Dawley rats (N=7) received i.p. injections of 1.0 μg/kg CCK-8 (Sincalide, Squibb) or saline immediately following 0, 1, 2, or 5 min access to 30% sucrose. Beginning 10 min after injections, rats ran 6 runway trials for 0.3 ml of 30% sucrose. Mean running speeds (cm/s ± S.E.) were as follows:

	0'	1'	2'	5'
Saline	56.4±1.8	57.0±1.8	45.1±3.7	49.1±5.2
CCK-8	53.6±2.7	53.9±2.4	35.7±7.6	14.9±4.3
Prefed (ml)	0	2.0	4.6	9.7

After 5 min prefeeding (mean intake = 9.7 ml), CCK-8 produced a 70% decrease in running speed compared to tests on which rats were prefed the same amount but received saline injections ($P<.01$). Following shorter intervals of prefeeding or no prefeding, CCK-8 did not significantly affect runway performance. Mean 20 min consumption of 30% sucrose after runway trials with no prefeeding was 15.4±1.0 ml after saline and 12.6±1.4 ml after 1.0 μg/kg CCK-8 ($P<.01$).

Subsequent tests showed that after 5 min prefeeding. 0.50 μg/kg CCK-8 produced a 59% decrease in running speed compared to saline tests ($P<.01$). A dose of 0.25 μg/kg produced a marginally significant 16% decrease ($P<.10$).

Thus, in conjunction with prefeeding, low doses of CCK-8 produced substantial decrements in runway performance. These results contrast with the lack of effect without prefeeding of the same doses and the previously reported ineffectiveness of much larger doses. Inhibitory effects of CCK administration arise, therefore, though interaction with other signals generated by ingestion of food. The nature of these signals is not known, but after 1.0 μg/kg CCK-8 they apparently reach threshold after consumption of between approximately one-third and two-thirds (2 and 5 min prefeeding) of a full meal of 30% sucrose. (Supported by NIH grant AM31805).

CLINICAL NEUROPHYSIOLOGY: EPILEPSY

17.1 AGE RELATED SEIZURE FACILITATION. E.S.Sperber, R. Okada, B. Wong*, S.L. Moshe, Dept. of Neurology and Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461 and Dept. of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan.

Recent evidence indicates that bilateral muscimol infusions in the substantia nigra (SN) protect adult rats from a variety of experimentally induced seizures. In the present study we report the effects of muscimol infusions into the SN on seizures in developing rats.

Two week old rat pups were implanted with bilateral chronic cannulae in or above the SN. After a two day recovery period, the pups were injected with muscimol (in a volume of .25 ul per site) and their susceptibility to the development of flurothyl induced seizures was compared to that of saline infused rats. The latency to the onset of a generalized clonic seizure was considered as the convulsive threshold.

Rats injected intranigrally with 100 ng of muscimol per site and tested 30 min later, developed flurothyl seizures significantly faster than saline controls or rats whose cannula tips were 1-1.5 mm above the SN ($p<.001$). A time response curve was also generated. The proconvulsant effect of 100 ng of muscimol was already present at 15 min post infusion, became maximal at 30 min and decreased somewhat at 60 min. At all 3 times the muscimol infused rats differed from the two control groups ($p<.001$).

To evaluate the possibility that nigral muscimol infusions may have a biphasic effect on seizures in rat pups, a dose response curve was created, 30 min after the completion of the infusions. Rat pups infused with 12.5 ng/.25 ul/per site of muscimol into the SN had similar latencies to the onset of a flurothyl induced seizures as controls. However, doses of muscimol ranging from 50-200 ng/per site facilitated the development of flurothyl seizures.

These results indicate that the effects of nigral muscimol infusions on seizures are age dependent. The altered responsivity of the nigral GABA sensitive system may account for the increased seizure susceptibility of the immature CNS.
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17.2 RESERPINE REVERSES THE ANTICONVULSANT EFFECT OF GAMMA-VINYL GABA MICROINJECTED INTO THE SUBSTANTIA NIGRA OF KINDLED RATS. R.L. Gellman & J.O. McNamara. Duke & VA Med. Ctr., Durham, NC.

Microinjection of GABA agonists bilaterally into the substantia nigra (SN) suppresses both clonic motor and limbic seizures produced by kindling of olfactory structures, amygdala or entorhinal cortex. The suppression of limbic seizures behaviorally is paralleled by suppression of focal afterdischarge at the site of stimulation, thereby indicating that the SN can regulate intrinsic neuronal excitability of multiple forebrain sites. The anatomic pathways mediating this regulation are unknown. Direct connections from the SN to all three structures have not been elucidated. The widespread influence of SN on forebrain excitability raised the possiblity that this action was mediated by a population of neurons with diffuse projections. We considered monoaminergic neurons to be likely candidates both because of their diffuse projections and because of their profound influence on seizure susceptibility in the kindling model. To begin to test the hypothesis that the anticonvulsant effect of intranigral γ-vinyl GABA (GVG) is mediated by one or more of the monoamines, we determined whether reserpine, a monoamine-depleting drug, would reverse the anticonvulsant effect.

Sprague Dawley rats were stereotactically implanted with a bipolar electrode in the right amygdala and bilateral guide cannulas overlying the SN. They were stimulated 2x/day until 7 class 5 seizures were evoked. Following determination of the generalized seizure threshold, GVG (5 ug/0.5 ul saline in 2.5 min) was microinjected into the SN bilaterally and the response to a test stimulation was determined at 16 and 36 hrs thereafter. Either reserpine (2.5 mg/kg) or vehicle was administered subcutaneously to the animals 24 hours after GVG treatment. The afterdischarge duration (ADD) in seconds was recorded on EEG. All electrode and injection placements were histologically verified. Preliminary experiments indicated that reserpine produced >90% depletion of monoamines in multiple brain regions.

Reserpine, but not vehicle, reversed the anticonvulsant effect of intranigral GVG. Prior to GVG, both groups had similar baseline ADDs (86 ± 10 and 89 ± 10 secs respectively, mean ± SEM). GVG shortened ADD in both groups at 16 hrs (39 ± 11 and 50 ± 19, $p<0.05$, student's t-test). Reserpine (n=9) returned ADD to baseline (80 ± 10), while vehicle (n=8) left the GVG effect intact at 36 hrs (54 ±12, $p<0.05$ vs. baseline). Additional experiments demonstrated that reserpine alone did not lengthen the ADD in animals receiving intranigral injections of saline.

We conclude that reserpine, but not vehicle, reverses the anticonvulsant effects of intranigral GVG. This suggests that one or more of the monoamines may mediate nigral influence on intrinsic neuronal excitability in forebrain structures.

17.3 ELECTROPHYSIOLOGICAL EVIDENCE THAT SUBSTANTIA NIGRA TRANSMITS SEIZURE ACTIVITY IN KINDLED RATS. D.W. Bonhaus, J.R. Walters and J.O. McNamara. Depts. Med. (Neurology) and Pharm. Duke Univ. and V.A. Medical Centers, Durham, NC and NINCDS, Bethesda, Md.

Kindling, an animal model of limbic epilepsy, is achieved by repeatedly administering initially subconvulsive electrical stimuli to specific brain areas until generalized clonic seizures are evoked. Previous work in our laboratory has demonstrated that intranigral injection of muscimol or destruction of the substantia nigra (SN), treatments which presumably decrease the neuronal output of the SN, suppress both clonic motor and limbic seizures in kindled rats. These findings indicate that the SN is involved in the propagation of seizures in kindled rats, but they do not specify a mechanism. We proposed two hypotheses regarding the role of the SN in the propagation of kindled seizures: 1) the SN directly transmits seizure activity from rostral sites of origin to target structures; and/or 2) the SN produces a tonic seizure facilitating action on other structures which themselves directly transmit the seizure activity.

To begin testing these hypotheses we recorded single unit activity of both dopamine and pars reticulata neurons in the SN during electrical seizures induced by stimulation of amygdala in paralyzed ventilated rats. We recorded electroencephalographic (EEG) activity with electrodes in amygdala and caudate.

The most striking finding was that SN neurons in the kindled animals exhibited a dramatic change in firing pattern during the electrical seizure. This pattern consisted of the cells firing in bursts; moreover the bursts were temporally correlated to specific components of the EEG recorded in the caudate or amygdala during the evoked seizure. This firing pattern was found in the majority of both dopamine and pars reticulata neurons of kindled animals, but was found uncommonly in SN neurons of naive animals. In kindled rats 88% of the SN pars reticulata and 88% of the SN dopamine neurons fired in bursts time-locked to the EEG waveform during seizures while in naive rats only 18% of the SN pars reticulata and none of the SN dopamine neurons fired in this pattern. The firing of neurons in bursts temporally correlated to components of the the EEG waveform in kindled animals is not a global phenomenon since only one of 30 cells recorded in the pontine tegmentum of kindled animals exhibited any clear change in firing pattern during seizure.

This change in firing pattern, to firing time-locked to specific components of the EEG, indicates that the SN is directly transmitting seizure activity. Although these results do not exclude the possibility that the SN exerts a tonic seizure facilitating action to promote seizure propagation, the observed change in firing pattern of nigral neurons during the seizure seems sufficient to account for its role in seizure propagation.

17.4 ALPHA$_2$, BUT NOT ALPHA$_1$, ADRENERGIC RECEPTOR ANTAGONISTS FACILITATE KINDLING DEVELOPMENT. J.A. Kallianos*, R.L. Gellman and J.O. McNamara (SPON: S. Schiffman). Depts. of Medicine (Neurology) and Pharmacology, Duke and VA Medical Centers, Durham, NC 27710.

Kindling, an animal model of epilepsy, is a phenomenon in which periodic administration of an initially subconvulsive stimulus eventually results in prolonged limbic and motor seizures. Selective depletion of forebrain norepinephrine (NE) markedly facilitates kindling development; this suggests that endogenous NE normally mediates an anticonvulsant action. The receptor subtype with which endogenous NE interacts to produce this anticonvulsant action is unknown. The anticonvulsant effects of an alpha$_2$ agonist against audiogenic and pentylenetetrazol (PTZ) seizures together with the proconvulsant effects of alpha$_2$ antagonists in the PTZ model led us to hypothesize that the anticonvulsant effects of NE in the kindling model are mediated through alpha$_2$ receptors. To test this hypothesis, we examined the effects of three different compounds on the rate of kindling development: yohimbine and idazoxan (RX 781094), two structurally dissimilar alpha$_2$ antagonists and corynanthine, a stereoisomer of yohimbine with selective alpha$_1$ antagonist properties.

Male Sprague Dawley rats implanted with a bipolar electrode in the right amygdala were pretreated daily with antagonist or vehicle and stimulated until 3 class 5 seizures were elicited.

TREATMENT	DOSE	# STIMS 3 CL 5 SZ (mean ± SE)	P value
Control		19.6 ± 1.6	
Yohimbine	1 mg/kg	9.5 ± 1.9	P<0.05
Idazoxan	1 mg/kg	7.8 ± 0.5	P<0.001
Corynanthine	1 mg/kg	18.0 ± 2.1	NS

The facilitatory effects of yohimbine and idazoxan were dose dependent over a range of 0.1 - 10 mg/kg and 0.05 - 10 mg/kg respectively. Corynanthine had no effect on kindling development at 0.1 mg/kg or 1 mg/kg. No difference was observed between drug and vehicle treated animals in terms of the behavioral or electroencephalographic patterns of seizures. The drugs did not produce any overt behavioral effects nor did the drugs alone produce seizures.

We propose that alpha$_2$ adrenergic receptors mediate the anticonvulsant action of NE since: 1) antagonists of these receptors facilitate kindling development; 2) the degree of facilitation is equivalent to that observed after elimination of NE. The proconvulsant effect of alpha$_2$ receptor antagonists seems paradoxical in view of the historical perspective that alpha$_2$ receptors are located on presynaptic terminals and that alpha$_2$ antagonism results in increased NE release. Hence, our results suggest that the alpha$_2$ receptors mediating the endogenous anticonvulsant effects of NE are located on targets of NE neurons, not on NE neurons themselves.

17.5 THE ROLE OF SUBSTANTIA NIGRA IN THE DEVELOPMENT OF KINDLING. C. Shin, J.M. Silver*, D.W. Bonhaus and J.O. McNamara. Depts. Med. (Neur.) and Pharm. VA and Duke Univ. Med. Ctrs. Durham, NC 27710.

Kindling, an animal model of temporal lobe epilepsy, refers to the phenomenon whereby repeated administration of an initially subconvulsive electrical stimulus eventually results in intense limbic and generalized motor seizures. Previous work from our laboratory has demonstrated that both intranigral injection of γ-vinyl-GABA (GVG), a GABA transaminase inhibitor, and lesioning of substantia nigra (SN) with the neurotoxin N-methyl-DL-Aspartate (NMDA) can block motor and limbic seizures in previously kindled rats. This led us to hypothesize that the same manipulations in SN would retard the development of kindling. We tested this hypothesis using both intranigral GVG and NMDA and thermocoagulative lesions of SN.

SN of male Sprague-Dawley rats were lesioned bilaterally by either microinjection of NMDA or thermocoagulation. Stimulating electrodes were stereotaxically implanted in the right amygdala. After 1 week, both lesioned and sham-operated control rats were stimulated 4 times a day at 100 μA above their afterdischarge threshold (ADT) until kindled (3 clonic motor seizures). Another group of animals was implanted with amygdala electrodes and guide cannulae overlying SN. After 1 week, GVG (5μg/side) was injected into the SN bilaterally. These rats and their respective controls were stimulated 7 times per day at 100 μA above their ADT until kindled. Nissl stained frozen sections were examined to determine electrode and cannula placements and extent of lesion.

Animals with SN lesioned by either method were found to kindle significantly faster than sham-operated controls: NMDA lesioned animals were kindled after 15.0 ± 1.7 (mean ± SEM) stimulations compared to 22.5 ± 2.2 for controls ($p<0.05$). Thermocoagulative lesioned animals were kindled after 13.5 ± 2.0 stimulations versus 22.2 ± 1.6 in the controls ($p<0.05$). Conversely, animals receiving GVG required 77% more stimulations (28.8 ± 3.8) to kindle than those receiving intranigral saline (16.3 ± 1.7) ($p<0.05$, 2-tailed Student's t test). GVG 2 mm dorsal to the SN did not delay kindling development (17.3 ± 2.0 stimulations).

Thus kindling development is retarded by intranigral GVG and facilitated in animals with lesions of SN. One explanation for these findings is that an intact SN is part of the network of kindling development and GVG, by presumably inhibiting SN output, retards kindling. However, in the absence of SN, kindling may take place through an alternate network. Kindling through this alternate network is apparently faster than that which exists in the unlesioned brain. Once kindling is established through an intact SN, the SN becomes an important structure in seizure propagation; thus do both SN lesions and intranigral GVG suppress kindled seizures.

17.6 METHOD OF BRAIN LESIONING DETERMINES LIKELIHOOD OF SEIZURES. J. M. Silver*, D.W. Bonhaus and J. O. McNamara. Departments of Medicine (Neurology) and Pharmacology, Duke University and VA Medical Centers, Durham, N.C. 27710.

Lesions of brain structures are used extensively to elucidate the anatomical substrates of brain function. Little is known, however, about the physiologic consequences of different lesioning techniques. Previous observations from our laboratory demonstrated that electrolytic lesions caused seizures. Since lesion-induced seizures are a serious confounding variable in our attempts to understand the functional anatomy of kindled seizures, we examined several lesioning techniques in order to find one that did not evoke seizures.

Brainstem structures were lesioned in male Sprague-Dawley rats using one of three different techniques. Lesions of similar size and location were obtained with the excitotoxin N-methyl-DL-aspartate (NMDA), electrolysis or thermocoagulation. All animals were monitored for electroencephalographic and behavioral seizures for a period of four to five hours following lesioning. Sham-operated animals served as controls.

Four of six animals receiving NMDA lesions and three of six animals receiving electrolytic lesions demonstrated seizure activity, whereas zero of six animals receiving thermocoagulative lesions did so. In a group of pooled controls, one of eighteen animals demonstrated seizure activity. Significant differences in the fraction of animals seizing were found between both the NMDA and electrolytic groups and controls ($p=.01$ and $p=.03$, respectively), but no significant difference was found between the thermocoagulative group and controls. The average NMDA treated animal had 4.8 seizures during the 4 hour monitoring period ($p<0.001$ compared to controls by Student's t-test) and the average electrolytic animal had 3.3 seizures ($p<0.02$ compared to controls). Again, thermocoagulative animals showed no seizures and these were not different from controls in average number of seizures.

These results demonstrate that of the three methods examined, thermocoagulation is least likely to produce seizures and is therefore the lesioning method of choice when lesion-induced seizures are a potential confounding variable. Recognition that seizures occur following brain lesions is crucial since seizures may alter brain function in a manner unrelated to the simple destruction of a specific anatomical structure.

17.7 Unilateral Chronic Foci in deep prepiriform cortex in Rat. G.G.Smith*, R.G. Fariello, G.T.Golden and P. F. Reyes. Neurology & Research, VAMC, Coatesville PA 19320 and Neurology & Psychiatry, Thomas Jeff. Med. College, Phil. PA 19107.

Recently a site deep in the forebrain of the rat has been identified from which generalized convulsions can be elicited by focal injection of a number of different epileptogenic agents including bicuculline, picrotoxin, carbacol, kainic acid and acetylcarnitine at doses many magnitudes lower than the convulsant threshold dose for other cerebral structures (Piredda, Lim & Gale, Life Sciences, 1985. 36 (13):1295).

In the present study we have examined the electrographic and behavioral results of unilateral placement of cobalt chloride ($CoCl_3$) or ferric chloride ($FeCl_3$) in deep prepiriform cortex in adult Long Evans hooded male rats. Placement of either $CoCl_3$ or $FeCl_3$ into left deep prepiriform cortex was accomplished by a micropipette and a nanoliter pump. $CoCl_3$ 25 µg to 150 µg or $FeCl_3$ 135 µg was injected over a period of 15 to 30 minutes. Coordinates for placement of foci were 4 mm anterior to bregma, 2.5 mm lateral to midline and 6.5 mm below the dura, with the incisor bar 5 mm above the interaural line. EEG recording electrodes were placed within 1 mm of the focus i.e. left prepiriform area, in the right mirror prepiriform area, in left and right frontal cortex and left and right parietal cortex. Animals had EEG activity recorded for 4 - 6 hours each day for over 80 days.

Early epileptiform activity consisted of interictal spikes in the right mirror prepiriform area. Several days later the EEG pattern consisted of electrographic seizures with onset from the focal area in left deep prepiriform area often with spread to the ipsilateral frontal and parietal cortex. Occasionally this spread to cortex was accompanied by mild behavioral manifestation of seizure activity e.g. facial myoclonus and or forepaw twitches. At no time were generalized electrographic or behavioral seizures observed.

Large injections of $CoCl_3$ produced extensive necrosis in prepiriform and adjacent areas. In $CoCl_3$ animals with large injections (150 µg) interictal and ictal discharges were very transient. $CoCl_3$ animals with small injections (25 µg) showed more consistent and longer lasting electrographic discharges. Interictal spikes and ictal electrographic discharges were observed up to 80 days post focus in some animals.

17.8 MPTP protects against picrotoxin induced seizures. M.DeMattei*, R.G. Fariello, G.T. Golden. (Spon: L.A. Marco). Neurology & Research VAMC, Coatesville PA 19320 and Thomas Jeff. Med. College, Phil. PA 19107

The substantia nigra has been implicated as the site of anticonvulsant activity mediated by GABA (Iadarola & Gale, Science, 1982,218:1237) and as a site crucial to the generation of seizures (McNamara et al., Epilepsia, 1983, 24:522). In addition, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine has been shown to produce nerve cell destruction in the substantia nigra (SN).

In the present study we have investigated the effect of destruction of SN cells, by the selective neurotoxin MPTP, on picrotoxin induced seizures. Albino male swiss mice were injected ip with either 30 mg/kg of MPTP or injection vehicle for ten days. On the eleventh day all mice were given sc injections of picrotoxin 7.0 mg/kg and observed and rated on behavioral seizure activity. A four point seizure scale was used, 0 indicating no seizure activity and a score of 4 indicating maximal seizure activity with tonic forelimb extension.

Ninety percent of the control vehicle injected mice experienced convulsions and death following a 7.0 mg/kg dose of picrotoxin whereas only 25% of the MPTP mice died following picrotoxin injection.

The mean latency to first seizure after picrotoxin injection was 8 min 32 sec for the control vehicle mice whereas MPTP treated mice had a mean latency of 16 min 32 sec MPTP treated mice had fewer seizures ($\bar{x}$ = 1.75), than control animals ($\bar{x}$ = 4.25). The mean seizure score for the control group was 14.8. The MPTP group had a mean seizure scores of 5.8. These results will be discussed with regards to the histopathological findings.

These preliminary results suggest that the substantia nigra is an essential area for picrotoxin induced seizures, and provides further evidence for the substantia nigra in GABA mediated seizure mechanisms.

17.9 **TRANSIENT EXPOSURE TO NOREPINEPHRINE INCREASES NEURONAL EXCITABILITY IN HIPPOCAMPAL AREA CA3.** D.M. Taylor*, A.C. Bragdon and W.A. Wilson. Depts. of Pharmacology and Medicine (Neurology), Duke University and VA Medical Centers, Durham, NC 27705.

Norepinephrine (NE) is recognized as having an anticonvulsant effect in a variety of in vivo and in vitro models of epilepsy. We observed that in addition to an acute inhibition of epileptiform activity in area CA_3 of the hippocampal slice, transient exposure to NE had the effect of increasing neuronal excitability in CA_3 following NE removal.

Male Sprague-Dawley rats, 270-380 g, were decapitated, and the hippocampi rapidly dissected into chilled (15 C), oxygenated ACSF. ACSF contained (mM): NaCl 120, $NaHCO_3$ 25, dextrose 10, KCl 3.3, NaH_2PO_4 1.23, $MgSO_4$ 1.2, and CaCl 1.8. 625 micron slices were cut perpendicular to the hippocampal long axis at the temporal end using a McIlwain chopper. Slices from temporal hippocampus were chosen (the second through fifth slices cut, starting at the temporal pole). After incubation for at least an hour, slices were placed in an immersion chamber with a continuous flow of warmed (32 C), oxygenated ACSF. Stimulating electrodes were placed in stratum radiatum of area CA_3, adjacent to the fimbria--extracellular recording electrodes in stratum pyramidale of CA_3b. Slices were stimulated every 100 seconds at the current intensity found to yield a maximum orthodromic population spike, in order to evaluate the slice response. After a given slice produced stable, healthy evoked potentials for 15 min., ACSF containing 100 uM NE was bath applied for 15 min.,followed by 15 minutes of washout. If the slice was not spontaneously bursting after this regimen, stimulus trains of a type previously shown to induce bursting (60 Hz, 0.01 ms duration, 2 s train; Stasheff et al., Brain Research, 1985, in press) were applied every 10 minutes until spontaneous bursting occurred. Control slices were treated identically, except that no NE was applied.

Evoked responses in the presence of NE were similar to those in control slices. Following NE washout, responses became more "epileptic"; a single stimulus resulted in multiple orthodromic population spikes. Following NE aplication and washout, 1.0 $\pm$0.3 SEM stimulus trains were required to obtain spontaneous population bursting in CA_3; in controls 2.3 $\pm$0.4 SEM were needed to reach the same criterion (significant @ $p<.025$, Student's t-test).

NE modulates population firing of CA_3 pyramidal cells. The excitatory component of this modulation is not seen during drug aplication, perhaps due to simultaneous activation of alpha and beta recptors. Thus NE acutely may be inhibitory, but the long term effect of NE is to increase neuronal activity in hippocampal area CA_3. This suggests that NE may modulate the development and expression of epilepsy in the whole animal.

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17.10 EFFECTS OF ANTICONVULSANT DRUGS ON SINGLE UNIT ACTIVITY OF SUBSTANTIA NIGRA (SN) PARS RETICULATA NEURONS. B.L. Waszczak, E.K. Lee* and J.R. Walters. Pharmacology Section, Northeastern Univ., Boston, MA 02115 and NINCDS, Bethesda, MD 20205.

Recent reports have suggested that enhancement of GABAergic transmission within the SN prevents the motor manifestations of both chemically-induced and kindled seizures. Thus, it has been proposed that inhibition of SN pars reticulata output function specifically suppresses the propagation of seizure activity to motor effector sites. The current studies were undertaken to evaluate the effects of a diverse group of anticonvulsant drugs on the firing rates of SN pars reticulata neurons, and to correlate these effects with the drugs' anticonvulsant profiles and/or presumed mechanisms of action.

Extracellular, single unit activity of pars reticulata neurons was monitored in male rats which were paralyzed and locally anesthetized. Drugs were administered i.v. at 2 min intervals over a range of logarithmically-increasing doses which included doses effective in suppressing experimentally-induced seizures in rats. Phenytoin (1.25-160 mg/kg; n=9) and carbamazepine (1.25-40 mg/kg; n=13) produced no changes in reticulata cell firing at any dose. Conversely, diazepam (31.25-8000 µg/kg; n=10) and clonazepam (2-500 µg/kg; n=10) consistently produced partial inhibitions of cell firing. Both drugs had similar efficacies, with diazepam producing an average maximal inhibition to 45.6$\pm$10.6% of the baseline rate, and clonazepam producing a maximal inhibition to 59.3$\pm$5.9% of baseline. Clonazepam, however, was approximately 16 times more potent in eliciting equivalent degrees of inhibition. Two other anticonvulsant drugs, phenobarbital (1.25-80 mg/kg; n=10) and valproic acid (5-640 mg/kg; n=9), also partially inhibited cell firing, but only at doses several time greater than those required to block seizure activity in experimental models. In fact, for phenobarbital doses up to 20 mg/kg produced slight increases in firing (ranging from about 10-25% above baseline), whereas only at the highest dose administered (80 mg/kg) was firing inhibited (to 64.9$\pm$9.3% of the baseline rate). Similarly, valproic acid caused no significant changes in firing until administration of the highest dose (640 mg/kg) whereupon firing was slowed to 69.5$\pm$5.8% of the baseline rate. Finally, ethosuximide (12.5-800 mg/kg; n=11) differed from all of the above drugs in its ability to markedly stimulate firing of pars reticulata neurons. The average change in firing was 27.2$\pm$7.7% over baseline after 50 mg/kg and increased to 92.0$\pm$29.7% over baseline after a dose of 200 mg/kg.

These results reveal that the effects of anticonvulsant drugs on the firing rates of SN pars reticulata neurons in normal rats does not correlate closely with either their clinical profiles or effectiveness in inhibiting seizures in various experimental models. However, the ability of some drugs to partially inhibit reticulata cell firing does correlate with the known or proposed ability of those drugs to potentiate inhibitory responses to GABA, a transmitter these cells are sensitive to and physiologically influenced by. (Supported by a Pharmaceut. Manufact. Ass. Grant and NIH-BRSG #S07 RR05830-04 to B.L. Waszczak.)

17.11 EFFECT OF NOREPINEPHRINE DEPLETION ON KINDLING-BASED STATUS EPILEPTICUS. D.C. McIntyre and N. Edson.* Department of Psychology, Carleton Univ., Ottawa, Ont., Canada K1S 5B6

The progressive development of kindled seizure activity, resulting from the low intensity stimulation of discrete forebrain sites, is suppressed by norepinephrine (NE). This suppression is inferred by the dramatic rapid genesis of kindling following NE depletion with the neurotoxin 6-hydroxydopamine (6-OHDA) (e.g., Arnold, et al., Exp. Neurol., 40: 457, 1973).

We have described previously a model of status epilepticus (SE), based on antecedent kindling of the amygdala, which results in convulsive behavior lasting 24 h or more and which is associated with extensive pathology in the pyriform cortex, its projections and CA_1 hippocampus (McIntyre et al., Brain Res., 250: 53, 1982). The SE develops from continuous stimulation of the kindled amygdala, for 60 min, with the original kindling stimulus. The sustained activity precipitated by this procedure consists largely of partial motor seizures with afterdischarges at 2-3 Hz.

In the present experiments, we examined NE involvement in SE development by continuous stimulation of the amygdala in NE-depleted or control rats having either (a) no previous kindling, (b) one kindled amygdala, or (c) two kindled amygdalae. The NE depletions were achieved by bilateral intraventricular 6-OHDA infusions (125 µg x2) three weeks before kindling or stimulation per se.

Good depletion of NE was observed in all the 6-OHDA pretreated rats compared to controls. As previously reported, NE depletion resulted in a significant facilitation in the rate of kindling, prior to the SE treatment. In none of the SE experiments, however, did NE depletion affect the probability of developing SE, its severity, duration or the extent of associated brain pathology compared to controls. Clearly NE has little obvious involvement in the etiology or offset of kindling-based SE from an amygdala focus.

OTHER BIOGENIC AMINES I

18.1 SEROTONIN MIGRATION IN BRAIN CELL MICROENVIRONMENT. M. E. Rice* and C. Nicholson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Serotonin (5-HT) is implicated in a vast range of neuronal functions ranging from control of neuronal development to modulation of brain arousal. Some of these functions are thought to be mediated by local release of serotonin into the brain cell microenvironment. To understand such functional modes it is essential to clarify how serotonin migrates when released into the extracellular space. Using quantitative methods based on iontophoresis and ion-selective microelectrodes (ISMs) sensitive to serotonin and electrochemical microsensors (ECMs) we showed that this indolamine can migrate readily within the extracellular space, but that its behavior deviates from that associated with small non-neuroactive cations.

Methods were based on those described by Nicholson & Phillips (J. Physiol. 321:225, 1981) in the rat cerebellum and consisted of the iontophoretic release of serotonin from a micropipette in the isolated turtle cerebellum and subsequent monitoring of extracellular concentrations of serotonin at distances of 75-250 µm using ISMs and ECMs. Diffusion properties were analyzed using an on-line microcomputer. Tetramethylammonium (TMA) was used as a control cation in a similar experimental paradigm with ISMs and all measurements were compared to diffusion in an adjacent agar substrate.

Measurements with TMA indicated that this ion diffused normally in the turtle cerebellum and revealed volume fraction and tortuosity values similar to those reported in the rat cerebellum. In contrast, serotonin showed small but consistent deviations from ideal diffusion: during iontophoresis $[5\text{-}HT^+]_o$ increased more slowly than TMA but at the cessation of the pulse $[5\text{-}HT^+]_o$ decayed more rapidly.

These results may indicate uptake of serotonin from the extracellular space, despite the fact that classical serotonin uptake mechanisms (Km <10 µM) (Shaskan & Snyder: J. Pharmacol. Exp. Therap., 175:404, 1970) are likely to be saturated at the extracellular concentrations achieved in our experiments (0.02 - 2mM at the ISM). Moreover, the cerebellum has only a sparse serotonergic afferent system. The putative uptake revealed by our studies is thus likely to be relatively non-specific. [Supported by USPHS Grants NS-13742 and NS-07745 (MER).]

18.2 TWO POPULATIONS OF SEROTONERGIC AXONS IN CAT CEREBRAL CORTEX. K.A. Mulligan* and I. Tork* (SPON: P. Huttenlocher). School of Anatomy, University of New South Wales, Kensington, Sydney, Australia

Recent studies of the serotonergic (5-HT) innervation of the mammalian cortex reveal regional and laminar variations in the density of 5-HT axons. However, little information is available on the morphology of cortical 5-HT axons in any species. In the present study, quantitative analysis and serial reconstructions at the light microscopic level provide the first detailed characterization of 5-HT axons in the cat cerebral cortex.

Three types of 5-HT axon segments can be distinguished in immunohistochemical preparations of cat visual cortex (area 17) on the basis of the presence and nature of varicosities.

(1) Axons with small, fusiform varicosities (< 1 um in diameter) constitute the largest population (90%) of cortical 5-HT fibres. They are distributed throughout all layers, but are most abundant in layer I. They branch frequently, giving rise to daughter fibres of similar morphology, and end as single terminal boutons in the upper layers of cortex.

(2) Non-varicose axons (7% of the cortical 5-HT fibres) are usually about 1 um in diameter and course parallel to the pial surface in layers I, V, VI and the white matter. They branch infrequently.

(3) Fine axons bearing large (one or more microns in diameter), round varicosities constitute only 3% of the population and are located predominantly in layer I, where they form dense, basket-like arrays around single neurons. In Nissl-counterstained preparations the varicosities of these axons contact somata and appear to extend along the unstained dendrites of the neurons.

It was found in partial serial reconstructions that the fine, basket-forming fibres (3) arise as branches of non-varicose 5-HT axons (2), and that a single non-varicose axon can give rise to fibres forming several baskets. 5-HT axons with small, fusiform varicosities (1) do not appear to be related to the latter two types of 5-HT axon. Hence, it appears that the 5-HT innervation of the cat cortex is organized into two parallel subsystems. One system, distributed widely through the cortex, is composed of fibres with small varicosities and may play a role in the diffuse function normally associated with the 5-HT system. The second system, represented by the non-varicose axons and their basket-forming branches, has a more restricted distribution and may exert a strong influence over a particular population of cortical neurons.

18.3 A COMPARISON OF BIOCHEMICAL INDICES OF 5-HYDROXYTRYPTAMINERGIC NEURONAL ACTIVITY FOLLOWING ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS. N.J. Shannon , J.W. Gunnet* and K.E. Moore. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

The activity of 5-hydroxytryptaminergic (5HT) neurons has been estimated from measurements of: 1) concentrations of 5-hydroxyindoleacetic acid (5HIAA), 2) 5HIAA/5HT concentration ratios, 3) rate of accumulation of 5-hydroxytryptophan (5HTP) following the administration of an aromatic L-amino acid decarboxylase inhibitor such as NSD 1015 and 4) rate of accumulation of 5HT and decline of 5HIAA after administration of a monoamine oxidase inhibitor such as pargyline. There has been no systematic comparison of these techniques under conditions of elevated 5HT neuronal activity, although electrical stimulation of 5HT neurons has been reported to increase 5HIAA concentrations (Aghajanian et al., 1967) and 5HTP accumulation following NSD 1015 (Duda and Moore, 1985). The purpose of the present study was to compare these different methods with changes in neuronal impulse traffic produced by electrical stimulation of these neurons and, to determine which of these methods is the most appropriate for use in selected brain and pituitary regions.

Male Long-Evans rats anesthetized with chloral hydrate (400 mg/kg, i.p.) were sacrificed following 0, 15 or 30 minutes of electrical stimulation of the dorsal raphe nucleus (monophasic pulses; 0.3 mA; 1 msec duration; 0, 5 or 10 Hz). Concurrent with the onset of stimulation, the appropriate groups of animals received either saline (1 ml/kg, i.v.), a supramaximal dose of NSD 1015 (25 mg/kg, i.v.) or a supramaximal dose of pargyline (30 mg/kg, i.v.). HPLC with electrochemical detection was used to quantify concentrations of 5HTP, 5HT and 5HIAA in selected brain regions (e.g., amygdala, nucleus accumbens, suprachiasmatic nucleus). Because the content of 5HIAA in the intermediate lobe of the pituitary gland was below the sensitivity of the assay (30 pg), only the 5HTP accumulation method could be applied to this region. In each brain region, electrical stimulation elicited an increase in the concentration of 5HIAA and the 5HIAA/5HT concentration ratio in saline-treated animals and an increase in 5HTP accumulation in NSD 1015-treated rats. The magnitude of response to stimulation varied among these methods. In the amygdala, for example, 30 minutes of stimulation at 5 Hz produced increases in 5HIAA, 5HIAA/5HT and 5HTP concentrations that were 125%, 150% and 170% of sham-stimulated (0 Hz) values, respectively. However, in pargyline-treated animals, electrical stimulation failed to alter the concentrations of 5HT or 5HIAA. The results of this study indicate that while the concentrations of 5HIAA, 5HIAA/5HT and 5HTP each increase in response to accelerations in 5HT nerve firing, and thus serve as valid biochemical indices of 5HT neuronal activity, the sensitivities of the various methods and their applicability to specific brain and pituitary regions differ. In contrast, increases in 5HT and decreases in 5HIAA concentrations following administration of pargyline were not significantly altered following stimulation of 5HT neurons. (Supported by USPHS grant NS15911.)

18.4 EFFECTS OF TRYPTOPHAN ADMINISTRATION ON THE SYNTHESIS, STORAGE AND METABOLISM OF 5-HYDROXYTRYPTAMINE IN THE HYPOTHALAMUS AND THE POSTERIOR PITUITARY GLAND OF THE MALE RAT. K.J. Lookingland*, N.J. Shannon, D.S. Chapin and K.E. Moore. Dept. of Pharmacology/Toxicology, Michigan State Univ., East Lansing, MI 48824.

The effects of tryptophan administration on neurochemical estimates of synthesis (5HTP accumulation following NSD 1015), storage (5HT concentrations) and metabolism (5HIAA concentrations) of 5HT in selected regions of the hypothalamus and in the intermediate and neural lobes of the pituitary gland were determined using HPLC coupled to an electrochemical detector. Tryptophan (30-300 mg/kg, i.p.) produced a dose-dependent increase in 5HTP accumulation throughout the hypothalamus and in the intermediate but not the neural lobe of the pituitary. Peak 5HTP levels were attained by 30 min following tryptophan administration (100 mg/kg) and were maintained for an additional 60 min. Tryptophan also produced concomitant dose-dependent increases in 5HT and 5HIAA concentrations in these same regions without change in the ratio of 5HIAA/5HT. These results indicate that exogenous tryptophan administration increases the synthesis, storage and metabolism of 5HT in the hypothalamus and intermediate lobe of the pituitary. Since an increase in release of 5HT is typically accompanied by an increase in the ratio of 5HIAA/5HT, the lack of change in this ratio following tryptophan suggests that this precursor does not alter 5HT release.

5HIAA measured in brain tissue for the most part represents a combination of 1) newly synthesized 5HT that is directly metabolized within the neuron, and 2) 5HT that has been released, recaptured and subsequently metabolized by intraneuronal MAO. To determine the effects of precursor loading on the metabolism of newly synthesized 5HT, the effects of chlorimipramine (5 mg/kg, s.c.), an inhibitor of 5HT uptake, on 5HIAA concentrations were examined in control and tryptophan-treated rats. Chlorimipramine caused only a modest (10-35%) reduction in 5HIAA concentrations throughout the hypothalamus of control and tryptophan-treated animals indicating that only a minor portion of 5HIAA is derived from released 5HIAA while the major portion of this metabolite may reflect direct intraneuronal metabolism of unreleased 5HT. In chlorimipramine-treated rats 5HIAA concentrations were significantly increased by tryptophan administration suggesting that the increase in synthesis of 5HT following precursor loading is accompanied by an increase in the intraneuronal metabolism of 5HT. (Supported by USPHS grant NS15911).

18.5 5-HYDROXYTRYPTAMINE SYNTHESIS IN DISCRETE RAT BRAIN NUCLEI IS AFFECTED BY 5-HYDROXYTRYPTAMINE AGONISTS AND ANTAGONISTS. J.A. Nielsen. Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

A procedure for studying 5-hydroxytryptamine (5-HT) synthesis by determining the rate of accumulation of 5-hydroxytryptophan (5-HTP) after administering m-hydroxybenzylhydrazine (NSD 1015), an inhibitor of aromatic-L-amino acid decarboxylase (LAAD), and large doses of L-tryptophan (L-trp) was characterized. The utility of this method as an index of 5-HT neuronal activity was studied by determining the effects on 5-HTP accumulation after administering 5-HT agonists (viz., quipazine, chlorimipramine and fenfluramine) and 5-HT antagonists (viz., metergoline, pirenperone and pizotifen). The effects of these drugs on the accumulation of the dopamine (DA) precursor dihydroxyphenylalanine (DOPA) and L-trp were also studied.

The in vivo rate of 5-HT and DA synthesis was determined in the nuclei caudatus putamen, accumbens, amygdaloideus centralis and septi lateralis of male rats (175-300 g) by measuring the concentration of 5-HTP and DOPA after the administration of NSD 1015 (100 mg/kg, i.p.). 5-HTP, DOPA and L-trp were analyzed by high pressure liquid chromatography coupled to an electrochemical detector.

In the absence of NSD 1015 pretreatment, 5-HTP and DOPA were not readily detectable in any brain region studied. However, both of these neurochemicals accumulated after NSD 1015 treatment in a time-dependent manner. Administration of L-trp 60 minutes before sacrifice increased 5-HTP, but not DOPA, in a dose-related manner. Chlorimipramine, fenfluramine and quipazine all decreased 5-HTP, but had no effect upon DOPA in NSD 1015 and L-trp-treated animals. Chlorimipramine produced these effects in a dose-related manner only after L-trp loading and without affecting brain concentrations of L-trp. The three 5-HT antagonists each had a unique effect. Pizotifen increased 5-HTP in a dose-related and time-dependent manner, but did not alter DOPA or L-trp. Metergoline increased 5HTP and DOPA, and decreased L-trp in a dose- and time-dependent manner. Pirenperone increased 5-HTP and DOPA, and had no effect on L-trp. These effects were also time- and dose-related.

These results suggest that: 1) the measurement of 5-HTP after L-trp administration and LAAD inhibition might serve as a useful index of 5-HT synthesis; 2) three 5-HT agonists all decreased 5-HTP, but neither DOPA accumulation nor L-trp concentrations; and 3) three 5-HT antagonists affected 5-HTP, DOPA and/or L-trp. (This work was supported in part by USPHS Grant NS 15911).

18.6 IMMUNOCYTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF THE BULBOSPINAL SEROTONINERGIC PATHWAY AFTER NEONATAL 5,7-DHT LESION. EFFECTS OF MONOSIALOGANGLIOSIDE GM_1. G. Vantini*, M. Fusco*, B. Figliomeni*, R. Zanoni*, H. Hallman*[1], G. Jonsson [1] and A. Gorio (SPON: A. Cangiano). Fidia Research Laboratories, 35031 Abano Terme, (PD), Italy and [1]Dept. of Histology, Karolinska Institutet, Stockholm, Sweden.

The potential role of the monosialoganglioside GM_1 in promoting regeneration and restoration of functions following lesions to the nervous system has derived recent impetus from positive results obtained in many laboratories. In this study, the effect of GM1 on the alterations of the bulbospinal serotoninergic system induced by 5,7-dihydroxytryptamine (5,7-DHT) was investigated. 5,7-DHT (50 mg free base/Kg s.c.) was administered within 4-8 h after birth, thereafter the rat pups received four injections of saline or GM_1 (30 mg/Kg s.c.) daily from postnatal day 1 to 4. All the analysis were performed in two month old rats. Neonatal administration of 5,7-DHT caused a marked decrease of 5-HT and 5-HIAA contents in the thoracic and lumbar spinal cord (-40%), conversely, in the medulla-pons, 5-HT and 5-HIAA were increased (+30%; "pruning effect"). These alterations detected by HPLC-ED were reflected by decreases and increases respectively of the number of 5-HT nerve terminals as demonstrated by quantitative immunocytochemical analysis using antibodies against 5-HT. Moreover, in thoracic and lumbar cord sections of lesioned animals 5-HT fibers showed swollen varicosities and their general organization seemed disrupted. Neonatal administration of GM1 did not have significant effects on 5-HT neurons. However, GM_1 showed a pronounced counteracting effect on the alterations induced by 5,7-DHT. In thoracic and lumbar segments, the levels of 5-HT and 5-HIAA were almost normal as if regeneration of 5-HT fibers was stimulated by GM_1 treatment. Preliminary data indicate that 5,7-DHT lesion produce apparent changes in binding parameters of the high affinity component of $5\text{-}HT_1$ binding sites in the lumbar spinal cord. GM1 administration was still effective in reducing such alterations. These data indicate that GM_1 may have normalizing and/or regenerative effects and further support the hypothesis that GM_1 may play a role in CNS repair processes after injury.

18.7 IMMUNOCYTOCHEMICAL ANALYSIS OF SEROTONINERGIC REINNERVATION IN THE VENTRAL HORN OF THE RAT SPINAL CORD, AFTER NEONATAL 5,7-DIHYDROXYTRYPTAMINE LESIONING. EFFECT OF GM_1 GANGLIOSIDE TREATMENT. M.G. Nunzi, D. Guidolin*, P. Polato*, M. Fusco* and A. Gorio. Lab. Of Neurobiology, Fidia Research Labs., 35031 Abano Terme, Italy.

The capability of GM_1 monosialoganglioside to enhance regenerative growth of the bulbospinal serotoninergic system was evaluated, at various times after birth, following neonatally degeneration induced by 5,7-dihydroxytryptamine (5,7-DHT, 50 mg/kg s.c.). In particular, morphometric changes in serotonin (5-HT)-positive varicosities were quantified, via computerized image analysis, in the laminae VIII and IX of the spinal cord, following immunocytochemical staining with monoclonal antibody to 5-HT and PAP tecnique. The mean areas of 5-HT varicosities in non-lesioned control and 5,7-DHT lesioned animals, either treated with saline or GM_1 (30 mg/kg s.c. from postnatal day 1 to 4) are reported in the Table. Results indicate that the post-natal development, from day 17 to day 60, of 5-HT fibers is characterized by a 60% increase in the size of the 5HT varicosities. However, in the animals injected with 5,7-DHT no varicosities were detectable at postnatal day 17. In addition, at longer post-natal times, these animals displayed a statistically significant reduction in the size of the 5-HT varicosities. Treatment with GM_1 did not interfere with the primary degenerative action of 5,7-DHT. However, at postnatal day 30 and 60, a significant increase in the size of 5-HT varicosities occurred in GM_1 treated animals.

Electron microscopy analysis of the 5-HT varicosities, in adult rats, indicated that the above varicosities were mainly presynaptic terminals associated with motoneurons. As such, the decreased size of 5-HT varicosities observed in the lesioned saline-treated animals may represent a structural immaturity of the regrowing 5-HT-fibers. This immaturity is not present in 60 day old animals treated with GM_1, thereby indicating a facilitatory effect of GM_1 treatment on synaptic maturation of the regrowing 5HT axons.

	CONTROL	LESIONED	TREATED
17 days	1.20 ± .04 μm^2	------	------
30 days	1.24 ± .04 μm^2	.90 ± .03 μm^2	1.11 ± .04 μm^2
60 days	1.87 ± .08 μm^2	1.20 ± .05 μm^2	1.80 ± .04 μm^2

18.8 CHARACTERIZATION OF [3H]KETANSERIN (SEROTONIN-2) BINDING SITES IN CHICK EMBRYO BRAIN. J.S. Soblosky, G.D. DuMontier* and I. Jeng. Neurochemistry Unit, Missouri Institute of Psychiatry and Department of Biochemistry, University of Missouri-Columbia, School of Medicine, St. Louis, MO 63139.

Utilizing [3H]ketanserin and 2 µM cinanserin to define nonspecific binding, saturable, specific high affinity binding sites were detected in the chick embryo brain. When measured at three different times (days of incubation) during embryonic development the binding sites were found to develop linearally: 13 days: B_{max} = 1.57 pmol/g., K_D = 3.7 nM; 16 days: B_{max} = 2.30 pmol/g, K_D = 4.4 nM; 20 days: B_{max} = 4.50 pmol/g, K_D = 5.2 nM. In the "20 day old" chick embryo, regional investigations revealed the following proportions in the number of specific binding sites - cerebellum:optic lobes:cerebral cortex:remainder = 1:2:2.4:3.

The specificity of various displacing ligands, in terms of IC_{50} values, suggested that [3H]ketanserin binding sites in the chick embryo brain may be similar, but not identical, to the serotonin-2 receptors reported in the rat frontal cortex (Leysen et al. (1982) Mol. Pharm. 21: 301). IC_{50} values: ketanserin = .9 nM, spiperone=.2 nM, pizotifen (BC-105) = 30 nM, cinanserin = 100 nM, methysergide = 100 nM, 5-HT = 1 µM.

When compared to our previous results regarding [3H]5-HT binding site development (Soblosky & Jeng (1985) J. Neurochem. 44: 544), the present results suggest that there are also serotonin-2 binding sites in the chick embryo brain, which are present in a smaller number and appear later in embryonic development than the [3H]5-HT binding sites.

18.9 **INNERVATION OF EMBRYONIC CEREBRAL CORTEX BY SEROTONERGIC NEURONS IN VITRO.** A. R. Aitken* and I. Törk* (SPON: C. Straznicky), School of Anatomy, University of New South Wales, Kensington 2033, Sydney, Australia.

The serotonergic system is one of the earliest developing neurotransmitter systems in the mammalian brain. The axons of brainstem serotonergic neurons reach the cerebral cortex a few days after the establishment of neurotransmitter identity. In the present study some of the guidance mechanisms by which serotonergic fibres find their way to cerebral cortex were examined.

Explants of embryonic serotonergic neurons from the dorsal raphe were cultured separately and co-cultured with embryonic cerebral cortex on a substratum of poly-L-lysine. The interactions of serotonergic neurons with cerebral cortex were observed using early to late embryonic material (E12-19) that was maintained for 7 days in vitro. Serotonergic neurons were identified using a monoclonal antibody against 5-hydroxytryptamine in conjunction with the avidin-biotin peroxidase procedure.

Serotonin immunoreactive fibres displayed spontaneous radial outgrowth from explants of dorsal raphe, forming a vast anastomotic plexus of varicose fibres in the explant and within the outgrowth zone. The outgrowth of serotonergic fibres was accompanied by a concomitant migration of non-immunoreactive neurons and glia from the perimeter of the explant which formed a carpet of cells beneath the serotonergic fibres. Within the outgrowth zone serotonergic fibres maintained preferential contact with these migrating cells avoiding direct contact with the culture substratum. Finally, many non-immunoreactive cells within the outgrowth zone appeared surrounded by a dense plexus of serotonergic fibres and varicosities.

When embryonic dorsal raphe and cerebral cortical explants were co-cultured the outgrowing serotonergic fibres did not show preferential growth towards the cortical explant. Instead, they continued their radial outgrowth observed in single cultures of dorsal raphe, growing in all directions including into the cortical explant. After reaching explants of embryonic cerebral cortex, serotonergic fibres were observed to colonize the surface of the explant before growing further inwards. No special enhancement of serotonergic fibre proliferation was noticed after contact with the cortical explant.

These observations indicate that embryonic serotonergic neurons possess an intrinsic capacity for spontaneous neurite extension and elongation. This urge for outgrowth is not influenced by soluble target factors produced by cerebral cortex. In contrast the evidence suggests that the guidance of serotonergic fibres depends on local recognition of specific membrane constituents apposing the serotonergic growth cone.

18.10 EVIDENCE FOR A 5-HT INVOLVEMENT IN VASOPRESSIN RELEASE FROM NEUROHYPOPHYSIS OF RATS. L. Steardo*[1,2,3] M. Iovino*[1], E. Hunnicutt[3], Dept. Neurology, 2nd Medical School, Naples, Italy[1], Dept. of Neurology, Harvard Medical School[2], and Dept. of Neurology, Mass. General Hospital, Boston, MA 02114[3]

There is evidence that serotonin (5-HT) may play a role in the mechanisms controlling thirst and water balance. In fact electrolytic lesions of the dorsal raphe nucleus (DR), in rats an important site of 5-HT neuronal cell bodies in the brain, produce significant increases of daily urine output and water intake, whereas electrical stimulation of DR results in a diminished diuresis. The present experiments were designed to pharmacologically investigate whether brain 5-HT participates in the regulation of vasopressin (ADH) secretion. For this purpose plasma ADH levels were measured in rats treated with drugs enhancing 5-HT transmission, such as d-fenfluramine and quipazine, and with 5-HT depleting drugs, p-chlorophenylalanine (PCPA) and 5,7-dihydroxytryptamine (5,7-DHT). Forebrain 5-HT, noradrenaline (NA) and dopamine (DA) were also measured.

1. In a first group of rats, plasma ADH concentrations were measured after quipazine maleate (5,10 or 20 mg/kg) or d-fenfluramine hydrochloride (5,10 or 20 mg/kg) administration. Controls received saline injections alone.
2. In a second group of animals, plasma ADH concentrations were determined 60 min after quipazine (10 mg/kg) or 30 min after d-fenfluramine (10 mg/kg) administration in rats pretreated with PCPA (300 mg/kg), or saline.
3. In a third group of rats, plasma ADH concentration and forebrain catecholamines and 5-HT were determined 14 days after i.c.v. administration of 150 ug of 5,7-DHT. Controls received desipramine (25 mg/kg i.p.) and an equal volume of vehicle i.c.v.
4. In a fourth group of rats, plasma ADH levels, forebrain catecholamines and 5-HT were measured 60 and 40 min after PCPA (300 mg/kg) administration. Controls received saline. The rats of the third and fourth groups were also water-deprived 24 h before sacrifice to induce ADH release.

Quipazine and d-fenfluramine induced dose-related increases in plasma ADH levels in normally hydrated rats. The stimulatory effect of both quipazine (10 mg/kg i.p.) and d-fenfluramine (10 mg/kg i.p.) on ADH release was completely prevented in normally hydrated rats by pretreatment with PCPA. In water-deprived rats receiving PCPA or 5,7-DHT, 5-HT forebrain levels decreased while NA and DA were unchanged. Both these treatments blocked the elevation of plasma ADH normally induced by water deprivation. The results summarized imply that serotonin is a part of the apparatus for the physiological release of ADH from neurohypophysis.

18.11 SEROTONERGIC DORSAL RAPHE NEURONS: REDUCED SENSITIVITY TO AMPHETAMINE WITH LONG-TERM TREATMENT. B.A. Heidenreich*, A.E. Basse and G.V. Rebec (SPON:R.M. Wightman). Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Dopamine (DA)-containing neurons in the substantia nigra and ventral tegmental area respond to amphetamine with a dose-dependent inhibition of firing rate. This response is attenuated during long-term treatment. In fact, multiple amphetamine injections produce a clear shift to the right of the inhibitory dose-response curve and, in some cases, shift the neuronal response to an excitation. This change in responsiveness, which appears to be mediated in part by a subsensitivity of DA autoreceptors, has been implicated in the behavioral alterations associated with chronic amphetamine treatment (Rebec, G.V., Monogr. Neural Sci.,10:207, 1984). Serotonin (5-HT) also has been implicated in these behavioral changes although relatively little information is available concerning the effects of this drug on the activity of 5-HT neurons. In the present series of experiments, therefore, we characterized the actions of both acute and long-term amphetamine administration on the firing rate of 5-HT neurons in the dorsal raphe nucleus (DRN).

Adult, male rats received twice daily injections of saline, 1.0 or 5.0 mg/kg d-amphetamine sulfate for 6 consecutive days. On the following day, the animals were prepared for single-unit recording and 5-HT neurons were identified according to previously described electrophysiological and histological criteria (e.g., Wang, R.Y. & Aghajanian, G.K., Brain Res.,132:186, 1977). Following the isolation of single-unit discharges, d-amphetamine was administered intravenously in increasing incremental doses beginning with 0.125 mg/kg until unit activity was inhibited by more than 50%. In control rats, this degree of inhibition was achieved with a mean cumulative dose of 1.6 mg/kg. Rats pretreated with amphetamine, on the other hand, were progressively less responsive. In fact, the mean cumulative dose required to suppress DRN activity in rats pretreated with the high dose of amphetamine was 3.0 mg/kg. These results indicate that, like midbrain DA cells, 5-HT neurons in the DRN lose their sensitivity to amphetamine with long-term treatment. To the extent that this drug facilitates the local release of 5-HT and that this effect is responsible for the amphetamine-induced inhibition in the DRN, the decreased responsiveness to amphetamine may reflect, at least in part, a reduction in the sensitivity of 5-HT autoreceptors. This effect would have important implications for understanding the role of 5-HT in the behavioral alterations produced by long-term amphetamine treatment.

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18.12 THE CENTRAL ACTIVITY OF TR2515 A QUINAZOLINEDIONE DERIVATIVE. *Barragán,LA.;*Galindo-Morales, J.A.;and *Hong,E.-(Spon:*Pacheco Carrasco, M.).- Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Apdo.Postal No.199, Colima,Col.,México;Depto de Farmacología,Fac.de Medicina,U.N.A.M.,México; Unidad de Terapéutica Experimental, Depto.de Farmacología y Toxicología, CINVESTAV, I.P.N., México.

It was previously reported (Barragán, etal., Proc.West.Pharmacol. Soc.27:329,1984) that the 2,4(1H,3H) quinazolinedione, 3-3-4(phenyl -1-piperazinyl)-propyl hidrochloride (TR2515) was highly effective in antagonizing the so called serotonin (5-HT) behavioural syndrome. Sveral lines of evidence had relate this 5HT syndrome with the specific activation of S_2 subtype of 5HT receptors (Peroutka et al, Science 212:827,1981). With the aim to evaluate the antagonist activity of TR2515 at different functional levels, experiments were undertaken on well established 5-HT agonist effects in several parallel series: 1)Behavioural open field observations of rats under harmaline (20mg/Kg) or quipazine (20 mg/Kg) effects and its interaction with methysergide (0.5 mg/Kg) or TR2515 (0.1mg/Kg); 2)Rat EEG analysis of the wake sleep cycle under same experimental conditions was carried out; and 3) At the neuronal cell level by means of microiontophoretic application of 5HT, quipazine, or harmaline as agonists and their interactions with methysergide and TR2515. Sites explored for unit neuronal activity were: the 5HT containing neurons of the dorsal raphe nucleus (DRN), 5HT target cells of the cerebral cortex, and the medial and dorsal subnucleai of the inferior olive (dION, mION) of the rat. We followed conventional techniques for each manipulation serie and animal care as established.(NIH Guide for Grants and Contracts, Vol.7,No.17,Nov.1978, App.1). The results confirm the antagonism exerted by TR2515 to the behavioural effects induced by quipazine and harmaline, in addition, this drug led to a significant diminution of the spontaneous activity not presented with methysergide. The sleep suppressive effects of quipazine and harmaline were prevented by TR2515 being of a higher effectiveness on the quipazine interaction although REM sleep was delayed on its onset, total REM time was not modified. DRN total inhibition of the cell firing induced by 5-HT (20nA) and quipazine (20nA) was prevented and reverted by continuously ejection of TR2515 (5nA). dION and mION cell rhythmic activation by harmaline and quipazine were also antagonized by TR 2515. This drug effectively antagonized cortical cell excitation elicited by 5-HT or quipazine, however, it was ineffective over inhibitory cell responses. In contrast, methysergide exerted its antagonism in both cell response types, but inconsistently. This results led us to assume that the quinazolinedione derivative antagonizes 5HT by a possible specific interaction with the S_2 5HT receptor subtype.

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18.13 **SIMILARITIES IN THE STIMULUS EFFECTS OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OHDPAT), BUSPIRONE, AND TVX Q 7821: IMPLICATIONS FOR UNDERSTANDING THE ACTIONS OF NOVEL ANXIOLYTICS?** K. A. CUNNINGHAM, P. M. CALLAHAN and J. B. APPEL. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.

The "partial ergoline" 8-OHDPAT has effects characteristic of a serotonin agonist: that is, it decreases serotonin turnover, produces the "serotonin syndrome" and is a potent inhibitor of serotonin-1, especially serotonin-1A, binding. In contrast to other serotonin agonists, however, 8-OHDPAT releases punished responding, stimulates male sexual behavior and, in animals trained to discriminate either d-lysergic acid diethylamide (LSD), trifluoromethylphenylpiperazine (TFMPP) or MK 212 from saline, elicits saline-lever responding. To further clarify the mechanism(s) of action of 8-OHDPAT, rats (N=24) were trained to discriminate this agent (0.4 mg/kg, i.p.) from saline and were given various neuroactive compounds during substitution (generalization) test sessions. Of the serotonin agonists tested, neither LSD (0.08-0.24 mg/kg), 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (0.5-4.0 mg/kg), MK 212 (0.25, 0.5 mg/kg), quipazine (0.5-2.0 mg/kg), Ru 24969 (0.5, 1.0 mg/kg) nor TFMPP (0.25-1.0 mg/kg) substituted for the training drug; the dopamine agonist apomorphine (0.0625-0.25 mg/kg) and the alpha-2 noradrenergic agonist clonidine (0.2, 0.4 mg/kg) also engendered saline-lever responding. However, the atypical anxiolytics buspirone (0.5-4.0 mg/kg) and TVX Q 7821 (1.0-4.0 mg/kg) completely mimicked the 8-OHDPAT cue. In combination tests, the serotonin antagonists metergoline (0.5-2.0 mg/kg), methiothepin (0.125-0.5 mg/kg) and pirenperone (0.02-0.08 mg/kg) as well as the dopamine antagonist haloperidol (0.25 mg/kg) failed to attenuate responding on the drug-appropriate lever.

Since the similar stimulus properties of 8-OHDPAT, buspirone and TVX Q 7821 are mirrored by the common abilities of these agents to potently inhibit serotonin-1 but not serotonin-2 binding, to produce the serotonin syndrome and to release punished responding, modulation of serotonin-1 (serotonin-1A?) neuronal systems may account for the similar behavioral effects of novel anxiolytics. However, such a conclusion must be tempered by the lack of sufficient antagonism data and the fact that possible interactions of all of these agents with other neuronal systems have not yet been investigated extensively.

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18.14 SEROTONIN AUTORECEPTORS: BIOCHEMICAL CHARACTERIZATION AND EFFECTS OF ANTIDEPRESSANTS, M.P. Galloway, and technical assistance from E.A. Novak* & R.A. Lodhi*, Neurochemical Pharmacology Research Unit, Lafayette Clinic, and Dept. of Psychiatry, Wayne State University, Detroit, MI 48207

To understand those factors that influence presynaptic activity of serotonin (5HT)-containing neurons, the neurochemical pharmacology of 5HT synthesis and metabolism has been studied in the rat CNS by determining the acute effects of 5HT agonists,antagonists, and uptake blockers on 5HTP accumulation and 5HIAA levels in different 5HT terminal fields. To measure the effect of agonists on 5HTP synthesis, animals were treated with the decarboxylase inhibitor NSD-1015 and different doses of 8-OHDPAT (8-hydroxy-2-(di-n-propylamino)tetralin), MDMT (5-methoxy-dimethyl-tryptamine), or TFPP (1-(m-trifluoromethyl-phenyl) piperazine) and levels of 5HTP, 5HT, and 5HIAA then determined by HPLC-EC. It was found that each agonist produced a dose-dependent (10-1000 ug/kg) decrease in 5HTP levels in the striatum, hippocampus, brain stem, medial prefrontal, piriform, and temporal cortices. In terms of regional responses to the synthesis-suppressant effect of 8-OHDPAT (100 ug/kg), the most sensitive area was the prefrontal cortex (-62%) whereas the least sensitive area tested was the brain stem (-19%). Acute administration of zimelidine (ZIM, 5 mg/kg), an antidepressant preferentially blocking 5HT reuptake, uniformly decreased 5HTP synthesis by 40-50%. Striatal, but not hippocampal, DOPA was increased (64%) after ZIM, a phenomena that was reversed by coadministration of 8-OHDPAT (500 ug/kg). Depletion of monoamines with reserpine pretreatment did not substantially alter basal 5HTP synthesis. These findings suggest that 5HTP synthesis in vivo is subject to modulation after stimulation of somatic or terminal 5HT autoreceptors and that regional differences in sensitivity may exist.

Chronic administration of 5HT uptake inhibitors has been reported to decrease the rate suppressant effects of LSD on dorsal raphe 5HT neurons (de Montigney et al 1984). To assess the biochemical correlate of this phenomenon, the effect of 8-OHDPAT on 5HTP synthesis was determined after ZIM treatment (13 days, 5 mg/kg/day ip). In preliminary studies, basal rates of synthesis were increased in the cortex (20%) and hippocampus (36%) after ZIM, however the inhibitory effects of 8-OHDPAT (10 or 100 ug/kg) on 5HTP, expressed as percent of relative control, was not significantly different in these areas. The decrease in 5HIAA levels after 8-OHDPAT, however, was less in subjects treated with ZIM. Thus, basal synthesis rates and the 5HIAA response indicate a decreased responsivity whereas the similar 5HTP response to agonist may indicate differences between LSD and 8-OHDPAT or between autoregulation of firing rate and 5HTP synthesis. Supported by Dept. of Mental Health, State of Michigan.

18.15 A PARTIAL CHARACTERIZATION OF THE RAT SPINAL CORD SEROTONIN (5HT) AUTORECEPTOR. L.M. Brown and D.J. Smith. Dept. of Pharmacology/Toxicology and Anesthesiology. WVU Med. Cent., Morgantown, WV 26506

Serotonergic compounds capable of interacting with the multiple subtypes of the 5HT-1 receptor were examined for their ability to directly alter ^{3}H-5HT release or to effect depolarization-induced (K^+-evoked) release from rat spinal cord synaptosomes. The compounds included the 5HT-1B preferring drugs 1-(m-trifluoromethylphenyl) piperazine (TFMPP) and 1-(m-chlorophenyl) piperazine (mCPP) (Sills et al. JPET 231:480, 1984) and the 5HT-1A preferring drug 8-hydroxy-2 (di-n-propylamino) tetralin (8-OH-DPAT)(Middlemiss and Fozard EJP 90:151, 1983). Two nondiscriminative compounds serotonin and lysergic acid diethylamide (LSD) were also studied.

A preparation of isolated nerve terminals (synaptosomes) from rat spinal cord tissue was used. The synaptosomes were suspended in Tris-buffered Krebs medium at 37°C and the tissue serotonin was labelled by incubation with 100 nM 3H-5HT for 10 minutes. Aliquots were then transferred to columns containing Sephadex G-15 as a solid support and were continuously superfused with oxygenated buffer containing fluoxetine (1μM). After 50 min, the tissue was exposed to buffer containing various concentrations of drugs for 20 min. This exposure allowed an assessment of the ability of the drugs to directly induce serotonin release. Then the tissue was superfused with buffer containing elevated K^+ (15 mM) as well as the drugs to determine if depolarization induced transmitter release might be modified.

None of the drugs tested were found to directly induce ^{3}H-5HT release. However, several of the compounds did alter depolarization induced release. For example, LSD, in a manner similar to that previously observed for 5HT (Monroe & Smith, Abs. Soc. Neurosciences 10:419, 1984), produced a dependent depression of K^+ stimulated 5-HT release that was reversed by quipazine, a putative 5 HT-1B antagonist (LSD at 0.1 and 1μM reduced ^{3}H-5HT release to 77.8 ± 6.9 and 70 ± 5.8% of control, no drug; while in the presence of quipazine the values were 93 ± 8.1 and 78 ± 4.1% respectively. The ability of a 1B antagonist to reverse autoreceptor effects supports the proposal that the 1B site may function as the autoreceptor.

Additional support comes from preliminary studies with the drugs that preferentially interact with specific receptor subtypes. For example, mCPP and TFMPP, 1B preferring drugs, depress K^+-evoked 5-HT release to about 76 & 80% respectively at 1μM drug. The 5HT-1A preferring drug, 8-OH-DPAT, did not alter evoked release except in concentrations that non-specifically altered serotonergic nerve function, as indicated by a drug-induced increase in both ^{3}H-5HT and ^{3}H-5HIAA effluxes.

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18.16 BIOCHEMICAL CHARACTERIZATION OF SEROTONIN (5HT) STIMULATED PHOSPHOINOSITIDE (PI) HYDROLYSIS. P.J. Conn and E. Sanders-Bush. Dept. of Pharmacology, Vanderbilt University Sch. of Medicine and Tennessee Neuropsychiatric Institute, Nashville, TN 37232.

Neurotransmitter induced hydrolysis of phosphoinositides has been proposed to be a multifunctional transducing mechanism for generating several important intracellular signals including calcium fluxes, increased arachidonate metabolism, increased cyclic GMP, and protein kinase C activation. 5HT stimulates PI hydrolysis in blowfly salivary gland, rat cerebral cortex, and rat aorta. This response is apparently mediated by the S_2 binding site in rat cerebral cortex and aorta. While it is generally assumed that stimulus induced increases in PI hydrolysis are the result of direct receptor mediated activation of phospholipiase C, there are a number of other mechanisms by which an agent could indirectly increase PI hydrolysis. These include: 1) Stimulus induced increases in arachidonate metabolism resulting in production of an arachidonate metabolite which stimulates PI turnover. 2) Stimulus induced release of another neurotransmitter which stimulates PI hydrolysis. 3) Stimulus induced increase in de novo synthesis of phospholipids resulting in a mass action increase in PI hydrolysis. We have initiated a series of studies aimed at elucidating the biochemical mechanism of 5HT's effect.

The accumulation of ^{3}H-inositol phosphate (IP) was measured in rat aorta and cerebral cortex in the presence of lithium. The cyclooxygenase inhibitor, indomethacin, did not inhibit the response to 5HT suggesting that this effect is not dependent upon formation of a cyclooxygenase product of arachidonate. Selective muscarinic, alpha-adrenergic, and H_1-histaminergic antagonists did not block 5HT induced increases in PI turnover. The presence of a cocktail of protease inhibitors consisting of phenylmethylsulfonyl fluoride (0.2 mM), leupeptin (1 μg/ml), and pepstatin (1 μM) did not potentiate the response to 5HT, suggesting that 5HT does not exert its effect by stimulating the release of a peptide neurotransmitter. Furthermore, the addition of 1 μM tetrodotoxin, a sodium channel blocker, did not inhibit 5HT induced PI hydrolysis, therefore, it is likely that this effect is independent of neurotransmitter release. These data suggest that 5HT does not exert its effect on PI hydrolysis by stimulating production of a cycloxygenase product or by stimulating release of another transmitter. It has yet to be determined if 5HT's effect upon PI turnover is dependent upon production of a lipoxygenase product of arachidonate or stimulation of de novo phospholipid synthesis. If these mechanisms are excluded, it can be concluded that 5HT elicits direct receptor mediated activation of phospholipase C.

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18.17 AFFINITY OF INDOLE AND PIPERAZINE AGONISTS FOR 5-HT_{1A} RECEPTORS IN FRONTAL CORTEX OF THE RAT. L.S.Y. Tyau* and A. Frazer, Departments of Psychiatry and Pharmacology and VA Medical Center, Philadelphia, PA 19104.

Recent studies of the 5-hydroxytryptamine$_1$ (5-HT_1) receptor indicate that multiple subtypes, termed 5-HT_{1A} and 5-HT_{1B}, exist. Structurally dissimilar serotonin agonists, namely indole-and piperazine-type drugs, were found to have different selectivity for these subtypes; indole-agonists were either non-selective or showed selectivity for the 5-HT_{1A} subtype whereas piperazine-type agonists showed selectivity for the 5-HT_{1B} subtype (J. Pharmacol. Exp. Therap. 231:480, 1984). In the study cited, the subtype selectivity of these drugs was determined by measuring the inhibition they produced of the binding of the non-selective ligand ^{3}H-5-HT in the presence of both GTP and drugs that preferentially inhibited one of the subtypes.

An alternative way to evaluate the selectivity of these serotonin agonists would be to measure their inhibition of selective ligands. Recently, evidence was presented that ^{3}H-8-hydroxy-2-(di-n-propylamino) tetralin (^{3}H-DPAT) is selective for the 5-HT_{1A} receptor (Nature 305:140, 1983). Consequently, competition experiments using indole- and piperazine-agonists were conducted using this ligand. Saturation experiments indicated that the binding of ^{3}H-DPAT in the frontal cortex was to a single population of sites having an affinity for ^{3}H-DPAT of about 1nM. GTP produced a dose-dependent inhibition of ^{3}H-DPAT binding (IC_{50}, 20uM) such that specific binding was non-existent at concentrations of GTP greater than 0.5 mM. Because of this, competition experiments (15 concentrations) with the indole- and piperazine-agonists for ^{3}H-DPAT binding were done in the absence of GTP. The indole compounds, 5-HT, lysergic acid diethylamide (LSD), N,N-dimethyltryptamine (DMT), and 5-methoxy-N-N-dimethyltryptamine (5-MeODMT) inhibited ^{3}H-DPAT binding with IC_{50} values ranging from 1-100nM. The piperazine-agonists, quipazine, 1-(m-chlorophenyl) piperazine (mCPP) and 1-(m-trifluoromethylphenyl) piperazine (TFMPP), had IC_{50} values ranging from 150 to 5000nM. The order of potency of these drugs for inhibiting ^{3}H-DPAT binding - LSD = 5-HT > 5-MeODMT > DMT > TFMPP > mCPP > quipazine - is identical to their order of potency for the 5-HT_{1A} receptor as assessed previously by their inhibition of ^{3}H-5-HT binding. This separate study confirms that indole-agonists do have greater affinity for the 5-HT_{1A} subtype than piperazine-agonists do. (Supported by Research Funds from the VA and USPHS Grant MH29094).

18.18 IN VIVO AND IN VITRO INVESTIGATION OF NEWLY SYNTHESIZED DRUGS FOR 5-HT_1 AND 5-HT_2 RECEPTORS. R.A. Lyon*, M. Titeler, J.D. McKenney*, and R. Glennon* (SPON: D. POULOS). Dept. Pharmacol.Toxicol., Albany Medical College, Albany, New York 12208; Dept. Pharmaceutical Chem., Virginia Commonwealth University, Richmond, Va. 23298.

Brain serotonin receptors have been subdivided into 5-HT_1 and 5-HT_2 receptors based on their differing pharmacological properties (1). Recent data have revealed that the potencies of a series of phenylisopropylamine hallucinogens are strongly correlated with their potencies in animal drug discrimination tests; also there is a very strong correlation between hallucinogenic potency and affinity for the radiolabeled 5-HT_2 receptor(2). In order to design compounds with selectivity for 5-HT_2 and 5-HT_1 receptors, derivatives of phenylispropylamines and phenylpiperazines, which have been implicated as having a potential serotonergic structure, have been synthesized. These compounds are being screened for their ability to interact with brain 5-HT_1 and 5-HT_2 receptors labeled in vitro with ^{3}H-serotonin and ^{3}H-ketanserin respectively (table one).

Drug	K_i (nM) ^{3}H-serotonin	K_i (nM) ^{3}H-ketanserin
DM-4-72 (phenylisopropyl.)	1,710+/-249	207+/-34
DM-4-61 (phenylpiperazine)	148+/-7	17,170+/-1189
DM-1-50 (phenylpiperazine)	6+/-3	18+/-2

In general, it appears that phenylisopropylamines have higher affinity for 5-HT_2 than 5-HT_1 receptors. One of these, DOB(3), is currently being radiolabeled to be used as an agonist probe of the 5-HT_2 receptors. Among the phenylpiperazines there appear to be compounds with higher affinity for 5-HT_1 receptors than 5-HT_2 receptors. Animals are being trained to discriminate selective 5-HT_1 receptor and 5-HT_2 receptor drugs as they are developed. The results of further in vivo and in vitro screening of new compounds will be presented. It is anticipated that the functional roles of 5-HT_1 receptors (and possibly 5-HT_{1A} and 5-HT_{1B}, ref.4) and 5-HT_2 receptors will be revealed through the development of such selective drugs. (Supported by PHS grant DA-01642 and BRSG grant SO7RR05394-23)

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18.19 SEROTONERGIC DORSAL RAPHE NEURONS: ELECTROPHYSIOLOGICAL RESPONSES IN RATS TO 5-HT_{1A} AND 5-HT_{1B} RECEPTOR SUBTYPE LIGANDS. J.S. Sprouse* and G.K. Aghajanian. Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508.

Radioligand binding studies have suggested the presence of multiple 5-HT_1 recognition sites termed 5-HT_{1A} and 5-HT_{1B} (Pedigo et al., J. Neurochem., 36: 220, 1981). Compounds selective for these "receptor subtypes" have been identified (Sills et al., J. Pharmacol. Exp. Ther., 231: 480, 1984). Autoradiographic localization of subtypes has disclosed high receptor densities within the septal area, the prefrontal and frontal cortex, the hippocampus and certain areas of the midbrain including the raphe nuclei (Deshmukh et al., Brain Res., 288: 338, 1983). However, little is known about the physiological significance of the multiple 5-HT_1 binding sites and the pharmacology of subtype-selective ligands.

Two long-chain substituted piperazines, TVX Q 7821 (Dompert et al., Naunyn. Schmied. Arch. Pharmacol., 328: 462, 1985) and LY 165163 (Asarch et al., Life Sci., 36: 1265, 1985) were chosen for use in the present study for their selective 5-HT_{1A} binding activities; two short-chain substituted piperazines, trifluoromethylphenyl piperazine (TFMPP) and m-chlorophenylpiperazine (mCPP) were chosen for their selective 5-HT_{1B} binding activities (Sills et al., 1984). Extracellular recordings were made from spontaneously active 5-HT neurons in the dorsal raphe nucleus of chloral hydrate anesthetized rats. Compounds were administered intravenously and the effect on firing rate observed.

The 5-HT_{1A} ligands uniformly inhibited raphe cell firing in a dose-dependent manner. For TVX Q 7821, the dose which reduced spontaneous activity by 50% was 9 ± 3 µg/kg (N = 5); for LY 165163, 42 ± 12 µg/kg (N = 5). In contrast, the 5-HT_{1B} ligands displayed weak or irregular actions: TFMPP in repeated doses ranging 25 - 400 µg/kg either had no effect on raphe cell firing, partially suppressed activity or occasionally caused a facilitation (N = 6). Similar responses were observed following repeated 250 µg/kg doses of mCPP (N = 7).

The efficacy of 5-HT_{1A} ligands was further demonstrated by intracellular recordings of dorsal raphe neurons in coronal brain slices maintained in vitro. TVX Q 7821 and LY 165163 mimicked the effects of 5-HT in hyperpolarizing cell membrane potential and decreasing input resistance (cf., Aghajanian and Lakoski, Brain Res., 305: 181, 1984).

Taken together, these data suggest that (1) dorsal raphe 5-HT neurons are highly responsive to 5-HT_{1A} ("autoreceptor agonists") but not 5-HT_{1B} ligands and (2) the biochemical, physiological and behavioral effects of the 5-HT_{1A} compounds may stem in part from their inhibitory action on raphe neurons.

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18.20 SPIPERONE ALTERS THE EFFECT OF 5-HT ON POPULATION SPIKE AMPLITUDE IN RAT HIPPOCAMPAL SLICES. S. G. Beck, W. P. Clarke, and J. Goldfarb. Department of Pharmacology, Mount Sinai School of Medicine, New York, N. Y. 10029

Superfusion of rat dorsal hippocampal slices in vitro with serotonin (5-HT) reversibly decreases the amplitude of CA1 population spikes evoked by stratum radiatum stimulation. The magnitude of the 5-HT effect is stimulus-dependent and concentration-dependent with an EC_{50} of 3.2uM. In some experiments the decrease in population spike amplitude is preceded by a transient increase within the first 5 min of drug perfusion (Beck & Goldfarb, Life Sciences, 36, 1985, 557). In order to characterize the effects elicited by 5-HT we have been testing the actions of 5-HT antagonists in this system.

The 5-HT elicited changes in population spike amplitude were altered in the presence of 0.1 and 1.0 uM spiperone, although the effect of 0.1uM spiperone was not as pronounced. At either concentration, spiperone antagonized the 5-HT induced decrease in population spike amplitude, and it was possible to surmount at least partially the antagonism by increasing the 5-HT concentration (10-100uM). In addition, 10uM 5-HT invariably caused a transient increase in the population spike amplitude that lasted from 5-8 min, even when no increase was observed before perfusion with the antagonist. When the increase was present in control 5-HT responses, it was augmented during spiperone superfusion. The transient increase in population spike amplitude decreased as the concentration of 5-HT was increased (10-100uM). In the absence of 5-HT, spiperone did not affect the population spike amplitude.

The ability of spiperone to block differentially the 5-HT elicited decrease but not the increase in population spike amplitude is consistent with the hypothesis that these effects of 5-HT may be mediated by a heterogenous population of 5-HT receptors. In preliminary experiments 1.0uM ketanserin did not alter the 5-HT induced decrease in population spike amplitude. Therefore, we suggest that the decrease in population spike amplitude may be mediated by the 5-HT_{1A} receptor.

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MONOAMINES AND BEHAVIOR II

19.1 SPONTANEOUS ACTIVITY OF STRIATAL NEURONS IS CORRELATED WITH RECOVERY OF BEHAVIORAL FUNCTION AFTER DOPAMINE-DEPLETING BRAIN LESIONS. E.S. Nisenbaum*, M.J. Zigmond, E.M. Stricker, and T.W. Berger. Psychobiology Program and Center for Neuroscience, University of Pittsburgh, Pgh, PA 15260. (SPON: A.R. Caggiula).

Near-total depletions of striatal dopamine (DA) in rats produced by intraventricular (ivt) administration of the neurotoxin 6-hydroxydopamine (6-HDA) result in profound behavioral deficits from which animals can gradually recover. Such lesions also induce presynaptic compensatory mechanisms, including increased DA synthesis and release, which may underlie the observed behavioral recovery (Zigmond, et al., Arch. Neurol., 41, 1984). The nigrostriatal DA system is inhibitory in nature, and electrophysiological studies have revealed increases in the spontaneous firing rate of striatal cells shortly after 6-HDA treatment (e.g., Orr, et al., Soc. Neurosci. Abstr., 8, 1982). Schultz and Ungerstedt (Exp. Brain Res., 33, 1978) also have demonstrated that striatal spontaneous activity in DA-depleted animals returns to normal more than 1-year later. The present study investigated two hypotheses: 1) that recovery of striatal activity is correlated with behavioral recovery, and 2) that striatal cell recovery is dependent on DA neurons remaining after the 6-HDA lesion.

Rats were injected ivt. with 200 ug of 6-HDA, dissolved in 0.9% NaCl and 0.1% ascorbic acid and extracellular single unit activity of Type II neurons was recorded from the striatum of these animals either 4-8 days or 27-38 days post-lesion. A control group of animals was injected ivt. with the vehicle solution and striatal activity was recorded 4-8 days later.

The spontaneous firing rates of striatal neurons recorded 4-8 days post-lesion from animals exhibiting behavioral deficits were significantly increased relative to neurons recorded from control animals. In contrast, striatal activity was not increased when recorded from animals that had recovered behaviorally by 27-38 days after the lesion. However, the spontaneous activity of striatal cells recorded from non-recovered animals 27-38 days post-lesion was significantly higher than levels seen in recovered and control animals, but not different than striatal activity recorded 4-8 days post-lesion. These results indicate that the recovery of striatal activity is positively correlated with behavioral recovery, and can occur much earlier than one year after the lesion. Finally, in an additional group of animals that had recovered from an earlier 6-HDA injection, a second injection of 6-HDA (100 ug) was administered. Firing rates of striatal neurons recorded from these animals were increased significantly relative to control levels, in association with marked behavioral deficits. The latter demonstrates that neural recovery is dependent on residual, intact elements of the DA system. Supported by NS19608.

19.2 DIFFERENT EFFECTS OF D-1 AND D-2 AGONISTS IN RATS WITH UNILATERAL STRIATAL LESIONS. P. Barone*, T.A. Davis*, A.R. Braun*, T.N. Chase. Experimental Therapeutics Branch, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD 20205.

The injection of excitotoxins into rat striatum may provide a behavioral, histological and biochemical model of Huntington's disease (HD) (Coyle J.T., Schwarcz, R., Nature 263: 244-246, 1976). Two weeks after the administration of kainic or quinolinic acid into one striatum, ipsilateral rotation can be induced by systemically administered dopamine agonists (L-dopa, apomorphine, pergolide). Here we report the characterization of this turning behavior through the use of relatively selective D-1 and D-2 agonists, SK&F 38393 and LY 171555, respectively.

Sprague-Dawley rats (200 gm) were placed in stereotaxic apparatus and lesioned with quinolinic acid (300 nM in 1 µl) injected into the left striatum (coordinates: 8.2 A, 2.6 L, 4.8 V). Two weeks later they were tested with apomorphine (0.5 mg/kg s.c.). Rats performing 200-230 turns/hr were selected for further experiments allowing one week intervals between treatments for wash-out.

The D-1 agonist, SK&F 38393 failed to induce rotation even at doses which produce behavioral effects such as grooming, sniffing, rearing, locomotor activity, non-object chewing. The D-2 agonist, LY 171555, induced only rotation, whose duration was dose-dependent, but none of the behavioral effects, characteristic of the D-1 agonist. After completion of the behavioral observations, acetyltransferase activities in the striatal tissues were measured to evaluate the amount of lesioning.

The present observations indicate a clear distinction between the behavioral effects of D-1 and D-2 stimulation in rats with intact DA receptors. In a different model where supersensitivity of DA receptors is produced by 6 OH-DA lesion of the median forebrain bundle, both D-1 and D-2 agonists induce rotation (Gerhanik, O. et al., Neurology 33, 1489-92, 1983).

SK&F 38393 seems to require supersensitivity of DA receptors to produce effects that do not occur in intact preparations. This observation is supported by neurophysiological evidences: SK&F has no significant effect on pallidal firing rates in normal animal. However, in the supersensitive rat, SK&F induces changes in pallidal firing comparable to those observed with apomorphine (Carlson, J.H., et al., Soc. for Neuroscience Abstract # 124.8, 1984).

19.3 DOPAMINE-INDUCED CONTRALATERAL CIRCLING FROM RAT OLFACTORY TUBERCLE. M.R.Szewczak*, M.Cornfeldt* and S.Fielding. Pharmacology Dept., Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876.

Postural asymmetry and circling commonly result from manipulation of the nigrostriatal dopamine tract; the same has not been reported for the mesolimbic dopamine system. Unilateral lesions of the ventral tegmental area or the nucleus accumbens, as well as direct injections of dopamine agonists into the nucleus accumbens have all failed to cause postural asymmetry or circling.

In addition to the nuc. accumbens, the olfactory tubercles serve as terminal areas for mesolimbic dopamine neurons. We are now reporting that unilateral injection of dopamine (50 and 25 μg in 1 μl) into the olfactory tubercles of nialamide pretreated rats results in contralateral circling. A dose of 12.5 μg was ineffective. Circling developed gradually over a 2 hour period and then persisted for 3-4 hours. Norepinephrine (50 μg) was without effect. Systemic treatment with haloperidol (0.2 mg/kg,ip) and clozapine (20 mg/kg,ip) but not phentolamine (2 mg/kg,ip) or propranolol (5 mg/kg,ip) blocked the circling. These results suggest that the mesolimbic dopamine system shares control with the nigrostriatal system over the direction of locomotion. Circling induced from this area may also provide a valuable method for the evaluation of site specificity of antipsychotic agents.

19.4 LATERALIZED BEHAVIORAL AND BIOCHEMICAL EFFECTS OF UNILATERAL LESIONS OF DOPAMINE AND SEROTONIN NEURONS. R. J. Carey, Department of Psychiatry, SUNY and VA Medical Centers, Syracuse, NY 13210.

In an effort to determine the functional inter-relationship between dopaminergic and serotonergic innervation of the striatum, separate and combined denervations of these two neurotransmitter systems were performed. In adult male sprague-dawley rats unilateral injections of the neurotoxins 6-hydroxydopamine HBr (2µl of 3 µg/µl, base) and 5-7 dihydroxytryptamine creatnine sulfate (2 µl of 3 µg/µl, base) were made separately or together in animals pretreated with 25 mg/kg desmethylimipramine. Tests for lateralized sensory-motor disturbances included tests of responsivity to nociceptive stimulation applied to the ipsilateral versus contralateral body surface and measurements of turning tendencies. Also, measures of global behavior were evaluated by measurement of spontaneous motor activity. Unilateral lesions of dopamine neurons produced lateralized deficits in responsivity to nociceptive stimulation, persistent turning tendencies and decreased spontaneous motor activity. Combined lesions of dopamine and serotonin lesions attenuated the lateralized deficit in responsivity to nociceptive stimulation produced by the dopamine lesion alone but the motor asymmetry and decreased spontaneous motor activity was not affected. The unilateral serotonin lesions generally were without effect on the behavioral tests. The serotonin lesions both separately and in combination with the dopamine lesions produced marked effects on dopamine metabolism in the contralateral non-lesional hemisphere. Striatal dopamine and dopamine metabolites HVA and DOPAC were substantially increased by the destruction of serotonergic neurons in the contralateral hemisphere. These studies indicate both behavioral and biochemical influences of serotonin on dopaminergic systems in the brain.

19.5 A FOREBRAIN MAP OF DOPAMINE'S RELEVANCE TO LATERAL HYPOTHALAMIC STIMULATION REWARD AS BASED ON INTRACRANIAL NEUROLEPTIC INJECTION. J.R. Stellar, D. Corbett and A.L. Hamilton*. Department of Psychology and Social Relations, Harvard University, Cambridge, MA, 02138.

A great deal of evidence now exists to implicate dopamine in the reward effect generated by lateral hypothalamic (LH) brain stimulation. For example, sophisticated behavioral measurement techniques have revealed that systemic neuroleptic treatment degrades LH stimulation reward, apart from any motor/performance effects. As a refinement of the systemic treatment technique, brain injections of neuroleptic drugs into some discrete brain areas have been shown to disrupt self-stimulation behavior. However, in many of these brain injection studies reward degradation and general performance impairment effects of brain injection are confused due to the use of behavioral measures which do not separate these factors.

In this study, a high quality behavioral measure, the runway-based reward summation function (RSF) technique, was employed to quantitatively assess shifts in reward pulse effectiveness independently of impairments in performance capacity. (Stellar, Kelley, and Corbett, Pharmac., Biochem., Behav. 18: 433-442, 1983). The neuroleptic cis-flupenthixol was injected bilaterally into specific brain sites through chronic guide cannulae in conscious animals which were then immediately tested with the RSF technique. Two doses were employed: 0.5 ug/0.5 ul and 1.0 ug/0.5 ul dissolved in isotonic saline. Trans-flupenthixol (0.5 ug/0.5 ul saline), an inactive isomer, served as a control. As of this writing, 47 sites have been investigated in the caudate, putamen, the nucleus accumbens, the amygdala, and the frontal cortex.

By far the largest reward impairing effects were obtained from injections into the accumbens and surrounding ventral forebrain (N = 20). No effect was found from dorsal caudate injections (N = 5). The central caudate-putamen (N = 6), amygdala (N = 3), and frontal cortex (N = 6) injections produced small changes in reward pulse effectiveness. Very few motor impairments were seen following brain injection from any region, perhaps due to the small quantities of drug injected. Other brain sites of neuroleptic injection and the effects of selective 6OHDA lesions of dopamine in some animals cited above will also be presented.

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19.6 EFFECTS OF LY163502, A SELECTIVE D_2-DOPAMINERGIC RECEPTOR AGONIST, ON COPULATORY BEHAVIOR OF MALE RATS. M. M. Foreman and J. L. Hall*. The Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN. 46285.

LY163502, trans(-)5,5a,6,7,8,9,9a,10 octahydro-6-propyl-pyrimido (4,5g) quinolin-2-amine dihydrochloride, is a potent and selective D_2-type dopaminergic receptor agonist that we have used to study the effects of dopaminergic receptor stimulation on sexual behavior. The male rats used in these studies were either sexually inactive (noncopulators), unable to achieve ejaculation (nonejaculators) or capable of ejaculation in a 30 min exposure to a receptive female rat. Subcutaneous injections of solutions containing various concentrations of LY163502 were made 30 min prior to behavioral evaluation. At doses of 0.25-25 µg/kg, 75% or more of the noncopulators displayed mounting behavior and at doses of 0.025-25 µg/kg, 50% or more of the nonejaculators were able to achieve ejaculation. In both cases, the effects of LY163502 were dose related. At doses of 0.025-25 µg/kg, statistically significant reductions in ejaculatory latency were observed in rats capable of ejaculation. At doses of 0.10 - 25 µg/kg, significant reductions in the number of mounts required for ejaculation were observed. The minimum effective doses for potentiation of sexual behavior in each of these experiments were at least 10 X lower than any other detectable response. At doses lower (2.5 ng/kg) or higher (25 mg/kg) than this dose range, inhibition of sexual behavior was observed. The inhibiton of sexual behavior observed at the low doses of LY163502 are characteristic of behavioral changes associated with autoreceptor activation. Dopaminergic autoreceptor activation is thought to be linked with the inhibition of endogenous dopamine secretion and thereby a suppression of dopamine-mediated behaviors. The inhibition of sexual behavior observed at 25 mg/kg was accompanied by profound stereotypic behavior, which was assumed to have disrupted sexual behavior by a nonselective overstimulation of CNS dopaminergic receptors. These data are supportive of the view that within a defined dose range D_2 dopaminergic receptor stimulation can induce the expression of sexual behavior and lower the response thresholds for erectile and ejaculatory reflexes.

19.7 DOPAMINE VARIATIONS ASSOCIATED WITH ACUTE AND CHRONIC STRESSORS. P. Ahluwalia*, R. M. Zacharko and H. Anisman. Dept. Psychology, Carleton University, Ottawa, Ontario, K1S 5B6, Canada.

Several experiments assessed the consequences of stressors of varying severity on norepinephrine (NE) and dopamine (DA) neuronal activity in several brain regions. Consistent with other reports, alterations of DA activity associated with stressors varied considerably across brain regions. In frontal cortex footshock appreciably increased the accumulation of the DA metabolite, DOPAC, and markedly reduced DA concentrations. Likewise, in the ventral tegmentum reductions of DA concentrations were readily provoked by stressors. In the nucleus accumbens DA utilization was increased, but a greater amount of aversive stimulation was necessary to provoke reductions in DA concentrations. In other DA rich regions, such as the caudate and substantia nigra, DA utilization and concentrations were unaffected by the stressor. The most pronounced stressor induced NE variations occurred in hypothalamus and locus coeruleus. As in the case of stressor provoked NE variations, controllability over the aversive stimulation appeared to influence DA activity; however, this effect appeared to vary with the brain region examined. While escapable shock provoked a small decline of DA in frontal cortex, a more pronounced DA reduction was evident after inescapable shock. In contrast, both escapable and inescapable shock were equally effective in altering DA concentrations in the nucleus accumbens. Thus, it appeared that the psychological dimension of coping contributed to the DA variations in the frontal cortex, while the stressor per se played a greater role in affecting the DA variations in the nucleus accumbens. Finally, adaptation was evident after repeated application of a stressor such that the reductions of DA and NE concentrations were absent in mice that had received 15 footshock sessions applied on successive days. These data paralleled several of the behavioral changes associated with stressors, thus provisionally supporting a role for DA variations in accounting for the stressor induced behavioral alterations.

19.8 PERIPHERAL ADMINISTRATION OF NOREPINEPHRINE FACILITATES RECOVERY FROM BRAIN DAMAGE. J.P. Ryan, C. Hardy,* J. Johnston,* J. Thomas,* and R.L. Isaacson. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901

A role for catecholamines in neural plasticity and behavioral recovery have been proposed by several researchers (Feeney, Gonzalez, & Law, Science 217: 855, 1982; Kasamatsu, Pettigrew, & Ary, J. Comp. Neurol. 185: 163, 1979). The various catecholaminergic treatments in these studies have involved intracerebral injections or drugs that readily cross the blood-brain barrier. Others, using nonlesioned animals, have demonstrated behavioral alterations following peripherally administered catecholamines that appear to have limited access to the brain (McCarty & Gold, Horm. & Beh 15: 168, 1981; Gold & van Buskirk, Horm. & Beh. 7: 509, 1976). The present study examined the effects of intraperitoneal injections of norepinephrine (NE) on the behavioral anomalies of hippocampally lesioned animals both before and after the induction of adrenergic supersensitivity.

Male Long-Evans Hooded rats were prepared with sham, neocortical, or hippocampal lesions by aspiration. Pharmacological treatments and behavioral testing began 21 days after surgery. The T-maze and open field apparati were used to measure spontaneous alternation and activity/exploratory behavior, respectively. Bretylium tosylate, a drug that inhibits the release of peripheral NE, was administered in order to induce a condition of adrenergic supersensitivity. NE (4 μg/kg) and a solution of tartaric acid/distilled water were injected on separate days prior to and after bretylium treatment. At the completion of testing clonidine (.1 mg/kg) was administered and colonic temperatures checked at 40, 60 and 80 min intervals. An enhancement of the clonidine-induced decrease in body temperature has been interpreted as an indicator of α-adrenergic supersensitivity (Isaacson, Youngue, & Carrera, Physiol. Psych 6: 236, 1978).

Animals with hippocampal lesions exhibited hyperactiviy in the open field as well as impaired ability to alternate between arms in the T-maze. The administration of NE following 4 days of Bretylium treatment significantly increased the amount of spontaneous alternation of these lesioned animals, however, NE was ineffective in normalizing the lesioned-induced hyperactivity. Both the activity level and the alternation were not affected by the initial NE administration prior to bretylium treatment. A condition of supersensitivity was established as indicated by the significant decrease in colonic temperature of the bretylium-treated animals in comparison to control animals. The results indicate that the rigid, perseverative behavior of hippocampally lesioned animals can be ameliorated by the peripheral administration of NE following the induction of supersensitivity.

19.9 EFFECTS OF DESIPRAMINE ON LOCUS COERULEUS NEURONS IN THE FREELY MOVING CAT. R.J. Ross,* G.L. Mann,* S. Yang,* J.H. Indik,* and A. R. Morrison (SPON: C.P. O'Brien). Dept. of Psychiatry, Vet. Admin. Med. Ctr. and Sch. of Veterinary Medicine, University of Penna., Philadelphia, PA 19104 U.S.A.

Effects of tricyclic antidepressant drugs on diverse forms of behavior, including paradoxical sleep (PS) architecture and the course of depressive illness, are often ascribed to noradrenergic (NA) mechanisms. With electrophysiological techniques, it has previously been demonstrated that the repeated administration of imipramine inhibits NA neurons in the locus coeruleus (LC) of the anesthetized rat (Svensson, T.H. and Usdin, T., Science, 202:1089-1091, 1978). While these results could have been influenced by the presence of anesthesia, use of the technique of single unit recording in the unrestrained, unanesthetized animal should eliminate such a source of variance. We have therefore begun a series of experiments in the unanesthetized cat, designed to compare the properties of LC neurons recorded following repeated desipramine (DMI) administration with those recorded under control conditions. The feline LC has been recognized as pharmacologically heterogeneous, but criteria have been suggested for identifying particular cells as NA on the basis of their prominent deceleration during PS (PS-off cells) (Parsons, T.D., et al., Soc. Neurosci. Abst., 9:560, 1983).

A microdrive assembly, for recording single cell activity, and EEG, EOG, EMG, and hippocampal electrodes, were implanted in 4 female cats under halothane anesthesia. Microwires were aimed at the region of the LC (P2.5, L3.0, V-3.0). The rate of discharge of each isolated neuron was studied over the entire sleep-wake cycle. Four PS-off cells, which were presumed to be NA, were recorded in 2 cats after the repeated administration of DMI (1 mg/kg p.o., b.i.d.) for periods of time ranging from 1 to 30 days (av. 18). Their average frequencies of discharge ranged from 0.51-1.39s^{-1} (mean 0.79s^{-1}) during the state of quiet waking (QW); from 0.41-0.77s^{-1} (mean 0.55s^{-1}) during slow wave sleep (SWS); and from 0.00-0.15s^{-1} (mean 0.05s^{-1}) during PS. These values compared with mean firing rates of 1.36, 0.60, and 0.08s^{-1}, respectively, of a control population of PS-off cells (Reiner, P.B., Ph.D. Thesis).

The activity of 1 neuron was recorded in both the undrugged and drugged states. Approximately 2 hours after the first dose of DMI, its frequency of firing was reduced by 10% of baseline during SWS. By the following day, the decrements in firing rate were 68% and 69% during QW and SWS, respectively.

These results, suggesting that chronic DMI administration may slow the firing of at least some norepinephrine-containing LC neurons, are consistent with earlier observations in the anesthetized rat.

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19.10 DIETARY MANIPULATION OF D-AMPHETAMINE SELF-ADMINISTRATION IN RATS L.S. Geis*, D.G. Smith* and W.H. Lyness. Dept. of Pharmacology, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

It has been well documented that the addition or omission of the large neutral amino acids in a diet can alter human and animal behavior as well as the neurochemistry of brain monoamines. Of the monoamines, serotonin (5-HT) and dopamine (DA) have been shown to be of import in the maintenance of stimulant self-administration. DA appears to be crucial to the subjective experience of euphoria (reward), while the role of 5-HT is unknown. Lesions of ascending 5-HT neurons increase self-administration while i.p. injection of L-tryptophan (TRY), the 5-HT precursor, decreases drug intake. With this in mind, experiments were designed to test the hypothesis that a diet high in TRY might attenuate drug self-administration.

Rats were implanted with i.v. cannulae and allowed access to a device which delivered 0.125 mg/kg d-amphetamine with each lever press. After achievement of a steady daily response rate (55-98 inj./8 hr. (0900-1700 hr), the rats were divided into 2 groups; normal rat chow and identical chow supplemented to 4.0% TRY. Access to food was from 1700 to 0900 hr. Animals on the supplemented diet, when allowed access to the self-administration apparatus, exhibited a profound decrease in drug intake. When the diet was continued for 3 days, on each successive day amphetamine self-injection was reduced (range; 0-40 injections/ 8 hr). With the reinstatement of the normal rat diet, the recovery to pre-diet drug intake appeared slow to return to normal (3 days) although whole brain 5-HT concentrations appeared normal much sooner (1 day post-diet). One animal which disconnected its i.v. line exhibited an increased rate of lever pressing while on the diet which is characteristic of an extinction response. This suggests that the diet does not produce sedation or decrements in motor performance which could lead to decreased responding. Experiments will be performed to: 1) establish a minimal TRY supplementation that reduces drug intake, 2) determine whether tolerance develops with long term dietary changes and 3) to determine possible mechanisms for the phenomenon.

Further experimentation includes dietary manipulation of L-tyrosine, precursor to both norepinephrine (NE) and DA, and the examination of both behavior and neurochemistry. In preliminary experiments, i.p. injection of tyrosine restores brain NE concentrations which were reduced by prior amphetamine injections. It remains to be determined whether dietary supplements will alter drug intake.

This work was supported by a grant from the Institute of Nutrition, TTU, and an Institute for Biomedical Research Seed Grant Award, TTUHSC.

19.11 LOCUS COERULEUS UNIT ACTIVITY IN BEHAVING CATS: HABITUATION OF RESPONSES EVOKED BY SIMPLE SENSORY STIMULI. B.L. Jacobs and K. Rasmussen. Prog. in Neurosci., Princeton Univ., Princeton, NJ.

Brain noradrenergic (NE) neurons in the area of the locus coeruleus (LC) are responsive to a variety of afferent inputs. In the course of studying NE-LC neurons in freely moving cats, we noted that their single unit response to repetitive presentation of clicks or flashes appeared to show rapid habituation. The present study examined this relationship in greater detail. For comparison, we also examined the response of serotonergic (5HT) neurons in the dorsal raphe nucleus and dopaminergic (DA) neurons in the pars compacta of the substantia nigra. Single unit activity was recorded by means of bundles of movable 32 and 64 μm dia, wires implanted in the LC. Neurons in the area of the LC were initially identified as NE by their long duration action potentials, slow and somewhat regular discharge pattern, excitation-inhibition response to pinch, and complete cessation of activity during REM sleep. This identity was confirmed further by the complete suppression of neuronal activity following injection of the α_2 agonist clonidine. Finally, all neurons displaying such characteristics were histologically localized within the LC (this characteristic activity was not encountered on penetrations outside of the LC). Cats were exposed to a series of 64 auditory or visual stimuli presented once every two sec. Auditory stimulation consisted of a 1 msec duration 113dB pulse. Visual stimulation consisted of flashes of approximately 1.5 x 10^6 candle power, 1.9 x 10^7 lumens peak intensity. Data were analyzed both with computer generated peristimulus time histograms and with raster outputs on an oscilloscope. Neurons in all three areas displayed similar latencies and durations of excitation in response to both the flash and the click. (The duration of excitation in NE cells appears to be slightly longer.) In contrast to the similarities

	STIMULUS TYPE			
	AUDITORY		VISUAL	
CELL TYPE	latency	duration	latency	duration
NE	61	130	69	120
5HT	40	64	53	76
DA	51	86	63	82

in response latency and duration across these three cell types, the NE neurons differed dramatically from the 5HT and DA neurons in terms of habituation. Neurons in the latter two groups displayed no evidence of habituation, whereas, the response strength of NE neurons often would begin to diminish within a few trials and often would show a complete abolition of response within 10-20 stimulus repetitions. We are currently attempting to determine whether this response decrement in NE neurons is attributable to shifts in behavioral state or attention. (Supported by grants MH-23433 and AFOSR 85-0034.)

19.12 LOCUS COERULEUS UNIT ACTIVITY IN BEHAVING CATS: RESPONSE TO AVERSIVE AND APPETITIVE CONDITIONING. K. Rasmussen and B.L. Jacobs, Prog. Neurosci., Princeton Univ., Princeton, NJ.

Noradrenergic (NE) neurons in the area of the locus coeruleus (LC) have been implicated in stress and anxiety responses. This is supported by a variety of studies including reports that in rats brain NE turnover increases in response both to acute stress and to environmental stimuli previously paired with acute stress. NE units in the LC have also been implicated in some aspects of learning. This is supported by a variety of studies including reports that transient posttraining decreases in brain NE concentration predict later retention performance of an avoidance response. In an attempt to shed light on both of these issues, we employed paradigms that involve anxiety and/or learning. Thus, we have examined LC unit activity in response to conditioned emotional response (CER) and conditioned food reward (CFR) training. The general methodology and the criteria for identifying neurons as NE are described in the preceding abstract. During CER training cats were exposed to 10 pairings, with a 3 min inter-trial interval, of a 5 sec tone (CS+, 2900 Hz, 74 dB) and a 1 sec duration air puff to the face (US). During the inter-trial-interval a CS- (5 sec lights off) was randomly delivered. During CFR training a separate group of cats was exposed to repeated pairings, with a 3 min inter-trial-interval, of a 5 sec tone (the same tone used in CER training) with the delivery of a highly preferred food. All animals rapidly learned the stimulus contingencies in both paradigms. Presentation of the CS+ prior to CER training resulted in a modest but significant increase in NE unit activity above a baseline level of activity of ∿1-2 spikes/sec. However following CER training, NE units showed a dramatic increase in activity upon presentation of the CS+. The increase in activity in response to the CS+ during CER training (173%) was significantly greater than the increase in response to the CS+ before CER training (54%). Conversely, the increase in activity in response to the CS- during CER training (53%) was not significantly different from the increase in activity in response to the CS- before training (33%). Contrary to this, the increase in activity in response to the CS+ during CFR training (76%) was not significantly different from the increase in activity in response to the CS+ before training (54%). Therefore NE units in the LC dramatically increase their activity in response to a tone that predicts the occurrence of a noxious event, but not to a tone that predicts the occurrence of a rewarding event. These data support the hypothesis that NE neurons in the LC play a role in stress or anxiety responses and also support the hypothesis that NE units play a role in aversive but not appetitive conditioning. (Supported by grants MH-23433 and AFOSR 85-0034)

19.13 INCREASES IN STRIATAL SEROTONIN ARE NOT NECESSARY FOR WEANING IN DOPAMINE-DEPLETED RAT PUPS. J.P. Bruno, D. Jackson*, M.J. Zigmond, and E.M. Stricker. Department of Psychology, Center for Neurosciences, University of Pittsburgh, Pittsburgh, PA 15260

Unlike adult rats given large dopamine (DA)-depleting brain lesions, neonatal animals given comparable lesions are spared from the initial deficits in ingestive and motor behavior. Moreover, there is a sprouting of striatal serotonin (5-HT) neurons only in response to the neonatal lesion. Two experiments were done to determine whether the 5-HT hyperinnervation is necessary for the sparing phenomenon seen in rats given brain lesions as neonates. First, three-day-old rat pups were injected ivt either with 6-hydroxydopamine (6-HDA; 150 ug in 10 ul, n=11), 6-HDA and 5,7-dihydroxytryptamine (5,7-DHT; 150 ug of each drug in 5 ul, n=18), or the vehicle solution (.1% ascorbic acid in .9% NaCl, n=7) 30-45 min after pretreatment with the norepinephrine uptake blocker desmethylimipramine (DMI; 25 mg/kg, s.c.). Despite >98% depletions of striatal DA (control, 12.77 ± 1.35 ug/g) and 5-HT (control, .60 ± .05 ug/g), 87% of these pups continued to suckle and grew at a rate that was slower than controls but comparable to that of pups with 99% DA depletions. Moreoever, they had no difficulty ingesting food and water when weaned on Day 27. In the second experiment, 15-day-old rat pups were injected ivt with 6-HDA (150 ug in 10 ul, n=14) or its vehicle solution (n=9) 30-45 min after pretreatment with DMI (25 mg/kg, s.c.) and the MAO inhibitor pargyline (45 mg/kg, s.c.). Despite 97% depletions of striatal DA (control, 10.42 ± 1.80 ug/g), the brain-damaged pups continued to suckle and could be weaned on Day 27. Unlike the younger pups, however, there were no significant lesion-induced increases in striatal 5-HT levels when measured 1-2 mo later (control, .36 ± .03 ug/g; 6-HDA-treated, .47 ± .08 ug/g).

These results suggest that increases in striatal 5-HT are not necessary for maintaining ingestive and motor behavior in rats sustaining near-total DA-depleting brain lesions as neonates. We are currently investigating the neural mechanisms underlying this age-dependent sparing phenomenon.

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19.14 ANALGESIA INDUCED BY INTRATHECALLY INJECTED CLONIDINE IN THE INFANT RAT. Harry E. Hughes* and Gordon A. Barr* (SPON: Wagner H. Bridger). Biopsychology Program, Hunter College, City University of New York, and Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461.

Various lines of evidence suggest that inhibition of dorsal horn afferents and consequent analgesia are under the control of descending noradrenergic (NE) pathways in the spinal cord of adult animals. Furthermore, pharmacological studies have suggested that it is the alpha receptor, rather than the beta receptor, that is involved. Recent developmental studies from several laboratories have shown that opiate-induced analgesia in the spinally mediated tail flick test develops largely during the second week of life and that kappa opioid receptors are functionally active prior to mu opioid receptors However, there are no data on the development of spinally mediated non-opiate induced analgesia. Because NE-stimulated cyclic AMP activity, the number and size of spinal NE terminals, and the concentration of NE in rat spinal cord increase postnatally, to peak near the end of the second week of life, it might be expected that NE induced analgesia would appear at that time. The present study addressed the contribution of the developing spinal alpha NE receptors in producing analgesia in the infant rat.

Long-Evans hooded rats were implanted with chronic intrathecal catheters at 9 days of age. Anesthesized pups were incised just above the thoracic and lumbar vertebrae and the underlying muscle and fascia gently scraped away. A laminectomy was performed at level T8 - T11 with care taken not to injure the underlying dura. The dura was then gently lifted and punctured with a needle and a five mm length of dialysis tubing (250 μm in diameter) was inserted under the dura. The dialysis tubing was fit to polyethylene tubing (PE-10) external to the entry into the dura, the PE tubing was anchored to the vertebrae and musculature, and the wound was closed. The entire procedure required approximately 15 minutes and there was no damage to the underlying spinal cord when examined histologically and no obvious impairment of motor function. Twenty-four hours later, at 10 days of age, pups were injected with 0, 25, 50, or 100 μg in 4 μl of diluent of the alpha NE agonist clonidine HCl. This volume of fluid perfused the cord from the lumbar/sacral segments rostral to the caudal cervical region. Spread was verified in each pup by injection of dye following testing. Following a 15 minute postinjection period, the forepaw, hindpaw, and tail were sequentially submerged in 47 ° C water and the latency to withdraw each appendage was recorded. The order of testing of each limb and the tail was randomly determined.

Clonidine produced significant dose dependent analgesia as evidenced by increased tail flick latencies. It had no effect on withdrawal of either the forepaw or hindpaw. Thus, the physiological organization of NE induced analgesia appears different for the limbs and the tail. This is consistent with other data that demonstrate developmental differences in the ability of opiates to produced analgesia in the paws compared to the tail. These data further suggest that, in response to a thermal stimulus, in the tail flick test, spinal NE circuitry associated with antinociception is functional by at least 10 days of age.

19.15 REPEATED STRESSOR OR DESMETHYLIMIPRAMINE EFFECTS ON FOOTSHOCK INDUCED DEPRESSION OF SELF-STIMULATION. W.J. Bowers*, R.M. Zacharko and H. Anisman, Psychology Dept., Carleton University, Ottawa, Ontario, K1S 5B6, Canada

It has been observed that acute exposure to inescapable footshock induces marked and long lasting reductions in responding for intracranial self-stimulation (ICSS). Such effects were noted in dopamine (DA) containing brain regions where stressors reliably influenced DA turnover and concentrations. In particular, inescapable shock disrupted ICSS responding from the nucleus accumbens, ventral tegmental area and the medial forebrain bundle in both rate dependent and independent paradigms. In contrast, inescapable footshock did not affect DA turnover in the substantia nigra, and likewise this treatment did not influence responding for ICSS. Contrary to the reduction of ICSS elicited by acute stressors, adaptation occurred with repeated exposure to footshock, such that high levels of responding for brain stimulation were maintained. Moreover the extent of the adapatation was directly related to the number of shock sessions mice received. Paralleling these findings, it was observed that the depressed response rate from the nucleus accumbens ordinarily elicited by acute footshock was prevented by repeated administration with the tricyclic agent, desmethylimipramine (DMI). It was observed that in animals where adaptation had occurred, withdrawal from the DMI treatment or termination of the chronic shock regimen resulted in a marked and relatively protracted depression of responding. These data were taken to support the contention that chronic stressor application results in the induction of adaptive neurochemical changes. Behavioral depression occurs when the adaptive variations do not occur. Under such conditions, antidepressants are necessary to engender the course of neurochemical changes that would ordinarily occur. However, since such effects persist only so long as the treatment is maintained, it is suggested that the antidepressants either provoke compensatory adaptive changes or mask the symptoms associated with the stressor, rather than affecting the etiology for the depressed behavior.

19.16 PERTURBATIONS OF SLEEP CYCLE PERIOD LENGTH PRODUCE PARALLEL INCREASES IN THE FREQUENCY OF DESYNCHRONIZED (D) SLEEP AND DORSAL RAPHE (DRN) DISCHARGE PROFILES. R. Lydic, R.W. McCarley, and J.A. Hobson. Harvard Medical School, Boston, Ma 02115

Studies of intact, undrugged, adult mammals uniformly report regular DRN firing which is positively correlated with behavioral arousal and a cessation of DRN discharge (D-off) during D sleep. The present experiment characterized the DRN and D sleep response to alterations in the period length of the sleep cycle. If DRN discharge is causally involved in generating the ultradian sleep cycle, then the relationship between DRN activity and behavioral state should not be easily dissociable by forced locomotor activity.

Adult, male cats were chronically implanted with electrodes for recording sleep and wakefulness (EEG, EOG, EMG, and PGO waves) and with moveable microwire electrodes aimed for the DRN. These polygraphic variables and DRN discharge potentials were recorded after: 1) 16 hrs. of forced locomotor activity; and 2) a similar interval during which sleep could occur ad libitum.

The results were derived from 177 sleep cycles, with each cycle operationally defined: from the end of one D sleep epoch thru the end of the subsequent D sleep epoch. For cycles ranging in period length (Tau) from 10 to 80 mins., DRN discharge profiles were phase-locked to the temporal organization of sleep. Although forced activity shortened the sleep cycle (control Tau=34.7 $\pm$ 13.1 mins., n=86; forced activity Tau=28.7 $\pm$ 11.3 mins., n=91; $p<0.0001$) it was not possible to dissociate the sleep cycle from the DRN discharge profiles. DRN discharge rate in each behavioral state was partly a function of sleep cycle Tau. Forced activity produced statistically significant reductions in the duration of wakefulness (W) and synchronized (S) sleep and increases in the duration of transition (T) and D sleep. There were no significant differences in DRN discharge rate during W, S, or T, but an enhanced DRN discharge rate during D epochs which followed forced activity. There was a significant increase in the number of D sleep epochs but not in D sleep duration after forced activity. The D-off DRN discharge profiles exactly paralleled the changes in D sleep.

These results are consistent with the hypothesis that the DRN is involved in sleep cycle regulation. Furthermore, they suggest that the homeostatic response to perturbations of the ultradian sleep cycle is mediated by an increase in the frequency rather than the period length of putative D sleep generating mechanisms.

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19.17 SIMILAR EFFECTS OF CLONIDINE AND HISTAMINE ON THE CENTRAL NERVOUS SYSTEM. B. Delbarre and G. Delbarre. Faculté de Médecine, 37000 TOURS, FRANCE.

The action of clonidine and histamine have been investigated in several animal species. In young chickens, mice and rats, clonidine (0.25 - 0.75 mg.kg-1 I.M.) and histamine (20 - 50 mg.kg-1 I.M. - 0.250 ug I.C.V.) induce behavior and E.E.G. sedation (a pattern of high slow waves appeared on the E.E.G.) This sedative effect is blocked by mepyramine (5 mg.kg-1 s.c) atropine (5 - 10 mg.kg-1 s.c) and alpha 2 adrenergic blocking agent : yohimbine (1 - 3 mg.kg-1 I.P.) and piperoxan (2 - 5 mg.kg-1 I.P.) but not by alpha 1 : dibenamine (15 - 20 mg.kg-1 I.P.) and prazosin (2 - 5 mg.kg-1 I.P.).

In rats, clonidine (0,15 mg.kg-1 s.c) and histamine (30 mg.kg-1 s.c) depress avoidance conditionned reflexes. Atropine (10 - 15 mg.kg-1 s.c) antagonizes the depression of conditionned avoidance response.

Clonidine (0,1 - 0,5 mg.kg-1 I.M.) and histamine (10 - 40 mg.kg-1 I.M.) induce hypothermia in mice, rats and chickens.

Sedative effect of chlorpromazine (4 mg.kg-1 I.M.) (N-Methyl transferase inhibitor) is blocked by atropine (10 mg.kg-1 s.c) and mepyramine (2 mg.kg-1 I.P.)

These data suggest the central action of histamine may be explained by release of catecholamines.

19.18 RADIOENZYMATIC DETERMINATION OF PHENYLPROPANOLAMINE IN PLASMA. A.A.Reid*, P.J.Fleming* and C.R.Lake* (SPON:H.Holloway). Dept. of Psychiatry, USUHS, Sch. of Med., Bethesda, MD 20814.

Phenylpropanolamine (PPA) is a drug which is widely used in over-the-counter diet aids and cold remedies and as an abusive substance. In recent years an increasing number of articles in the literature have reported mild to severe neuropsychiatric side effects associated with the use of PPA containing products. To date only a few controlled PPA studies aimed at understanding its effects, metabolism and mechanism of action have been conducted. Current methodologies capable of detecting plasma PPA concentrations from 5 ng/ml to 240 ng/ml include one gas chromatography (GC) and two high pressure liquid chromatography (HPLC) techniques.

We have developed a new radioenzymatic method for plasma PPA analyses with a 17 fold increase in sensitivity. In principle the assay involves conversion of PPA to tritium radiolabelled ephedrine ([3H]EPD) by the enzyme phenylethanolamine - N - methyltransferase (PNMT) and tritium radiolabelled S-adenosylmethionine ([3H]SAM). The product, [3H]EPD, is isolated from unreacted [3H]SAM and labelled side products by an organic extraction and a thin layer chromatography procedure. In addition, a pre-incubation organic extraction procedure is included to remove inhibitors of PNMT from plasma and to concentrate the sample for enhanced enzymatic conversion.

Each of the steps within the radioenzymatic assay has been optimized for PPA and the assay has been characterized in terms of sensitivity, linearity, reproducibility, recovery, accuracy and specificity. In order to accurately quantitate PPA across the wide range of possible physiological concentrations, the assay is conducted at two plasma volumes. PPA concentrations between 0.3 - 50 ng/ml can be detected with 1 ml of plasma, while concentrations between 4 - 1500 ng/ml can be detected with 0.1 ml of plasma. The intra (within) assay coefficients of variation (CV), which represent the reproducibility of the assay, are 9.3 % and 5.7 % at 0.5 ng/ml and 1500 ng/ml respectively, while the inter (across) assay CV is 9.7 %. Although the overall recovery of PPA as EPD (1 %) appears low, it is predictable because the enzymatic reaction is conducted at initial velocity conditions, which results in only 3 % conversion of PPA. Further, the accuracy of the assay was confirmed by a GC technique and the specificity analysis revealed that none of the compounds tested interferred with PPA determinations.

The new radioenzymatic assay is currently the method of choice for plasma PPA analyses, because it is superior to the HPLC and GC techniques in terms of sensitivity, range of linearity, and volume of plasma required.

19.19 INTERACTION BETWEEN HARMANE AND ADRENOCEPTORS IN THE MODULATION OF ANXIETY. G. Delbarre, B. Delbarre and A. Ferger*. Faculté de Médecine, 37000 TOURS, FRANCE.

Harmane is a potent endogenous inhibitor of benzodiazepine receptor binding (Rommelspacher, H., et al., Naunyn-Schmiedeberg's Arch. Pharmacol., 314 : 97, 1980).

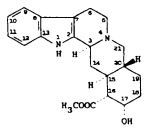
Harmane (50 mg.kg-1 I.P.) induced convulsions are antagonized by diazepam (ED 50 : 1,20 mg.kg-1 I.P.) and delorazepam (ED 50 1,09 mg.kg-1 I.P.).

Similarity of structure between harmane, yohimbine and raubasine prompted us to investigate anticonvulsive activity of these compoumds.

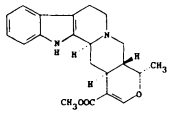
Yohimbine (2 - 5 mg.kg-1 I.P.) known to have anxiogenic activity (Goldberg et al., Pharma. Rev., 35 : 143, 1983) potentiates convulsive activity of harmane (50 mg.kg-1 I.P.). Raubasine (5 - 10 - 20 µg.I.C.V) alpha 1 blocking agent, known to have benzodiazepine agonist type activity (Charveron, M., et al., Eur. J. Pharmacol., 106 : 313, 1985) antagonizes convulsive activity of harmane. Moreover this effect is antagonized by yohimbine (5 mg.kg-1 I.P.).

These results suggest that alpha 1 and alpha 2 may be involved in the modulation of anxiety.

HARMANE



YOHIMBINE



RAUBASINE

19.20 HALOPERIDOL, THIAZIDES AND SOME ANTIHYPERTENSIVES SLOW RECOVERY FROM APHASIA. B. Porch*, J. Wyckes* and D.M. Feeney. Depts. of Communicative Disorders, Psychology and Physiology, University of New Mexico, Albuquerque, NM 87131.

This study was prompted by the report of haloperidol slowing recovery from hemiplegia in the rat (Feeney et al., Science, 217, 815-817, 1982). Since catecholamine antagonists are often given to stroke patients to lower blood pressure or reduce agitation they may slow recovery of function in man. Aphasia, despite its complexity, can be objectively scored and spontaneous recovery can be estimated using a mathematical model (Porch, Clin. Aphasiology Proc., 187-200, 1981). In this model untreated patients, based on the 1 month poststroke test with the Porch Index of Communicative Ability, have a predicted score for a second evaluation six months later. Scores reported here represent deviations from the predicted scores, and so a 0.0 indicates spontaneous recovery to the predicted test result. On the second test some patients receive scores markedly different from the predicted value and some of this variability may be due to medications. There were 32 patients who received speech therapy and aphasia testing at the Albuquerque VA hospital (between 1980-1984) and all cases with complete records were examined. There were 13 cases that received no drugs and their deviation score was +9.1. For patients in speech therapy the math model applies a correction factor of +10.0 above untreated cases, so this group had the expected recovery. The 19 patients on various drugs, mostly thiazides and antihypertensive medication, had a mean of -7.4 below the predicted value for spontaneous recovery (17.4 below the score expected for patients in therapy). The difference between the drug and no drug groups was significant (p less than .01). This result is likely due to medications rather than differences in hypertension, since 4 patients given propranolol, which does not slow recovery in the rat hemiplegia model (Harrington et al., MBRS Abst., 411, p.103, 1984), had a mean score of +15.5. There were 3 patients given haloperidol and they had a mean score of -16.0. We tentativly conclude that some drugs are contraindicated in patients with brain damage.

FEEDING AND DRINKING I

20.1 NALOXONE ATTENUATES DEPRIVATION-INDUCED DRINKING AND FEEDING IN A DESERT-DWELLING RODENT. D.A. Czech and C. McCrimmon* (SPON: K.A. Vaughn). Dept. of Psychology, Marquette Univ, Milwaukee, WI 53233.

Considerable evidence has accumulated suggesting that endogenous opioid mechanisms play a modulatory role in the regulation of consummatory behavior. Opioid antagonists, e.g., reliably attenuate both drinking and feeding in a number of animal species, and in a variety of experimental situations. Recently, pronounced species differences in opioid-linked consummatory behaviors have been reported. It was reported that the prototypic opioid antagonist, naloxone, attenuates food, but not water, intake in domestic pigeon (Deviche & Schepers. Psychopharm. 82:122, 1984) and that the longer acting antagonist, naltrexone, depresses drinking but appears to be without effect on feeding in male golden hamsters(Lowy & Yim. Life Sci.30:1639, 1982). In the present study, we investigated a potential effect of naloxone on deprivation-induced feeding and drinking in a desert-dwelling, murid rodent - the spiny mouse (Acomys cahirinus). In a first experiment, male mice were adapted to a 23-hr water-deprivation schedule with food available ad lib. Following stabilized drinking, mice were injected SC with 4 doses of naloxone hydrochloride (NX) (0.3, 1.0, 3.0 & 10.0 mg/kg as the salt) and normal saline vehicle, approximately 30 min prior to scheduled drinking, in a within-subject design. Injections were given at 3-4 day intervals, approximately 4 hours into the light period. Deionized water intake was automatically monitored at 1-min intervals throughout the hour drinking period in the home cage. Food was not available during tests. Intakes were converted to percent of intake on saline(control) day and these data were evaluated at 5, 15, 30 & 60 min into the drinking period with ANOVAs and Dunnett's procedures. Significance level was set at $p < .05$. All dosage levels used resulted in significantly attenuated drinking, as compared to the control condition, for all cumulative periods. Mice characteristically consumed all of their water within the first 5 min of access. In a second experiment, food intake was measured in the same group of mice, using the same doses of NX. This time, however, they were food-deprived for 20 hr prior to feeding tests, again at 3-4 day intervals. These animals do not adjust well to a feeding schedule, seeming unable to ingest adequate food during limited access. Water was not available during feeding tests. One-hour intakes were converted, again to percent of control, and analyzed with ANOVA and Dunnett's procedures. As with drinking, food intake was significantly attenuated at all dosage levels of NX. The spiny mouse appears to possess an opioid-sensitive mechanism for both feeding and drinking. Results are discussed.

20.2 FOOD HOARDING AND INGESTION IN THE DEER MOUSE: SELECTIVE MU AND KAPPA OPIATE INFLUENCES. M. Hirst* and M. Kavaliers. Depts. Pharmacology and Toxicology and Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7.

Many studies have suggested that endogenous opiates participate in the mediation of feeding. Little is known, however, about the influences of endogenous opioid systems on the components of natural feeding activity. The deer mouse, Peromyscus maniculatus, displays in the field and in the laboratory several behaviours associated with feeding. These include searching for food, transporting of food, hoarding or caching of food and ingestion. Since both mu and kappa agonists have been implicated in feeding behaviour in rodents, their influences on food hoarding and ingestion by deer mice were evaluated.

Male deer mice (22-25 g) were maintained at 20°C under LD 10:14 for several days. After being housed individually for three days, with powdered food available ad lib, determinations were made of food acquired by licking each hour in three hour blocks of time in the light period after injections (i.p.) of U-50,488H, morphine or saline. Uninjected mice were used as an additional control. Other mice were acclimated in cages which had a black polyethylene U-shaped tunnel attached from which they could remove food pellets and hoard them in a second polyethylene tube in the home cage. After five days determinations were made of food hoarded each hour in three hour blocks of time in the light period after animals received morphine, U-50,488H or saline. Uninjected mice were used as a second control.

The results of this study demonstrated that the kappa agonist, U-50,488H, caused a significant dose-dependent increase in food ingestion over three hours, whereas the mu agonist, morphine, had little impact. In contrast, morphine induced a dose-dependent increase in food hoarding while U-50,488H decreased hoarding, the animals ingesting the pelleted food. Morphine-treated animals did not hoard similar pellets of a non-food item.

These findings imply that endogenous opiate systems can selectively influence different components of food acquisition in this species.

20.3 EFFECTS OF NALMEFENE, AN OPIATE ANTAGONIST, ON ENERGY BALANCE AND GLUCOSE METABOLISM IN ZUCKER OBESE RATS. C.L. McLaughlin, C.A. Baile, R.L. Gingerich* and M.E. Michel. Washington Univ. Med. Sch., St. Louis, MO 63110 and Keys Pharmaceuticals, Miami, FL 33269.

Evidence suggests that opioid peptides are involved in the control of food intake and glucose metabolism. Since Zucker obese rats are hyperphagic and hyperinsulinemic, we investigated their responses to nalmefene, an opiate antagonist. SC administration of 2 mg/kg nalmefene twice daily to Zucker obese and lean rats for 21 days decreased food intake (20.8 vs 21.9 and 17.2 vs 19.6 g/day, respectively $p \leq .001$) and body weight (1.3 vs 1.5 and .4 vs .9 g/day, respectively, $p < .02$). On day 1 glucose concentrations were decreased 60 min after the first injection in obese (110 vs 124 mg/dl, $p < .002$) but not lean (118 vs 119 mg/dl rats). In addition, insulin concentration was decreased only in obese rats (15.0 vs 17.7, $p < .03$ and .67 vs .74 ng/ml, NS for lean rats). On the last day of injection glucose concentration was also increased only in obese rats (124 vs 114 mg/dl, $p < .001$ and 112 vs 106, NS for lean rats) and insulin was again decreased only in obese rats (17.5 vs 21.7, $p < .03$ and 1.11 vs 1.16, NS for lean rats). In nalmefene-treated compared with saline-treated rats B-endorphin content of the pituitary was increased (1341 vs 1178, $p < .07$) while concentration in the plasma was unaffected (49 vs 43 pmol/l). In a second experiment addition of nalmefene to food (100 mg/kg food) for 21 days also decreased food intake (19.3 vs 21.2 g/day $p < .01$) and body weight gain (.03 vs .59 g/day $p < .01$) in obese but not lean rats (18.0 vs 18.5 and .13 vs .42 g/day for food intake and weight gain respectively). In the same experiment addition of tolbutamide to food (670 mg/kg) did not influence food intake (19.4 vs 19.9 g/day) or body weight gain (.22 vs .50 g/day) in obese or lean rats; thus effects of nalmefene were greater than those of tolbutamide. Glucose and insulin concentrations were measured weekly after a 2-hr fast and only glucose concentrations were decreased in nalmefene-treated compared with saline-treated rats on day 7 (127 vs 136 mg/dl, $p < .03$). In a third experiment Zucker obese and lean rats were administered saline or nalmefene (2 mg/kg) orally with 2.0 ml/kg water or 50% dextrose. Nalmefene produced a smaller change in insulin concentration after 30 (11.5 vs 18 ng/ml, $p < .002$) and 60 (-12.7 vs -18.7 ng/ml, $p < .01$) min in obese but not lean rats (1.8 vs .9 and -1.4 vs -.1 ng/ml respectively, NS) and only after oral glucose, which increased blood glucose 66 and 33% respectively for obese and lean rats ($p < .001$) and which was not influenced by nalmefene. Thus, while nalmefene affected food intake and body weight in obese rats whether administered IV or in the food, and the effect was stronger than that of tolbutamide, effects on glucose metabolism occurred only after a glucose challange. (Supported by Keys Pharmaceuticals)

20.4 EFFECTS OF AMPHETAMINE MICROINJECTIONS INTO DOPAMINE TERMINAL AREAS ON FEEDING, DRINKING AND OPEN FIELD BEHAVIOR. G.D. Carr and N.M. White. Department of Psychology, McGill University, Montreal, Canada, H3A 1B1.

Systemic injections of amphetamine produce anorexia and adipsia but at lower doses have been shown to stimulate eating. In the open field amphetamine injections result in increased activity and stereotyped repetition of certain species-typical behaviors. All of these effects have been shown to depend to some extent on the ability of the drug to stimulate dopaminergic neurotransmission. The present study further examined these behavioral effects by microinjecting amphetamine into one of six dopaminergic regions and observing how the injections affected eating, drinking and open field behavior in rats. The drug was injected (10 ug in 0.5 ul, bilaterally) into the medial prefrontal cortex, nucleus accumbens, anteromedial caudate nucleus, lateroventral caudate nucleus, amygdala or the region subjacent to the area postrema. The rats were mildly food or water deprived, injected with the drug and allowed access to food or water respectively. Animals that had received injections into the nucleus accumbens or amygdala showed both anorexia and adipsia relative to saline control injections but no effects were observed from the other sites. It is suggested that amphetamine's actions on the accumbens and amygdala contribute to the anorexia and adipsia that are produced by systemic injections.

A detailed analysis of 12 behavioral categories in the open field showed that the amphetamine injections caused a slight increase in locomotion from the medial frontal cortex and anteromedial caudate. There was a large increase in the amount and speed of locomotion and amount of rearing produced by intra-accumbens injections accompanied by complementary decreases in lying down, standing still, grooming and sniffing of the floor. The amygdala-injected rats spent more time standing still and the sub-area postrema region group initially spent more time lying down. The stereotyped repetition of behaviors that is observed after systemic injections was not elicited from any of the intra-cranial injection sites suggesting that this component of amphetamine's effects may depend on simultaneous stimulation of more than one dopaminergic region.

20.5 CORTICOTROPIN-RELEASING FACTOR (CRF) INCREASES GROOMING IN THE PARAVENTRICULAR NUCLEUS (PVN). D.D. Krahn*, B.A. Gosnell, J.E. Morley, A.S. Levine (SPON: M.W. Dysken). Neuroendocrine Research Laboratory, VA Medical Center, Minneapolis, MN, 55417 and Geriatric Research Education and Clinical Care Center, VA Medical Center, Sepulveda, CA, 91343.

CRF decreases food intake and increases grooming and locomotor activity when administered intracerebroventricularly (icv) to rats. CRF also decreases feeding when administered locally in the PVN of the hypothalamus but does not decrease feeding when administered in the lateral or ventromedial nuclei of the hypothalamus, the striatum, or the globus pallidus. We decided to test whether CRF administered in the PVN of rats elicits the rest of the syndrome caused by CRF (icv) (i.e., increased grooming and movement).

Twenty-one male, Sprague-Dawley rats housed in a 12 hour light/12 hour dark environment in single cages were implanted with 23 gauge, indwelling cannulae directed at the PVN. Following a 5 day recovery period, the animals were treated with .5 µl of normal saline or CRF (1 µg/µl) in their home cages at 1600 hours in each of the following conditions: previous 24 hour deprivation, food available; 24 hour deprivation, no food available; no deprivation, food available. Each animal was observed once per minute by a rater blind to treatment and behavior was classified into one of six categories (grooming, resting, moving, eating, drinking or rearing). Food was collected and weighed after 1 and 2 hours.

ANOVA's showed that CRF increased grooming in the PVN ($F(1,20) = 43.93$, $p = .000$) but that neither deprivation ($F(1,20) = 2.68$, $p < .12$) or food availability ($F(1,20) = .04$, $p = .84$) significantly influenced grooming. CRF in the PVN also increased locomotion significantly ($F(1,20) = 37.26$, $p = .000$). CRF in the PVN did decrease food intake at these doses as well (mean 2 hour intakes; CRF = 4.86 g, NS = 2.45, $F(1,20) = 22.23$, $p = .0001$).

Thus, CRF in the PVN not only causes the decrease in feeding previously reported but also causes the increased grooming and locomotion seen with CRF (icv). Although these behaviors are linked following CRF injection into the PVN, injections into other areas of the brain may result in the dissociation of these behaviors. In the present study, we also observed that CRF-induced grooming is not influenced by either the presence of food or the degree of hunger, both of which obviously affect feeding.

20.6 GLUCOPRIVIC FEEDING AND VASOPRESSIN. P.F. Aravich* and C.D. Sladek (SPON: M. Blair). Departments of Anatomy and Neurology, University of Rochester Medical Center, Rochester, NY 14642.

One of the most popular theories of feeding behavior is the glucostatic theory. Major support for this hypothesis has come from the orexigenic effects of 2-deoxy-D-glucose (2DG) and insulin. However, the phenomenon of delayed glucoprivic feeding reveals that ongoing glucoprivation is not necessary for the feeding response, suggesting the contribution of other factors. One neuroendocrine consequence of glucoprivation is the release of vasopressin (VP) (Endoc. 107:1970, 1980), which has metabolic as well as pressor and antidiuretic actions. We now report that the acute release of VP may play a role in 2DG feeding.

Female Brattleboro rats (DI) lacking VP (n=6) and body-weight matched Long-Evans (LE) rats (n=6) served as subjects. The animals were maintained and tested with access to both a high-sucrose diet and a high-fat diet. Four-hour caloric intakes were determined following counterbalanced i.p. injections of varying doses of 2DG. The DI rats were found to have a substantially attenuated dose-response function relative to LE rats, which was particularly evident at higher doses. The DI rats were then given chronic VP replacement therapy (0.1 µg/hr). Such therapy corrects many of their abnormalities, including those related to diabetes insipidus. Replacement therapy failed to facilitate 2DG feeding in DI rats. Thus, their feeding deficit is not due to the complications of diabetes insipidus.

VP may contribute to 2DG feeding by promoting endogenous energy mobilization. This could occur via routes that include a direct action on liver glycogenolysis (V1 receptors) or by a direct action on glucagon release (V2 receptors) (Peptides 5:871, 1984). A specific V1 antagonist was selected for evaluation using the diets and normal LE rats noted above. Rather than reducing 2DG feeding specifically, the V1 blocker increased overall food intake (main effect) and was not associated with a glucoprivic-by-antagonist interaction effect. Thus, V1 VP receptors do not mediate 2DG feeding.

It is concluded that VP release from the hypothalamo-neurohypophysial system participates in 2DG feeding by affecting VP receptors related to glucagon release. This would in turn promote lipolysis and VP-insensitive glycogenolysis. Alternatively, VP within the suprachiasmatic nucleus may alter circadian rhythms of catobolism and anabolism, which influence 2DG feeding. It should be noted, however, that circadian ingestive rhythms in the DI rat are reported to be normal. Regardless, the contribution of VP to 2DG feeding suggests one mechanism by which glucoprivation induces secondary changes, which then elicit feeding.

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20.7 ENVIRONMENTAL CONTROL OF LIPOPROTEIN LIPASE ACTIVITY. J. A. Stidham*, S. M. Mena*, and A. W. Knehans*, (SPON: J.P. Farber). Departments of Psychology and Human Development, University of Oklahoma, Norman, OK 73019.

Continuous feed-starve cycles sometimes promote adiposity (Hollifield & Parson, 1962). Several lines of reasoning have led us to suspect that the phenomenon depends upon classical conditioning. This possibility was investigated by cyclicly feeding rats in a context distinctly different from the one in which they were deprived. Later, when the rats had been refed for a week, the ability of the separate environmental contexts to modify some indices of metabolism was assessed.

Sixteen male rats were placed on a variable time deprivation-feed schedule that prescribed daily 2 hr food access that was separated from the previous day's feeding by an average of 22 hr. The 2 hr feeding period, which occurred in a different context from deprivation (F vs. NF), was thus unpredictable on the basis of time alone. This assured that only the physical context could exert stimulus control and that circadian rhythms were not entrained. Following 35 conditioning days, the rats were allowed to feed freely. This took place in a separate context and lasted 7 days.

The test trial consisted of a 60 min exposure to the F or NF context and was followed by immediate decapitation. Blood was collected for blood urea nitrogen (BUN), free fatty acids (FFAs), and blood glucose (BG) determinations. The epididymal fat pads were removed and prepared for lipoprotein lipase (LPL) assay.

Rats exposed to the F context displayed a significant depression of FFAs and a significant elevation of LPL activity relative to the NF controls. BUNs and BGs were undifferentiated across groups. The results indicate that certain aspects of metabolism can be brought under external stimulus control. Under ordinary circumstances this anticipatory response would help to dispose of a forthcoming flood of nutrients and inhibit mobilization. That the conditioned responses were restricted to fat metabolism suggests that insulin was probably not the mediating hormone. The rise in aldosterone titers with repeated deprivation (Wright & Schultz, 1982), their role in the promotion of lipogenesis (Devenport, et al, in press), and inhibition of energy mobilization (Devenport, et al, 1983) suggest these steroids as possible candidates.

Devenport, L.D., Goodwin, K.G., and Hopkins, P.M., (in press). Pharmacology, Biochemistry and Behavior.
Devenport, L.D., Goodwin, K.G., Hopkins, P.M., Manes, G.L., and Oltmanns, K.L., (1983). Society for Neuroscience Abstracts, 9:196.
Hollifield, G. & Parson, W. (1962). Journal of Clinical Investigation, 42:250-253.
Wright, J.W., & Schultz, E.M., (1982). Pharmacology, Biochemistry and Behavior, 16:697-699.

20.8 Neurotoxic Hypothalamic Lesions Alter the Content of Cholecystokinin in Anterodorsal Hypothalamus and Pituitary. A.C. Scallet, P.L. Faris, M.C. Beinfeld* and J.W. Olney. National Center for Toxicological Research, Jefferson, AR 72079 and Washington and St. Louis University Schools of Medicine, St. Louis, MO 63110.

Both monosodium glutamate (MSG) and bipiperidyl mustard (BPM) produce partially overlapping basomedial hypothalamic lesions that interact to alter the subsequent feeding behavior and/or insulin levels of treated animals. Administration of exogenous cholecystokinin also has been shown to effect feeding behavior and plasma insulin. To study the possible role of endogenous cholecystokinin in the effects of neurotoxic hypothalamic lesions, cholecystokinin content of the regionally dissected hypothalami and pituitaries of rats treated with MSG alone, BPM alone or with both toxins was measured by radioimmunoassay. BPM alone produced a 147% increase in posterior pituitary concentrations of cholecystokinin ($p<.01$) compared to controls. MSG treatment by itself had no effect on pituitary cholecystokinin, but prior MSG lesioning prevented the BPM effect. Preliminary data on cholecystokinin levels in regionally dissected portions of the hypothalamus suggest that either MSG or BPM alone produced a non-significant increase (20%) in the anterodorsal area ($p<.10$). Combined MSG/BPM treatment increased cholecystokinin by more than 60% ($p<.01$). There were no effects on cholecystokinin of other hypothalamic regions. Since the paraventricular nucleus (contained in the anterodorsal sample) has many cholecystokinin containing cell bodies, and axonal projections from these cells are the source of cholecystokinin in the pituitary, it is not surprising that changes associated with neurotoxic hypothalamic lesions were localized to the anterodorsal hypothalamus and posterior pituitary. It is difficult to interpret our cholecystokinin findings, however, in terms of the known effects of these neurotoxic lesions. The striking increase in pituitary cholecystokinin occurred only in rats lesioned with BPM alone, a group that displays no significant disturbance in food intake or plasma insulin. Our preliminary results suggest that the greatest change in paraventricular nucleus content of cholecystokinin occurred in MSG/BPM rats, a group that does display hyperphagic obesity and hyperinsulinemia. How these functional disturbances may relate to the increased cholecystokinin concentrations in paraventricular nucleus of these rats, however, awaits further study. Supported by NS1818335 and NS18667 (MCB) and ES07066.

20.9 INSULIN BINDING SITES ARE WIDELY DISTRIBUTED IN RAT BRAIN: IN VITRO QUANTITATIVE RECEPTOR AUTORADIOGRAPHY. E.S. Corp*, D.A. Davidson*, D.P. Figlewicz*, D.M. Dorsa, S.C. Woods, D. Porte, Jr.*, D.G. Baskin. Depts. of Psychology, Medicine, Pharmacology and Biological Structure, Univ. of Washington and VA Medical Center, Seattle, WA 98108.

Recent evidence suggests that insulin is widely distributed throughout the brain and may be involved in feeding and metabolism. However, the anatomical location of insulin's action is not completely understood. Our approach to this problem was to localize insulin specific binding (SB) sites by in vitro receptor autoradiography. Therefore, frozen-dried coronal sections of rat brain were labeled with ^{125}I-monoiodinated human insulin (0.5nM; S.A.=336 μCi/μg). The location of binding was determined by apposing radiolabeled sections to LKB Ultrofilm (5 days exposure). Concentrations of bound insulin in small anatomical regions were determined by computerized densitometry, using a standard curve of optical density (OD) vs. fmol/mm^2 of tissue slice which had been calibrated with tissue standards of known radioactivity. The results showed that low levels of SB (radioactivity displaced by 0.1μM unlabeled porcine insulin) were widespread throughout the brain (e.g., SB=5x10^{-3} fmol/mm^2 in septohippocampal region and ventromedial hypothalamus). However, high concentrations of insulin SB sites were present in the following regions:

Region	SB (fmol/mm^2)
Raphe nucleus	13.0 x 10-3
Arcuate nucleus*	7.5 x 10-3
Dorsomedial nucleus*	7.5 x 10-3
Inferior olivary nucleus	7.5 x 10-3
Nucleus of the solitary tract*	6.1 x 10-3
Paraventricular nucleus*	5.7 x 10-3
Tract of the trigeminal nerve	5.7 x 10-3

These results show the presence of insulin SB sites in brain regions known to be involved in feeding and metabolism (*), some of which have been shown to be uptake sites for blood-borne insulin. Our new findings are: (1) insulin SB occurs in the dorsomedial nucleus and paraventricular nucleus, both of which have been implicated in the regulation of feeding and metabolism; (2) insulin SB occurs in the raphe nucleus, tract of the trigeminal nerve and inferior olivary nucleus. This suggests either that these structures participate in energy homeostasis, or that insulin has broader responsibility in the CNS than has previously been suspected.

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20.10 MEAL PATTERN ANALYSIS AND THE HOMEOSTATIC MODEL OF FEEDING. Thomas W. Castonguay*, and Judith S. Stern* (Spon: M. McGinn). Food Intake Laboratory and Nutrition Department, University of California - Davis, Davis CA 95616

The meal patterns of 8 week old male Sprague-Dawley rats (n=12) were studied over a two week period. All animals were individually housed in custom made modules, and given ad libitum access to Purina rat chow and water. Each module was equipped with a food cup placed on a Mettler balance that was capable of measuring 0.01 g. Output from the balance was monitored once per second by an LSI 11/02 minicomputer. The data were then repeatedly analyzed using five end-of-the-meal (traditionally referred to as the intermeal interval or IMI) definitions: 2, 5, 10, 20, and 40 minute definitions were used. Minimum meal size was defined as 0.05 grams. Thus, in order for a meal to be recorded, a rat had to eat at least 0.05 g and stop eating for a minimum of 2 or a maximum of 40 minutes. Intervals that did not meet the required definition were scored as pauses within a meal, and food intake from bouts both preceding and subsequent to the pause were scored together as part of the same meal. The resulting meal patterns from each of the animals were then characterized with respect to daytime and nighttime meal frequency, size, and duration. Further, the relationships between these variables were examined using a correlational analysis. Finally, the intervals between meals were correlated with both meal size and meal duration.

These analyses showed that variations in IMI definition had an effect upon meal size, duration and frequency. Shorter IMI's promoted the observation of more frequent but smaller meals, that were also shorter in duration. The relationship between meal size and meal duration was surprisingly weak. Further, variations in IMI definitions strengthened the observed correlation between the size and duration of nighttime meals but weakened the same correlation within daytime meals.

Of note, too, was the observation that the correlation between meal size and either the interval preceding the meal (the premeal interval) or the interval subsequent to the meal (the postmeal interval) was not reliably correlated. Increasing the IMI definition promoted weaker correlations between premeal interval and meal size, but strengthened the correlation between postmeal interval and meal size. At best, the postmeal interval/meal size correlation was r=0.288 with the use of a 40 minute IMI, and at worst, r=-0.047, with the use of a 2 minute IMI.

These results are in marked contrast to those reported by LeMagnen and coworkers. Their implications on LeMagnen's homeostatic model of feeding behavior will be discussed.

20.11 OPIOID REGULATION OF NUTRIENT SELECTION AND INTAKE. B.A. Gosnell, D.R. Romsos*, J.E. Morley, A.S. Levine, Neuroendocrine Res Lab, Minneapolis, MN, 55417, Dept of Food Sci and Human Nutr, Michigan State University, East Lansing, MI, 48824, and Geriatric Res Educ and Clinical Care Center, VA Medical Center, Sepulveda, CA, 91343.

Several experiments were performed to evaluate the effects of opioid agonists and the antagonist naloxone on the intakes of diets of varying carbohydrate and fat composition. In the first experiment, rats were maintained on either a high carbohydrate (CHO) diet (69% CHO, 20% protein, 12% fat), an intermediate (INT) diet (40% CHO, 20% protein, 40% fat), or a high fat (FAT) diet (3% CHO, 20% protein, 77% fat). After a 20 hour fast, naloxone injections (s.c.) caused a greater reduction in caloric intake in rats fed the FAT diet than in rats fed the INT or CHO diets. Naloxone (10 mg/kg) reduced 1 hour mean caloric intakes by 53, 61 and 71% in the CHO, INT and FAT groups, respectively. These differences, however, are complicated by the fact that the baseline intakes in the three diet groups were different; 18, 23 and 30 Kcal, respectively, for the CHO, INT and FAT groups. When nocturnal intake was measured in the non-deprived state, naloxone reduced caloric intake to an approximately equal degree in all 3 diet groups. The feeding effects of the opioid agonists ketocyclazocine (KC) and butorphanol tartrate (BT) were also tested in rats maintained on either the CHO, INT or FAT diet. Both KC and BT were generally more effective in rats fed the FAT diet than in those fed the CHO diet.

In a second set of experiments, rats were given access to both CHO and FAT diets. After a 20 hour fast, naloxone (s.c.) reduced total caloric intake. A 10 mg/kg naloxone dose reduced 1 hour total intake by 52%; calories derived from the FAT diet were reduced by 72%, whereas calories derived from the CHO diet were reduced only 29%. This preferential reduction of fat intake was not found when nocturnal intake was measured in non-deprived rats. When daytime intake was measured after the administration of butorphanol tartrate (BT) to rats offered a CHO and FAT diet choice, overall caloric intake was increased by doses from 0.5 to 10 mg/kg. This increase was due primarily, though not exclusively, to an increased intake of the FAT diet. Six hours after injection, mean total caloric intake was increased from 2 Kcal in the control condition to 20 Kcal in the 10 mg/kg condition; 63% of this intake was derived from the FAT diet.

These studies generally support the work of Marks-Kaufman and Kanarek (Pharmacol. Biochem. Behav. 12:427, 1980; Psychopharmacol. 74:321, 1981) which suggests that opioids may partially regulate both the amount and the type of nutrients ingested.

20.12 EFFECT OF CHRONIC ADMINISTRATION OF MORPHINE AND NALMEFENE ON FOOD INTAKE AND BODY WEIGHT IN DIABETIC AND CONTROL RATS. A.S. Levine, M. Grace*, C. Billington*, B.A. Gosnell, D.M. Brown*, J.E. Morley. Neuroendocrine Res Lab and Geriatric Res Education and Clinical Center, VA Medical Center, Minneapolis, MN and Sepulveda, CA.

Diabetic animals respond differently than non-diabetic animals to various effects of opiates, including analgesia and food intake. We investigated the effect of chronic administration of morphine (10 mg/kg bid) and nalmefene (10 mg/kg bid), a long acting opioid antagonist, on food intake and body weight in diabetic rats (DB) and their non-diabetic (ND) controls. Food intake was also measured 2, 4 and 6 h following administration of morphine on days 1, 2 and 7. Morphine (2.3 $\pm$ 0.5 g/4h) failed to increase 4 h food intake above saline baseline (2.0 $\pm$ 1.0 g/4h) on day 1 in the DB rats, whereas morphine (1.9 $\pm$ 0.3 g/4h) did stimulate 4 h food intake above saline baseline (0.4 $\pm$ 0.2 g/4h, $p < 0.05$) in the ND rats. By day 7 morphine caused a significant increase of 4 h food intake compared to baseline in both ND and DB animals (ND: 0.3 $\pm$ 0.1 g vs 2.5 $\pm$ 0.4 g, $p < 0.05$; DB 1.8 $\pm$ 1.2 g vs 5.8 $\pm$ 0.7 g, $p < 0.05$). However, cumulative food intakes for 24 and 48 h were decreased on days 1 and 7. Changes in body weight and food intake were also measured over a 21 day period. Diabetic animals receiving either morphine or nalmefene gained significantly less weight than the saline injected DB rats (morphine: 8.4 $\pm$ 3.3 g, $p < 0.05$; nalmefene: 20.1 $\pm$ 4.5 g, $p < 0.05$; saline: 31.2 $\pm$ 3.1 g). The ND animals receiving nalmefene gained slightly less weight than the ND rats injected with saline (18.0 $\pm$ 4.2 g vs 24.1 $\pm$ 7.7 g). Morphine resulted in a marked weight loss in the ND animals by day 21 (-13.2 $\pm$ 7.5 g). In the DB rats 21 day cumulative food intake was decreased following morphine administration (920.0 $\pm$ 38.4 g) or nalmefene administration (976.4 $\pm$ 23.3 g) compared to saline administration (1087.2 $\pm$ 18.6 g). In contrast, neither morphine (385.8 $\pm$ 13.2 g) nor nalmefene (464.0 $\pm$ 17.1g) effected food intake compared with saline injected (429.6 $\pm$ 22.1 g) in ND controls. Thus chronic administration of morphine and nalmefene to DB animals decreased both body weight gain and food intake, whereas ND rats were only sensitive to the morphine-induced reduction in body weight gain. The maximum weight loss for the animals receiving morphine was much greater in the ND controls (-56 $\pm$ 8 g) compared to the DB rats (-14 $\pm$ 2 g). These data demonstrate a differential effect of morphine and nalmefene on food intake and body weight change in DB and ND rats.

20.13 NEUROCHEMICAL CORRELATES OF FEEDING BEHAVIOR IN HYPEROSMOTIC WESTERN TOAD (BUFA BOREAS). K. H. Tachiki*, C. F. Baxter, J. W. Dole* and B. B. Rose*. V.A. Medical Center, Sepulveda, CA 91343, UCLA Sch. Med., Los Angeles, CA 90024, Dept. Biology, California State Univ., Northridge, CA 91330.

In western toads, acclimation to a hyperosmotic (HOA) environment (400mOs) alters prey-capture response. HOA acclimation also results in a change of many amino acid levels in brain tissue, including the levels of some putative neurotransmitters. This suggests that changes in behavioral responses may be related to these biochemical changes in the brain.

Feeding responses to mealworms by HOA toads were monitored and the following parameters quantitated: 1) time elapsed before feeding began (LT), 2) food consumption rate (FCR), and 3) the strike accuracy (SA), i.e. the consistency with which the toad's tongue hit targeted mealworms.

For amino acid analysis, toads were sacrificed following a behavioral test session. Brains were removed, divided into cerebral hemispheres (CH), optic lobs (OL), and olfactory bulbs (OB), and tissue extracts prepared of each brain area. Amino acids were quantitated employing an automated amino acid analyzer.

Pearson Correlation Analysis was used to assess the strength of association between the three behavioral measures and the levels of each of 22 amino acids in the three brain areas of responding toads. Of these, the levels of ten amino acids (GABA, ASP, PHE, GLU, GLY, SER, ASN, ALA, GLN, 3-METHYL-HIS) in one or more of the brain areas were significantly correlated ($p<0.05$ with at least one behavioral parameter. The levels of the first five amino acids were similar in the same brain areas of both normally-behaving HOA-acclimated toads and normally-behaving freshwater-acclimated toads. They were used for further analysis of neurochemical correlation with feeding behavior.

CORRELATIONS BETWEEN BEHAVIOR AND AMINO ACIDS IN BRAIN AREAS

BEHAVIOR	GABA	ASP	PHE	GLY	GLU
LT	CH*		OL* OB*	CH***	--
FCR	CH* OL*	CH* OL* OB*	OL** OB**	CH**	CH*
SA	--	CH* OL* OB*	OL** OB**	CH*	CH**

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

These five amino acids, in one or more brain regions, were directly correlated with one or more of the measured parameters of feeding behavior. Supported in part by the Medical Research Service of the Veterans Administration.

20.14 NEURAL TRANSPLANTS AND FEEDING BEHAVIOR. T.J. Collier, P.F. Aravich*, and J.R. Sladek, Jr. Department of Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

The development of techniques for neural transplantation has stimulated interest on the impact such grafts may have on the behavior of the host organism. Neural transplantation may be particularly useful in determining the roles specific transmitter-containing cells play in the elaboration of specific behaviors. One behavior that appears to be especially amenable to transplantation techniques is feeding behavior, which is influenced by a wide variety of established neurochemical systems. The feeding effect produced by central norepinephrine (NE) injections has been one of the most thoroughly studied responses, and can be obtained by intrahypothalamic or intraventricular NE infusion. Fetal locus coeruleus (LC) neurons were transplanted into the third ventricles of adult rats to determine if transplanted NE-containing neurons can mimic the feeding effect of intraventricular NE infusion.

Eight male Sprague-Dawley rats completed the experiment. Five subjects received intra-third ventricular implants of fetal (15 d. gestation) pontine tissue, including the LC, and three subjects received control implants of cerebellar tissue. The effects of the transplants on food intake (Purina chow) and body weight were determined across a 9-mo. period. The diet was then switched to a 0.1% quinine HCl (QHCL) diet, since NE infusions are associated with finickiness to bitter diets. Following testing, the rats were sacrificed and the presence of catecholamine-containing neurons in the pontine tissue verified utilizing the ALFA histofluorescence technique.

It was found that the LC rats were not hyperphagic on the chow diet, nor did they gain excess body weight. Epididymal fat-pad weights also indicated that the LC rats were not obese relative to controls. The LC rats were, however, abnormally reactive to the QHCL diet compared to controls (-59.7$\pm$5.8% vs. -43.6$\pm$3.0% reductions from baselines, respectively).

These data indicate that transplanted NE-containing neurons can induce finickiness to a bitter QHCL diet, but not hyperphagia and excess body-weight gain on a standard diet. This suggests either that the sensitivity of the finickiness effect is greater than that of the hyperphagic effect, or that different substrates mediate the two phenomenon. Transplantation of greater numbers of NE-containing neurons may be required to demonstrate an effect of LC implants on hyperphagia and body weight gain. Nonetheless, this investigation reveals that neural transplants can influence at least certain aspects of feeding behavior, and may be useful in lesion/replacement studies examining the role of transmitter-identified neurons in the neurobiology of feeding behavior.

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20.15 HEPATIC VAGOTOMY BLOCKS DIETARY OBESITY BUT NOT DEXAMETHASONE ANOREXIA. C.M. Woodbury*, F. Rodman* and R.M. Gold (SPON: A. Trehub). Department of Psychology, Univ. of Mass., Amherst, MA 01003.

Hepatic vagotomy has been shown to block glucagon anorexia. This suggests that glucagon anorexia is mediated by hepatoreceptors which send a satiety signal to the brain via the vagus nerve. The liver is known to contain glucocorticoid receptors. The present report examined the effect of hepatic vagotomy on another anorexic agent, the potent synthetic glucocorticoid Dexamethasone. We incidentally examined the effect of hepatic vagotomy on dietary obesity.

Prior to surgery, five days of a diet of 10% sugar water and Purina Mouse Chow increased the weight gain of female rats from 0.5 g/day to 6.2 g/day. Over the first 14 days following hepatic vagotomy the weight gain fell to only 0.6 g/day, whereas sham vagotomized controls continued to gain 2.5 g/day on the palatable diet. Thus, despite previous reports, hepatic vagotomy blocks dietary obesity when the rats are tested immediately after surgery. Perhaps the orosensory properties of good tasting food activate efferents to the liver which block hepatically mediated satiety in response to absorbed nutrients.

In contrast, despite the highly palatable diet, 100 ug/Kg of Dexamethasone, delivered for three days via the drinking water, produced rapid weight loss in both the hepatic vagotomized and sham vagotomized groups (12.3 and 19.1 g/day weight losses, respectively). The anorexic effect of dexamethasone must therefore be mediated via other glucocorticoid receptors such as those in brain, pituitary, and thymus.

20.16 ADRENAL SUPPRESSION FOLLOWING HYPOTHALAMIC KNIFE-CUTS THAT PRODUCE OBESITY. A. Sylvan*, J. Fecko*, R.M. Gold and J. McElroy*, Division of Neuroscience and Behavior, Psychology Department, University of Massachusetts, Amherst, MA 01003.

The hypothalamic knife cuts that elicit hyperphagia and obesity in rats are located alongside the paraventricular nucleus. The PVN has also been identified as the primary source to the pituitary of a 41AA corticotropin releasing factor. This suggests that knife cut obesity is produced via insult to the hypothalamo-pituitary-adrenal axis.

Ether stress tests were conducted prior to and 4 days after unilateral adrenalectomy and/or knife cuts. As compared with double sham-operated controls, the serum corticosterone response to ether stress after unilateral adrenalectomy was reduced by 39.2%. Hypothalamic knife cuts alone reduced the ether stress response 64.5%. Combined surgeries (knife cuts plus unilateral adrenalectomy) increased the suppression to 74.4%. Knife-cuts, but not unilateral adrenalectomy, also reduced basal (pre-stress) corticosterone levels.

Fifteen days after the initial surgery, all remaining adrenals were removed. In the previously double sham-operated rats, adrenal weights at 15 days were 37.9% greater than at initial surgery (vs. an 18.4% growth of body weight). Hypothalamic knife cuts without unilateral adrenalectomy completely suppressed this maturational adrenal growth (vs. a 71.5% increase in body weight). Using the same paradigm, sham knife cuts with unilateral adrenalectomy incerased the weight of the remaining adrenal by 90.5%. Hypothalamic knife cuts blocked 73% of this compensatory hypertrophy.

Adrenocortical secretions and adrenal weights were consistently suppressed by obesifying hypothalamic knife cuts. This supports the notion that hypothalamic knife cut obesity is mediated via interruption of the adreno-regulatory axis.

20.17 POTASSIUM-INDUCED NOREPINEPHRINE RELEASE FROM HYPOTHALAMUS OF RATS: MODULATION BY INSULIN. B.J. Mullen* and R.J.Martin(SPON: L.M.Proenza).Dept. of Foods & Nutrition, University of Georgia, Athens, GA 30602

Postprandial insulin release is associated with a satiated or anabolic state while preprandially the converse is true. Although insulin plays a major role in glucose metabolism, its influence on central feeding mechanisms is unclear. Since the activity of hypothalamic neurons has been shown to reflect feeding behavior, we investigated the possibility that insulin's effect on food intake was mediated by direct action at this brain site. The effect of insulin on in vitro hypothalamic norepinephrine (NE) release was measured. Ad libitum chow fed male Sprague Dawley rats were decapitated and brains were removed. The hypothalamus was dissected into a lateral (LH) portion and a ventral medial (VMH) portion. Control tissues were preincubated for 60 minutes in Krebs-Ringer buffer containing 10 mM glucose, 10 mg% sodium metabisulfite and 1 uM pargyline. Experimental tissues were preincubated in the same buffer containing 50 uU/ml insulin. In order to induce NE release, tissues were then depolarized for 10 minutes with buffer containing 100 mM potassium. Media were collected, concentrated over an alumina column, and assayed for NE using HPLC with electrochemical detection. In the VMH, insulin depressed release of NE while in the LH, a slight elevation of NE was noted. Considering NE's putative inhibitory role in the hypothalamus, a depression of NE in the VMH should result in satiety, while an elevation of NE in the LH should result in an inhibition of feeding. These data are in agreement with Porte et al. who has demonstrated a satiating effect of insulin when infused into lateral cerebral ventricles. Thus, these results suggest that that the peripheral effect of physiological levels of insulin are reflected in central mechanisms, however further research in this area is needed to confirm these findings.

[1]Y. Oomura and H. Kita. 1981. Diabetologia 20:290.

[2]D. Port and S. C. Woods. 1981. Diabetologia 20:274.

20.18 EFFECTS OF ALTERING FATTY ACID OXIDATION RATES IN THE HYPOTHALAMUS OF RATS ON FOOD INTAKE. J. L. Beverly* and R. J. Martin, Dept. of Foods and Nutrition, Univ. of Georgia, Athens, GA 30602

Metabolic profiles of the ventral hypothalamus are related to feeding status in rats (Kasser et al., Am. J. Physiol. 1985, in press) such that the rate of fatty acid oxidation (FAO) in the ventral lateral hypothalamus (VLH) is reciprocally related to level of food intake. The objective of this research is to determine if induced changes of the metabolic pattern in the VLH will influence the amount of food eaten. A two step approach has been undertaken, an in vitro evaluation of pharmacologic compounds followed by in vivo central infusions.

in vitro: Compounds which increase (HL-0654, clofibrate and L-carnitine) or decrease (4-pentenoic acid) hepatic FAO were evaluated at concentrations of .01 mM to 1 mM (except clofibrate, .1 mM to 10 mM). Compounds were added to incubation media (Krebs bicarb., 2% BSA, 1 mM palmitate) and $^{14}CO_2$ from ^{14}C-1-palmitate (1 uCi/umole) collected after 3 hr incubations with hypothalamic, cortical or hepatic tissue (collected at incubation from 200 g rats fed chow fed lib). Clofibrate stimulated hepatic FAO at 2 mM but had no effect on (.01 mM to 2 mM) or inhibited (2 mM to 10 mM) FAO in hypothalamic tissue. HL-0654 inhibited FAO in hypothalamic tissue at 1 mM but had no effect on hepatic FAO rates. 4-pentenoic acid and L-carnitine reduced or increased FAO, respectively at all concentrations measured.

in vivo: L-carnitine (1 mM) and 4-pentenoic acid (.1 mM) were compared to sCSF in rats with chronic 3rd ventricle cannulae. Compounds were administered dissolved in sCSF via osmotic pump (.5 ul/hr) for 14 d, after which rats were sacrificed. No differences in food intake or body weights during infusions were observed. In vitro evaluation indicated limited diffusion of compounds as, of tissues evaluated, only the ventral medial hypothalamus exhibited expected alterations in FAO. Evaluations with bilateral cannulae immediately dorsal to the VLH are presently in progress.

20.19 THE EFFECT OF SIX-HYDROXYDOPAMINE ON FOOD INTAKE AND BRAIN BIOGENIC AMINES IN CHICKS. W. J. Kuenzel. Dept. of Poultry Sci., Univ. of Maryland, College Park, Md. 20742.

Six-Hydroxydopamine (6-OHDA) was administered to chicks intracerebroventricularly (ICV) to study the effect of the neurotoxin on food intake and motor activity. The experimental design consisted of six groups in which two levels of pargyline (50 mg/Kg and 100 mg/Kg) and three concentrations of 6-OHDA (0, 200 and 300 µg) were examined. Six chicks were included in each of the experimental groups and pargyline (P) was injected intraperitoneally 15 min. prior to the ICV injection when two weeks of age. Food intake and body weight were monitored daily for 14 days and a subjective scoring of motor activity and behavior were determined daily. When four weeks of age all chicks were sacrificed and brains were dissected rapidly. While freezing, each brain was dissected into three pieces: striatum, hypothalamus and brainstem. All brain pieces were frozen in liquid nitrogen for later determination of dopamine, norepinephrine, epinephrine, 5-Hydroxyindole 3-acetic acid, and serotonin. Reverse phase high pressure liquid chromatography with electrochemical detection was used to quantitate biogenic amines in brain tissue. A dose-response effect was found; the greatest reduction in food intake and body weight loss occurred in the group injected with 100 mg/kg P followed by 300 µg 6-OHDA. The aphagic response, however, was transient and lasted only an average of 4 days in the highest drug treatment group. Several behavioral abnormalities were observed particularly in the two 300 µg 6-OHDA groups. The most commonly observed abnormalities included: hyperactivity, imbalance, head orientation problems, head shakes and pecking the air while attempting to feed. The largest differences in brain concentrations of biogenic amines occurred in the striatum. Striatal dopamine concentrations were found significantly depressed ($P < 0.05$) in the 6-OHDA treatment groups compared to controls. Similar to the response in chicks following large bilateral lesions made throughout the lateral hypothalamic-thalamic brain regions, aphagia persisted for only a few days in experimental groups. These data are in contrast to mammalian findings in which aphagia and adipsia last for several days and tube feeding is required to maintain rats subjected to bilateral hypothalamic lesions or ICV administration of 6-OHDA.

FEEDING AND DRINKING II

21.1 IMPACT OF FOOD DEPRIVATION ON HYPOTHALAMIC AND CORTICAL α1 AND α2-NORADRENERGIC RECEPTORS. M. Jhanwar-Uniyal*, M. Darwish*, G. Beitmirza* and S.F. Leibowitz. (SPON: L-M. Kow) The Rockefeller University, New York NY 10021.

Norepinephrine (NE) alters feeding behavior when injected directly into specific hypothalamic areas. The medial paraventricular nucleus (PVN) has been identified as the most sensitive brain site to NE, which stimulates food intake through α2-noradrenergic receptors. NE turnover has been found to be increased specifically in this nucleus in response to food deprivation. The present biochemical study was undertaken to investigate the impact of short- and long-term food deprivation on α1 and α2-noradrenergic receptors, in large brain areas and discrete hypothalamic nuclei.

Male albino rats, maintained on a 12:12 light/dark cycle, were deprived of food (water was provided ad lib) for either 0h, 3h (18.00h-21.00h), 6h (00.00h-06.00h) or 48h. The rats were sacrificed by decapitation, and 3 large hypothalamic areas (medial, lateral, and basal) and frontal cortex were microdissected. In other animals, 8 discrete hypothalamic nuclei were micropunched. Standard radioligand binding techniques were employed with the α2-noradrenergic agonist [^{3}H]p-aminoclonidine ([^{3}H]PAC, 0.05-1.0 nmoles; 3.0 nmoles for discrete hypothalamic areas), and the α1-noradrenergic receptor antagonist [^{3}H]prazosin (0.05-1.0 nmoles). Nonspecific binding was determined in the presence of phentolamine (50 µM). The specific binding was expressed in fmoles/mg protein.

The results demonstrated that, of the 3 large hypothalamic regions examined via Scatchard analysis, only the medial hypothalamus showed a significant down-regulation of its α2-noradrenergic receptors in response to 48h food deprivation. A 52% decrease ($P < 0.02$) in Bmax was demonstrated by the high affinity binding sites, in contrast to no change in low affinity sites. The K_D for both high and low affinity sites was unaffected. The α1-noradrenergic receptors did not exhibit a change as a result of food deprivation in any of the areas examined. Short-term food deprivation (3h), specifically during the onset of the dark period, also resulted in a down-regulation (-65%, $P < 0.001$) of the α2-noradrenergic receptor sites specifically in the PVN. However, 6h food deprivation during the second half of the dark period resulted in an up-regulation (+54%, $P < 0.05$) of PVN [^{3}H]PAC binding. These results indicate that food deprivation produces site-specific changes in α2-noradrenergic receptors in the medial hypothalamus, particularly in the PVN. Together with other biochemical evidence revealing deprivation-induced changes in PVN NE turnover, they support pharmacological results indicating a specific role for a PVN α2-noradrenergic system in the regulation of feeding behavior.

(This research was supported by NIH grant MH 22879)

21.2 DO INTESTINAL CALORIES DETERMINE THE INTERMEAL INTERVAL (IMI)? V. E. Mendel, J. Kobayashi and T. J. Kalogeris. Depts. of An. Physiol. and An. Science and, Food Intake Lab, Univ. of Calif., Davis, CA 95616.

Six rats, each with gastric and duodenal cannulas were individually housed and maintained on semi-purified diet and tap water. All were trained to sham-feed Vivonex™-high nitrogen (VHN) or 20% sucrose. During experimental periods the animals sham-fed liquid VHN with a caloric concentration of 1 Kcal/ml. Rats were fasted 13 to 16 hr, the gastric cannula were then opened and the stomach flushed 2-4 times with 0.9% saline. In experiment 1, ten ml of 20% sucrose at 0.25, 0.5 and, 1.0 Kcal/ml was duodenally infused at a rate of 0.42 ml/min beginning 10 min after sham-feeding was well established; thus, caloric loads of 2.5, 5.0 and, 10 Kcal were delivered in about 24 min. In experiment 2, 5 ml VHN was duodenally infused at 0, 1, 2, 3 and 4 Kcal/ml for total caloric loads of 0, 5, 10, 15 and 20 Kcal over 12 min. The results are shown in the table.

Caloric load	n	Vol. (ml)	IMI (min)
0.0	5	5	9.28 ± 5.19
* 2.5	4	10	16.35 ± 6.53
5.0	3	5	24.42 ± 4.12
* 5.0	4	10	52.47 ± 8.44
10.0	8	5	51.36 ± 10.18
*10.0	4	10	>120
15.0	2	5	>120
20.0	3	5	>180

*20% sucrose.

We conclude that IMI increases exponentially with increasing caloric load, however, 20% sucrose produces a significantly more rapidly rising curve than does VHN. It is not yet clear whether the volume infused or the duration of infusion interacts with IMI to influence its duration. It appears that the small intestine is capable of producing a signal in response to caloric load that results in satiety, although factors other than calories may be involved.

21.3 HEPATIC VAGOTOMY OR TOTAL LIVER DENERVATION CAN BLOCK PANCREATIC GLUCAGON'S SATIETY EFFECT. L. MacIsaac*& N. Geary (SPON: D. Hood). Dept. of Psychology, Columbia University, New York, NY 10027.

The inhibitory effect of pancreatic glucagon (PG) on meal size in rats has been blocked by selective hepatic vagotomy (HV) in some experiments[1,2], but survived HV or total liver denervation (TLD) in others[3,4]. We therefore reinvestigated the effects of liver denervations on the inhibitory actions of PG and of epinephrine (EPI), another hormone that may inhibit feeding via an hepatic neural mechanism[5]. First, either HV, selective hepatic denervations that spare only the hepatic branch of the vagus (PLD), or sham operations (SH) were done, and evaporated milk meal sizes compared after after i.p. injection of vehicle (VEH), PG, or EPI. PG's satiety effect was blocked by HV, but not by PLD. EPI inhibited meal size potently in both HV and PLD rats. Mean +SEM meal sizes:

		PG(mcg/kg)		EPI(mcg/kg)	
	VEH	100	400	12.5	50
SH	18.1	14.7**	12.3**	13.4**	9.3**
(n=20)	±0.7	±0.9	±1.0	±1.1	±1.2
HV	13.9	12.8	13.9	9.8**	8.5**
(n=14)	±0.9	±1.3	±1.3	±1.4	±1.4
PLD	14.9	9.8**	9.5**	11.7*	6.6**
(n=12)	±1.3	±1.2	±1.3	±2.0	±1.1

*p<.05 vs VEH, Dunnett's test after ANOVA; **p<.01.

The same rats were then reoperated to produce TLD; i.e. HV was done on PLD rats, PLD on HV rats, and SH on SH rats. TLD rats did not respond to PG, but EPI still inhibited meal size:

		PG(mcg/kg)			EPI(mcg/kg)	
	VEH	100	200	400	12.5	50
SH	14.7	12.3*	11.5**	11.4**	13.1	11.3*
(n=14)	±2.6	±1.3	±0.8	±0.9	±1.0	±1.1
TLD	12.3	11.2	12.0	11.7	7.8**	8.4**
(n=11)	±0.6	±1.2	±1.4	±1.4	±0.8	±1.2

These data indicate that the hepatic branch of the vagus is necessary for PG's satiety effect and fail to suggest a role for any other component of hepatic innervation in this effect. Further work is needed to determine the generality of this conclusion, however, because PG has continued to inhibit meal size after HV or TLD under apparently similar test conditions[3,4]. Finally, these data extend previous indications [2,3] that EPI's inhibitory effect on meal size is not mediated by hepatic innervation.

REFS: [1]PhysiolBehav31:391,1982. [2]SocNeurosciAbstr10:1109, 1985. [3]PhysiolBehav31:391,1983. [4]FedProc44:1164,1985. [5]JCCPsychol96:361,1982.

21.4 **SEROTONIN-INDUCED DRINKING IN RATS: FURTHER ANALYSIS** **N.E. Rowland and M.J. Fregly*. Depts. Psychology and Physiology, Univ. of Florida, Gainesville FL 32611.**

We have previously shown that serotonin(5HT) induces drinking in rats after peripheral (sc) administration. This drink may be inhibited by methysergide. Drinking is also induced by 5-hydroxytryptophan (5HTP), the precursor of 5HT, and this response is blocked by decarboxylase inhibitors as well as by methysergide. 5HT and 5HTP also induce the release of renin from the kidney, and it appears that the consequent formation of angiotensin II (AII) may mediate the drinking response.

To investigate this possibility, 5HT (4 mg/kg) and 5HTP (25 mg/kg) were administered sc to rats that were acutely nephrectomized, or laparotomized controls. The drinking response to 5HT was abolished by nephrectomy, but that to 5HTP was largely unaffected. Lower doses of 5HT also failed to induce drinking in nephrectomized rats. This suggests that the absence of drinking to 5HT in nephrectomized rats is not due to debilitating hypotension, and thus the peraistence of 5HTP drinking after nephrectomy is puzzling. We next showed that 5HTP drinking could be abolished by either peripheral or cerebroventricular (ic) injection of the 5HT antagonist metergoline. The role of angiotensin II in brain seems to be nonessential because ic saralasin had no effect on drinking induced by peripheral 5HT or 5HTP, but the same dose (250ng, ic) antagonized the dipsogenic action of AII (200 ug/kg, sc). It thus appears that no single theory can account for drinking induced by 5HT (which cannot cross the blood brain barrier) or 5HTP (which can enter the brain and be decarboxylated to 5HT).

In related experiments we measured water intake following peripheral administration of the 5HT agoniat, quipazine, and the 5HT- releasing drugs, fenfluramine and p-chloroamphetamine. In two studies, quipazine (10-20 mg/kg, ip) induced significant drinking, mostly in the 2nd hour after injection. The amount consumed also increased up to 5x with repeated daily administration. However, in two other studies quipazine produced no drinking; the reasons for this discrepancy are not clear to us. Quipazine has been reported to produce a short-lived rise in plasma renin activity, but we found no increases at the time (1-2 hr) at which any drinking normally would occur. P-chloroamphetamine, in doses which release renin, did not cause drinking and all of the rats were severely debilitated. Fenfluramine also failed to stimulate drinking. **Support: NIH AM31837-03.**

21.5 EFFECTS OF HYPOPHYSECTOMY ON HYPOTHALAMIC OBESITY IN RATS: ROLE OF CORTICOSTERONE. R. L. Smith* and B. M. King. Dept. of Psychology, University of New Orleans, New Orleans, LA 70148.

Recent studies have found that obesity induced by lesions of the ventromedial hypothalamus (VMH) in rats and mice is both reversed and prevented by complete adrenalectomy (ADX)(i.e., plasma corticosterone levels < 1.0 µg/dl) and restored by administration of glucocorticoid hormones (Debons et al., Endocrinology, 112: 1847-1851, 1983; King et al., Am. J. Physiol., 245: E194-E199, 1983). Similar results have been reported for the effects of complete hypophysectomy (HYPOX) in mice (i.e., plasma corticosterone < 1.0 µg/dl)(Debons et al., Physiol. Behav., 29: 695-699, 1982), but of the many older studies that have investigated the effects of hypophysectomy in VMH-lesioned rats, none have reported any substantial suppression of weight gain or body fat content. Most of these studies, however, failed to include a sham hypophysectomized (SHYPOX)-VMH lesioned control group and none measured plasma levels of corticosterone. The present study therefore directly compared the effects of hypophysectomy and adrenalectomy in female rats given VMH lesions.

Commercially prepared HYPOX, ADX, and SHYPOX-SADX rats (Charles River Breeding Labs) received sham or bilateral VMH lesions approximately two weeks after the initial surgery. Body weight was measured daily for 20 days after the lesions. The SHYPOX-SADX-VMH rats (n=8) displayed a mean weight gain of nearly 200 g over this time period, but the abnormal weight gain was totally absent in VMH rats with complete adrenalectomy (n=8). Adrenalectomy did not significantly affect intracranial self-stimulation and thus the absence of hyperphagia was probably not due to fatigue or a general loss of motivation. Six rats with incomplete adrenalectomies displayed a moderate obesity (97 g/20 days) even though stress-induced corticosterone levels were very low (mean=2.7 µg/dl). Hypophysectomy partially suppressed weight gain in VMH animals, but as reported by others, the HYPOX-VMH rats (n=8) gained more weight than HYPOX rats with sham lesions (n=10)(83.9 g vs. -12.3 g/20 days). The HYPOX animals were found, however, to have low levels of plasma corticosterone (from residual pituitary tissue or of diencephalic origin) similar to that found in incompletely ADX rats. It was concluded that hypophysectomy and adrenalectomy have similar effects on hypothalamic obesity and that glucocorticoid hormones play a permissive role in the obesity syndrome, i.e., obesity is prevented in their absence, but even small concentrations allow for some abnormal weight gain.

21.6 CIRCADIAN VARIATION OF HEPATIC GLYCOGEN: EFFECT OF GLUCAGON ON FOOD INTAKE AND GLYCOGEN CONCENTRATION. L.J. Stein*, L. O'Farrell, and D. Novin. Dept. of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

The mechanism by which peripheral glucagon reduces food intake is unknown. One possibility is that glucagon (GCN) may decrease intake by activating hepatic glycogenolysis. To test this hypothesis, GCN's ability to reduce meal size and to deplete hepatic glycogen were compared. Each of 4 groups of adult male rats was maintained on ad lib chow pellets and trained to eat a chow mash meal at a different time of the (12/12) light cycle: beginning light (BL, n=12), mid-light (ML, n=15), beginning dark (BD, n=13), and mid-dark (MD, n=13). Thirty-min food intakes were recorded following i.p. saline or GCN (600 µg/kg) and are expressed as % of baseline. Glucagon reliably reduced 30-min food intake only at ML.

Group	Saline	GCN	p
BL	-4.3 ± 17.7%	-12.8 ± 23.3%	n.s.
ML	+25.8 ± 11.7%	-46.8 ± 13.1%	<.005
BD	-13.3 ± 17.9%	-11.7 ± 5.4%	n.s.
MD	+19.6 ± 16.5%	+25.2 ± 30.2%	n.s.

One week later, rats were again injected with saline or GCN and food was removed. After 30 min, rats were decapitated, truncal blood collected, and liver samples removed for glycogen assay. Glycogen was influenced by time of cycle ($F(3,45)=10.5$, $p=.008$) and by GCN administration ($F(1,45)=10.5$, $p=.003$). The interaction was not significant ($F(3,45)=0.2$, $p=0.9$). Glycogen of saline rats was highest at BL and lowest at BD. At ML, glycogen did not reliably differ between saline (37.3 ± 1.2 mg/gm) and GCN (31.3 ± 4.1 mg/gm), even though food intake was significantly reduced by GCN. Conversely, at BD, glycogen was reliably reduced in GCN-injected rats (saline: 26.0 ± 2.3 mg/gm vs GCN; 16.8 ± 1.6 mg/gm, $p<.05$), while food intake was not affected. Plasma glucose levels did not differ as a function of either time of cycle or GCN administration. These data demonstrate that the ability of GCN to reduce food intake can be dissociated from its action to reduce hepatic glycogen, and suggest that glycogenolysis, systemic hyperglycemia, and reduction of total hepatic glycogen are not sufficient to explain GCN-induced reduction of meal size.

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21.7 Effects of the Narcotic Antagonist, Nalmefene, on Spontaneous and Insulin - Induced Food Intake and Body Weight Gain in Male Rats. J.W. Simpkins, M.E. Michel and R. Tuttle,* Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL 32610 and Key Pharmaceuticals, Miami, FL 33269.

Narcotic antagonists have been shown to acutely block the hyperphagic response to a variety of stimuli. However, their efficacy in chronically reducing food intake is less well defined. We undertook studies to determine the acute and chronic effects of the narcotic antagonist nalmefene, insulin, and their combination, on food and water intake and on the rate of body weight gain in male rats. Rats, at an initial body weight of 290±2.5 gm were treated twice daily (0800 to 0900h and 2000 to 2100h) with protamine zinc insulin (5 IU/KgBW), nalmefene HCl (10 mg/KgBW), nalmefene followed 30 min later by insulin, or the vehicle for both compounds. Body weights were determined daily and food and water intake was quantitated for a 24h period, beginning on the 1st, 4th and 8th days of drug treatment. Insulin increased mean daily body weight gain by 33%; increased food intake on the 1st and 4th but not the 8th day of treatment; and increased water intake at each of the observation times. Nalmefene decreased spontaneous mean daily body weight gain by 30% but this effect was diminished late in the 8-day treatment period. Nalmefene reduced food and water intake on the 1st, but not 4th and 8th, day of treatment. However, nalmefene antagonized the insulin-induced increase in mean daily body weight gain and antagonized the hyperphagic and dipsogenic effects of insulin treatment. In a second experiment, these treatments were initiated when the male rats weighed 181±2.0 gm. Nalmefene reduced food intake on 1st, 9th and 16th days of treatment and mean daily body weight gain was reduced throughout the treatment period. When nalmefene was administered 2-times daily for 8 days prior to the initiation of twice-daily insulin treatments, nalmefene antagonized the insulin-induced increase in food and water intake on the 1st but not the 8th day of insulin treatment. After 8 days of nalmefene pretreatment, narcotic antagonist was less effective in antagonizing the insulin-induced increase in mean body weight gain. These studies indicate that (i) nalmefene can transiently reduce weight gain in larger animals (BW = 290 gm) and can persistently reduce weight gain in lighter rats (BW = 181 gm), (ii) nalmefene can antagonize insulin-induced food and water intake and body weight gain for 8 days and (iii) the ability of nalmefene to antagonize these effects of insulin dissipates with increasing length of pretreatment with the narcotic antagonist.

21.8 SATIETY IN 6-DAY-OLD RAT PUPS: IS GASTRIC DISTENSION THE ONLY CUE? C.B. Phifer, C.R. Sikes and W.G. Hall. Dept. of Psychology, Duke University, Durham, NC 27706.

The relative roles of pregastric, gastric and intestinal food cues as mediators of satiety were investigated in 6-day-old rat pups using a non-suckling model ingestive system (Hall, W.G., J. Comp. Physiol. Psychol., 93:977, 1979).

Sham-feeding pups (i.e. animals with stomach contents spilling through open gastric fistulas), experiencing only pregastric and non-distension related gastric cues, ingested over twice as much milk as pups with closed fistulas (18.3±2.4 vs. 9.1±1.2% body weight, $p<0.01$). This difference in intake was not due to the absence of intestinal food cues. In a second experiment, pups whose pylorus was occluded by a noose, and therefore receiving no intestinal food cues, ingested less than pups with an open pylorus [$F(1,21)=28.7$, $p<0.01$]. Furthermore, when a 5% body weight gastric preload of milk (or a non-nutritive preload of saline) was given to pups with an open or closed pylorus, the two groups exhibited equivalent degrees of intake inhibition. The amount of suppression in each group was equal to the volume of the preload. Gastric distension appeared to be the critical factor for inhibition of feeding; intake was terminated when stomach content volume reached approximately 6.5% of body weight, regardless of pyloric noose or preload condition.

In a third experiment, two groups of pups, i.e. one group with open pyloric nooses and one group with closed nooses, were allowed to load their own stomachs by orally ingesting milk until they terminated intake. Subsequently, gastric fistulas were opened in half the pups from each group, allowing ingested milk to spill from the stomachs. All pups were then given a second opportunity to feed. Pups whose stomach contents had been emptied resumed ingestion and consumed over 12% of their body weight in milk, regardless of whether they had milk in their intestines or not. Pups whose stomachs had not been emptied ceased feeding after consuming only 3% of their body weight [$F(1,28)=56.1$, $p<0.01$].

We conclude that intake termination in 6-day-old rat pups ingesting independently is a function of gastric cues, specifically gastric distension. Pregastric cues, acting in the absence of gastric cues, are not sufficient to suppress intake. Intestinal cues, both pre- and postabsorptive, appear to be unnecessary. Only at later ages do intestinal signals become important.

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21.9 **PERIPHERAL ACTIONS OF FENFLURAMINE IN ANOREXIA AND TOLERANCE.** **J.Carlton and N.E.Rowland. Department of Psychology, Univ. of Florida, Gainesville FL 32611.**

The actions of fenfluramine (FEN) on central serotonin (5HT) systems have been well-established, but the relationship between these actions and the anorectic effect of the drug have been questioned. FEN has several peripheral actions including thermogenesis, metabolism (including futile cycles), and gastric clearance. We report a series of experiments investigating the role of peripheral factors in the production of anorexia and tolerance to FEN. 1. SHAM FEEDING. Rats with gastric fistulas were trained to sham feed a liquid diet. When intakes were stable, d,l-FEN (5 mg/kg) was given 30 min prior to daily sessions. FEN suppressed sham feeding on the first day by a % comparable to the inhibition of real feeding by this dose. Tolerance to the suppression of sham feeding developed within 5 days. Thus, anorexia and tolerance to FEN develop in the absence of normal accumulation of food in the upper digestive tract. 2. PRELOADS. Rats with indwelling gastric catheters were adapted to a deprivation schedule, and on test days were infused with loads of 0, 25 or 50% of the calories normally consumed. These preloads produced a graded suppression of the subsequent meal. FEN (0, 1 or 2.5 mg/kg) produced effects that were additive, but not interactive, with the preload. Thus, FEN does not seem to potentiate natural satiety signals. 3. CCK. The CCK antagonist, proglumide (200 mg/kg) reversed CCK (20 ug/kg) anorexia, but had no effect on FEN (5 mg/kg) anorexia. Thus, FEN does not appear to produce satiety indirectly by releasing CCK. 4. GUT 5HT. The concentrations of 5HT and 5HIAA were measured in rats given FEN (5 or 15 mg/kg) acutely or chronically (8 days). Brain, but not gut 5HT and 5HIAA were reduced in all treated groups. This suggests that FEN may not work via release of gut 5HT, although more sensitive measurements of release should be made. 5. EXERCISE. Syrian hamsters with free access to running wheels did not decrease their running (15 km/night) to FEN (20 mg/kg/day x 8 days). Both exercise and sedentary groups showed small but nonsignificant decreases in overall food intake and body weight gain, relative to vehicle-treated groups, but there was no selective enhancement of drug effect in the exercise group. FEN does not seem to affect muscular efficiency in this species. **Support: NSF BNS 82-16528.**

21.10 LESIONS IN THE VENTROMEDIAL HYPOTHALAMUS OR THE AMYGDALA DO NOT AFFECT NALOXONE'S SUPPRESSION OF WATER CONSUMPTION. G. A. Olson, M. F. Pignatiello*, A. J. Kastin, B. F. Geiger* and R. D. Olson. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

Previous work has indicated that opiate receptors of the ventromedial hypothalamus (VMH) do not mediate the suppression of feeding behavior produced by naloxone, since naloxone reduced eating in both VMH-lesioned and normal rats in deprivation-induced and appetitively-motivated feeding. One purpose of the present study was to determine if this finding also occurs with water consumption. A second purpose of this experiment was to test the effect of naloxone in rats with lesions in the amygdala. Previous studies had found that basolateral amygdala (BA) lesions produce either hypodipsia, hyperdipsia or no effect depending on their exact location. The effect of naloxone on water intake in these rats was not studied.

MIF-1 has been found to act like naloxone in some situations, including the suppression of fluid consumption by naloxone. Thus, both MIF-1 and naloxone were used here to evaluate their effects on drinking in these lesioned rats.

Four VMH-lesioned, six BA-lesioned, and five sham-operated adult female Long-Evans hooded rats were fed ad lib for several months after the operations, thus allowing ample recovery time. All animals were deprived of water for 12 hours before testing began and were deprived of food during testing. Amount of water intake was recorded every 30 min. for two hours starting immediately after the injections, which were given ip and consisted of either 2 mg/kg of naloxone, 2, 4 or 8 mg/kg of MIF-1, or diluent. Each rat received all five injections, one per day, every other day, over a nine day period.

There was a significant drug effect, with naloxone suppressing water intake relative to the diluent control group and to the 8 mg/kg dose of MIF-1. No other comparisons were reliable, and there were no interactions associated with the drug conditions. Although MIF-1 tended to reduce drinking, the effect was not statistically significant. There was no significant main effect for lesions. A significant lesion x time interaction occurred, however, with the BA-lesioned rats showing the greatest drinking for the first 30 min but slowed consumption in the 30-60 min interval relative to the other two groups, which continued high intake levels through 60 min. The VMH-lesioned rats also drank significantly more than the sham animals for the second interval. There were no differences between groups in consumption rate after 60 min.

These findings are in agreement with previous work on the suppression by naloxone of consumption in VMH-lesioned rats. Apparently the intact basolateral amygdala, like the VMH, is unnecessary for naloxone's effect on water consumption.

21.11 HYPERPHAGIA-INDUCING HYPOTHALAMIC AND PONTINE KNIFE-CUTS INTERRUPT OXYTOCIN PATHWAYS IN THE RAT BRAIN. A.L. Kirchgessner*, A. Sclafani and G. Nilaver. Downstate Medical Center of SUNY, Brooklyn Coll. of CUNY, Columbia Univ. Coll. Phys. & Surg., NY.

Bilateral parasagittal knife-cuts in the medial hypothalamus (MH) adjacent to the paraventricular nucleus (PVN) produce hyperphagia and extreme obesity in rats. A unilateral MH cut combined with a contralateral coronal cut in the caudal ventro- lateral pontine tegmentum also produces overeating. This indicates that fibers critical to hypothalamic hyperphagia extend into the hindbrain. At least some of these fibers may be PVN efferents as evidenced by retrograde labeling of PVN neurons following injection of HRP tracer into a pontine knife-cut (Kirchgessner and Sclafani, 1983). Since the axons which constitute the caudal projections from the PVN are predominantly oxytocinergic (Nilaver et al., 1980), we studied the effects of hyperphagia- inducing MH and pontine knife-cuts on oxytocin (OT) immunoreactivity.

Adult female rats received either (1) a unilateral parasagittal MH cut, (2) a unilateral coronal ventral pontine cut, or (3) a MH cut combined with a contralateral pontine cut. After 3 to 8 days of survival, the brains (100 um; coronal) were immunoreacted with specific rabbit antiserum to OT, employing the avidin-biotinylated peroxidase complex (ABC) method.

The results revealed that parasagittal MH and coronal pontine knife-cuts interrupt, although at different levels of the neuroaxis, OT fibers that course from the PVN in a caudo-lateral direction to reach the hindbrain. Distal to the unilateral MH cut, at the level of the midbrain, descending OT fibers that course dorsal to the substantia nigra were sparse compared to the contralateral unoperated side. In the medulla, OT immunoreactivity was also significantly reduced, on the cut side, in the ventrolateral tegmentum where fibers normally emanate from the main descending bundle to terminate in the nucleus of the solitary tract and dorsal motor nucleus of the vagus. Similar reductions in medullary OT were observed following unilateral ventral pontine cuts. However, although the unilateral MH and pontine knife-cuts clearly interrupted the ventro-laterally descending OT fiber bundle on the operated side, they did not affect the OT innervation of the vagus-solitarius complex. This suggests that OT fibers from the PVN to the hindbrain are crossed. Also, since a high density of OT fibers was seen in the vagus- solitarius complex following combined MH and contralateral pontine transections, bilateral innervation may be supplied by the periventricular bundle.

The present study demonstrates that hyperphagia-inducing knife-cuts interrupt PVN-hindbrain OT fibers, and suggest a role for OT in the hypothalamic hyperphagia syndrome. In addition, our findings indicate that the precise course of the descending PVN OT system is still only partially understood.

21.12 EFFECTS OF FOOD DEPRIVATION AND FOOD RESTRICTION ON PLASMA AND CEREBRAL TRYPTOPHAN AND TYROSINE CONTENT, ON PLASMA DBH ACTIVITY AND ON CEREBRAL BIOGENIC AMINES IN RATS. L. Thibault* and A.G. Roberge. Centre de Recherche en Nutrition et Département de Nutrition, F.S.A.A., Université Laval, Ste-Foy, Québec, G1K 7P4.

Adult rats were submitted to 4 days food deprivation or to 7 consecutive days on a 60% restricted energy intake, to obtain a weight loss of about 20%. Plasma and brain tryptophan and tyrosine content were measured, as well as brain biogenic amines in five different structures using a fluorimetric technique. Plasma tryptophan and tyrosine concentrations were lowered in starved and food restricted rats whereas plasma DBH activity was significantly increased in either group. Brain tryptophan and tyrosine content was decreased in starved rats whereas in food restricted rats to a significant increase in tryptophan content corresponds a significant decrease in tyrosine level. In food restricted rats, a significant increased DA level was observed in the brainstem, raphe nuclei and hypothalamus whereas a corresponding decrease was noted in the striatum. In starved rats brain, similar changes were observed in DA level except a normal value noted in the brainstem. In food restricted rats, the NA content was increased in the brainstem and decreased in the raphe nuclei whereas in starved rats brain, a decrease was observed in the raphe nuclei and thalamus but a significant increase was noted in the striatum. In starved rats brain, the 5-HT content did not change whereas in food restricted rats, a significant increase was observed in the brainstem and raphe nuclei and a significant decrease noted in the striatum. In the present study, starvation and food restriction induced similar effects in the plasma thus suggesting a common response to stressfull situations. In the CNS, the DA to NA ratio has revealed a DA mobilization in all structures studied except the brainstem in either group whereas the 5-HT to 5-HIAA ratio has demonstrated either a low 5-HT degradation or a high 5-HT utilization according to the situation and the neuroanatomic system. (Supported by NSERC of Canada and Fonds FCAR, Québec).

21.13 SINGLE-UNIT RESPONSES IN THE ROSTRAL ZONA INCERTA TO OSMOTIC STIMULATION OF THE ANTEROVENTRAL THIRD VENTRICULAR AREA (AV3V). D. Mok* and G.J. Mogenson (SPON: M.M. Robinson). Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Previous studies in the rat have shown that electrical stimulation of the zona incerta (ZI) elicits drinking (Huang and Mogenson, Exp. Neurol., 37:269, 1972) and that bilateral electrolytic lesions of this region produce water intake deficits in response to an osmotic stimulus (Walsh and Grossman, Physiol. Behav., 16:211, 1976). To further investigate the role of the ZI in the neural regulation of osmotic thirst, we made extracellular single-unit recordings from neurons in the rostral ZI of the rat while injecting hyper- and hypo-osmotic solutions into the anteroventral third ventricular area (AV3V), a region thought to contain osmoreceptive elements for thirst (Buggy et al., Am. J. Physiol., 236:R75, 1979).

Experiments were performed on urethane-anesthetized male Wistar rats. AV3V injections of hyper-osmotic solutions (1- or 2-µl of NaCl solutions having osmolarities of 1.2 or 0.6 osmol/l, respectively) and hypo-osmotic solutions (1- or 2-µl of distilled water) elicited 2 types of responses from ZI neurons. Cells which changed their firing rate relative to the baseline in response to either the hyper- or hypo-osmotic solution and whose firing rate remained unchanged in response to the alternate solution were designated Type I. The response patterns of Type I cells included an increase or a decrease in firing rate to a hyper-osmotic injection with no response to a hypo-osmotic injection and an increased or decreased firing rate to a hypo-osmotic injection with no response to a hyper-osmotic injection. Cells with firing rates that increased to one solution and decreased to the other, relative to the baseline, were designated Type II. Some Type II cells had firing rates that increased in response to a hyper-osmotic injection and decreased in response to a hypo-osmotic injection. Other Type II cells showed the reverse pattern. In a series of 16 animals we have been able to identify 13 Type I cells and 12 Type II cells in the ZI.

These results indicate that osmotic stimulation of the AV3V changes activity of neurons in the ZI and suggest that they have connections with osmo-receptive structures located near the AV3V. In addition, the findings provide further evidence that the ZI has a role in the central regulation of osmotic thirst.

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21.14 INTERACTION BETWEEN OVARIECTOMY-INDUCED OBESITY AND AREA-POSTREMA-ABLATION-INDUCED WEIGHT LOSS. Anita J. Bhatia*, Amina Bhatia*, Linda Stankovic*, Jon N. Kott and Nancy J. Kenney. Department of Psychology, University of Washington, Seattle, WA 98195.

Following ovariectomy (OVX), body weights of rats increase. In most cases, this is due to elevated food intakes although weights of OVX rats also increase when daily intakes are mildly restricted. Ablation of the area postrema and subjacent aspects of the caudomedial nucleus of the solitary tract (APX) results in transient hypophagia and weight loss. Although intakes of the lesioned rats do return to control levels, no compensation for the intial weight loss ensues.

To determine whether OVX would reverse, prevent and/or attenuate the weight loss due to APX, body weights and food intakes of rats which underwent OVX 2 or 5 wks before or 2 wks after APX were monitored. When OVX was performed 2 wks after APX, rate of weight gain of APX/OVX rats did not increase immediately. During the 2 wks following OVX, APX/OVX rats gained 20.1 ± 7.8 g and the APX/sham OVX rats, 20.1 ± 3.1 g. Sham APX/OVX rats gained 50.1 ± 4.1 g during that time ($F(1,14)=11.7$, $p<.01$ compared to APX/OVX). However, over the following 10 wks, APX/OVX rats gained more weight (42.6 ± 5.9 g) than did the APX/sham OVX rats (24.5 ± 5.6 g; $F(1,14)=5.0$, $p<.05$). By 12 wks after lesioning, weights of the APX/OVX rats (290 ± 19.2 g) did not differ from those of the sham APX/sham OVX rats (306 ± 10.4 g). Weights of the APX/sham OVX rats (267 ± 10.3 g) remained below those of the sham APX/sham OVX rats ($F(1,14)=7.1$, $p<.05$).

When OVX preceded APX by 2 wks, weight loss due to the APX was not attenuated. During the 2 wks following APX, the OVX/APX rats lost 42.4 ± 5.2 g and sham OVX/APX rats lost 33.9 ± 12.2 g. Weight changes of the OVX/APX rats remained indistinguishable from those of the sham OVX/APX rats for the next 10 wks. When OVX preceded APX by 5 wks, the OVX/APX rats lost 56.2 ± 5.1 g during the 2 wks following the ablation. At the end of that time interval, the OVX/APX rats did not differ in weight from the neurologically and ovarian intact controls.

Thus, ovariectomy, performed before or after area-postrema ablation has little effect on the weight loss due to the ablation. Ovariectomy may affect the weights of lesioned rats over the long-term such that APX rats without ovaries may weigh as much as neurologically and ovarian intact female rats. In no case, however, is the weight gain of the APX, ovariectomized rat as great as that of the non-lesioned, ovariectomized rat.

21.15 MICROINJECTED OPIOIDS INTO VTA OR PAG INDUCE OPPOSITE EFFECTS ON LH STIMULATION-INDUCED FEEDING. F. Jenck*, R. Quirion and R.A. Wise (SPON: A. Giovino). Center for Stud. in Behav. Neurobiol.,Dept. Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

Morphine was microinjected at the level of ventral tegmental area (VTA) or dorsal periaqueductal gray matter (PAG) and its effect recorded on latency for feeding in response to lateral hypothalamic (LH) electrical stimulation. Stimulation intensity was held constant and rats were stimulated at a range of different frequencies: for each stimulation frequency, latency to eat three 45 mg pellets was measured.

Morphine (0.8, 1.6, 3.2 and 8 nmoles) injected in 0.25 µl into the VTA was found to induce a dose-dependent facilitation of stimulation-induced feeding. Latency to feed as well as frequency threshold at which feeding started to appear, decreased; this facilitatory effect lasted for the 60-70 min duration of the test and was suppressed by an IP injection of 1.0 mg/kg naloxone. Conversely, morphine (1.6, 3.2, 8 and 16 nmoles) microinjected in a volume of 0.25 µl into the PAG induced a dose-dependent attenuation of stimulation-induced feeding. Increase in latency to feed as well as in feeding threshold has been found to last for the duration of the test; this effect was blocked by an IP injection of 2.0 mg/kg naloxone.

Morphine appears therefore to induce opposite and anatomically differentiated effects on stimulation-induced eating. Given the known differential distribution of the various subclasses of opioid receptors throughout the brain, these opposite effects of morphine might well be due to the activation of different opioid receptors at the level of two different brain regions. In order to test this hypothesis, the effects of microinjected U-50,488, a selective kappa opioid receptor agonist were compared to those of morphine. Equimolecular doses of morphine and U-50,488 were injected in a volume of 0.25 µl into the VTA and the PAG.

When microinjected into the VTA, the kappa-agonist U-50,488 induced a facilitation of LH-induced feeding; this facilitatory effect appeared even slightly more marked than the effect of morphine. Conversely, when microinjected into the PAG, U-50,488 did not produce any of the suppressive effects shown by morphine microinjected into this region.

These data indicate that the differential effects of morphine on LH stimulation-induced feeding are not only anatomically distinct but also pharmacologically distinct. Indeed, it is suggested that the facilitatory activity of morphine at the level of the VTA is subserved by kappa and perhaps also mu opioid receptors; at the level of the PAG however, mu and perhaps delta opioid receptors mediate the suppressive effect on LH-induced feeding exhibited by morphine.

21.16 GLUCAGON (GLG) AND EPINEPHRINE (EPI) EFFECTS ON MILK INTAKE (MI) AND PLASMA GLUCOSE (GLU) IN LIVER DENERVATED (LD) AND SHAM OPERATED (SHAM) RATS. L.L. Bellinger and F.E. Williams. Dept. Physiol., Baylor Coll. Dent., Dallas, TX 75246.

GLG and EPI injections suppress food intake in rats, however, it is unknown if this is a normal physiologic control. Where these hormones act to reduce food intake is uncertain, but the liver is a proposed site. It has been postulated that these hormones affect liver glycemia, which in turn influences neural afferents to the brain. In the present study male S.D. rats (260-270g BW) were SHAM (n=9) or totally LD (n=13) see Bellinger and Williams, Physiol. Behav. 30:463, 1983. Rats were kept on a L:D 12:12 (lights out at 1330h) and fed Purina chow ad lib. After operation chow intakes and BW of the groups did not vary significantly. Seven days after surgery chow was removed at 0830h and the rats injected I.P. with 0.5 cc of vehicle (VEH) at 0900h. Within one min. sweetened condensed milk (Eagle Brand), diluted 50% with water was given in Wahmann calibrated bottles. MI was recorded after 30 min. and then removed. After 8 daily VEH injections the rats were then injected with GLG, 400 ug/kg BW. Daily VEH injections were resumed. At 5-7 day intervals the rats were injected with GLG at 100 ug/kg (2 trials) and 50 ug/kg (2 trials) and EPI 30 ug/kg free base (2 trials). At weekly intervals, in a counter balanced design, the rats under ether anesthesia were bled (0.5cc) by heart puncture within 60 sec. of removal from their cages. Blood samples were obtained just prior to injection and at 10 min. after GLG (400 ug/kg)or VEH. At weekly intervals the rats were bled 10 min. after injection of 50 and 100 ug/kg GLG and 30 ug/kg EPI. Compared to VEH, GLG suppressed (pair T-test one tail, multiple trials averaged) MI (mls) of both SHAM and LD rats at 400 ug/kg (LD, 7.8±0.6 vs 6.5±0.6, P<0.04; SHAM, 8.5± 0.7 vs 6.4±0.8, P<0.05); 100 ug/kg (LD, 8.7±0.7 vs 7.7±0.7,P<0.05; SHAM 8.6±0.7 vs 6.4±0.9, P<0.01) but not at 50 ug/kg. Compared to VEH, EPI also reduced MI (mls) of both groups (LD, 8.3±0.3 vs 5.9±0.3, P<0.01; SHAM, 7.5±0.8 vs 4.9±0.6, P<0.01). Compared to VEH, GLG at 50-400 ug/kg raised (P<0.01) plasma GLU in both groups to a similar degree. Thus GLG at 50 ug/kg while not suppressing MI caused marked liver glycogenolysis. EPI increased GLU to a lesser degree than 50 ug/kg GLG, yet produced MI suppression. The data indicate that liver innervation is not necessary for GLG or EPI suppression of MI nor is MI reduction correlated to changes in liver glycogenolysis or plasma glucose. Concern is expressed since ug quantities of both hormones are required to suppress MI when they are normally found in only pg/ml concentrations.

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21.17 ELEVATION OF PLASMA AND BRAIN CATECHOLAMINES FOLLOWING MAJOR BURN TRAUMA. J.L. Nelson, W.T. Chance, M.W. Kim*, T. Foley-Nelson and J.E. Fischer*. Dept. of Surgery, Univ. of Cincinnati Medical School, Cincinnati, OH 45267.

Hypermetabolism is a characteristic of burn trauma, with the degree of hypermetabolism being directly related to the extent of the burn injury. A result of this hypermetabolic state is catabolism of lean body tissue. However, we have observed that major burn trauma induces anorexia for several days in experimental animals. This anorectic response in the presence of hypermetabolism suggests a dysfunction of CNS hunger and satiety mechanisms. Alterations in brain norepinephrine (NE) have been suggested to mediate the hypermetabolism following burn trauma. The present experiment was designed to evaluate changes in NE and other neurotransmitters associated with burn-induced anorexia and hypermetabolism. Nineteen rats were anesthetized and subjected to a 30% body surface area open-flame burn (25 sec). This treatment produces a full-thickness burn, destroying the free nerve endings that mediate pain from cutaneous sources. Thus, the animals experienced minimal pain after the burn trauma. An additional 9 rats served as nonburned controls. The burned (B) rats exhibited significantly reduced food intake for 4 days. Six of these animals were sacrificed at this time in order to assess, by HPLC, neurochemical alterations associated with the anorexia. The remaining B rats developed hyperphagia by day 10 and were eating 178% of the control intake values at sacrifice (day 28). However, these rats did not gain body weight, weighing 86% of control values at time of sacrifice. The assessment of metabolic rates on day 18 revealed that the B rats were experiencing hypermetabolism during this period. Plasma catecholamines were elevated in both B groups and catabolism was indicated by reduced gastrocnemius muscle weight. Plasma levels of glucagon were also elevated in the B anorectic rats, suggesting an increase in gluconeogenesis during the anorectic phase of burn trauma.

In the brains of the hyperphagic B rats NE levels were significantly elevated in the hypothalamus (21±3%) and nucleus accumbens (42±7%). Dopamine (DA) levels were significantly elevated in the amygdala of the anorectic B rats (56±13%). Concentrations of the DA metabolite, homovanillic acid, were significantly increased in the corpus striatum (49±11%), nucleus accumbens (26±6%) and amygdala (88±18%) of the anorectic B rats. Levels of the serotonin metabolite, 5-HIAA, were significantly elevated in the amygdala in both the B anorectic and hyperphagic rats, suggesting an increase in serotonin metabolism due to burn trauma. Together, these data suggest that there is an increase in DA turnover and gluconeogenesis during the anorectic phase of major burn trauma and elevated synthesis of NE when the rats are hyperphagic and hypermetabolic.

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21.18 CHANGES IN NEUROTRANSMITTER LEVELS ASSOCIATED WITH FEEDING AND SATIETY. W.T. Chance, T. Foley-Nelson, J.L. Nelson, M.W. Kim* and J.E. Fischer*. Dept. of Surgery, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267

Although previous reports have suggested that increased dopamine (DA) turnover in several brain areas is associated with feeding, the relationship of these changes to satiety is unclear. In order to investigate the role of DA and other neurotransmitters to food deprivation, the act of feeding and satiety, 21 adult male SD rats were deprived of food across an 18 day period. The amount of food allowed each rat was decreased by 25% of that consumed by ad lib. fed controls (n=8) across the first 6 days, 50% across the next 6 days and 75% during the last 6 days of the experiment. This gradual reduction was employed to mimic the weight loss that we have observed in anorectic tumor-bearing rats and resulted in a 31% reduction in body weight on day 18 as compared to the ad lib. fed controls. Seven of these rats were sacrificed while they were still food-deprived and 7 rats were presented with 2 grams of food (refed) and sacrificed 60 minutes later. The remaining 7 rats (satiated) were presented with 20 grams of food, allowed to feed to satiation (one meal $\bar{X}$ consumption = 5.2±0.5 g) and were sacrificed 60 minutes thereafter. The brains were dissected over ice into 8 regions (nucleus accumbens, amygdala, cortex, corpus striatum, hypothalamus, diencephalon, septal area, brainstem) and frozen in liquid nitrogen prior to analysis of neurotransmitter concentrations by HPLC. In the nucleus accumbens and corpus striatum, levels of the DA metabolites, homovanillic acid and 3,4-dihydroxyphenylacetic acid, were elevated significantly in the satiated rats as compared to the ad lib. fed or the food deprived groups. However, levels of these compounds were not significantly different from the deprived group in the rats that were fed 2 grams of chow. Therefore, only the rats that fed to satiation exhibited apparent increases in DA turnover. Concentrations of the serotonin (5-HT) metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were increased in the satiated rats in all brain areas and in the deprived and refed groups in all areas except the nucleus accumbens. These apparent changes in 5-HT turnover are probably due to increased precursor availability.

Therefore, these data suggest that increased DA turnover in the nucleus accumbens and corpus striatum may be associated with the termination of feeding within a single meal. The ubiquitous increases in brain 5-HT metabolism are probably reflective of overall nutritional status, being caused by malnutrition-induced decreases in blood concentrations of neutral amino acids that compete with tryptophan for transport into the brain.

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21.19 INSULIN AND SATIETY. D. A. VanderWeele. Dept. of Psychology, Occidental College, Los Angeles, CA 90041.

While it is well known that the acute and repeated administration of large, aphysiologic dosages of insulin can lead to obesity through increased eating, recent studies have suggested that more prolonged and physiological amounts of this hormone, given to rats that are feeding ad libitum, can result in decreased food ingestion and body weight gain. Furthermore, insulin has been shown to reduce sham food intake (these meetings, last year). The present study evaluated and reports the effects of small dosages of insulin upon meals when the insulin administration begins conincident with the initiation of the meal.

Rats were trained to press a bar for food pellets in a standard operant chamber. When taking all of their daily meals at a stable baseline rate (required 5 to 8 days of testing), subjects were anesthetized and received a hepatic-portal catheter threaded into a mesenteric collecting vein of the small intestine and terminating approximately 2 cm from the liver. Once recovered from surgery and eating normally (approximately 25 g per day), animals were returned to the operant chamber for the experimental phase.

Upon return to the operant chamber, all rats received 3 days of free feeding with each pellet contingent upon a single bar press and no infusions made; during this period, complete meal pattern analysis was carried out with a minimum meal definition used of 10 pellets (0.45 g) and minimum intermeal interval defined as 10 minutes of no feeding. After the 3 days, each meal during the daylight hours initiated an infusion pump which delivered injections of 1 or 0 mU of insulin. The insulin was regular Iletin insulin in diluting fluid provided by Lilly Co. The infusions of insulin or vehicle were delivered in 0.15 ml aliquots over 1 min which began 10 pellets into each meal.

Uninfused meals in the rats observed to date with this procedure averaged just over 2 g. Infusion of vehicle reduced this meal size by approximately 10%, not reliably different from no-infusion condition. Infusion of 1 mU of insulin, however, reduced the size of spontaneously ingested meals by 45%, $p < .005$. Most animals show an increase in meal frequency when being infused with insulin (compared to vehicle and no-infusion condition), but this effect has not reached statistical significance in the number of subjects evaluated to date.

The present findings are consistent with the hypothesis that insulin's effects on nutrient substrate utilization are part of the total complex of stimuli arising from the ingestion of food which produce satiety.

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SPECIFICITY OF SYNAPTIC CONNECTIONS I

22.1 CELLULAR BASIS OF THE RETURN OF FUNCTION OF REGENERATED GIANT AXONS OF LUMBRICUS TERRESTRIS. A.W. Lyckman and G.D. Bittner, Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Earthworms exhibit an escape response to novel, potentially threatening tactile stimuli. This response is mediated by the electrical activity of the three (one medial and two lateral) dorsal giant axons of the ventral nerve cord, and consists of a rapid contraction of longitudinal muscles which pulls the animal away from the stimulus. The transection of the nerve cord results in a blockade of the escape response at the site of the lesion. Both halves of a severed giant axon survive for weeks or months, and continue to conduct APs up to the lesion site. Return of the propagation of the escape response across the lesion site can be correlated with the appearance of APs in the giant axons which cross the lesion site. Furthermore, APs originating in the medial giant axon on one side of the lesion appear only in the medial giant axon on the other side of the lesion. Likewise, APs originating in the lateral giant axons on one side of the lesion appear only in the lateral giant axons on the other side. Through-conduction is initially slow and labile and often unidirectional, i.e., an AP may cross the lesion in one direction but not the other. Over time, through-conduction becomes more rapid and reliable, and unidirectional through-conduction becomes bidirectional (Birse and Bittner, 1981, J. Neurophys. 45: 724). Additionally, some APs approaching the lesion site may rebound from the lesion site (Balter, Drewes and McFall, 1980, J. Exp. Zool., 211: 395).

In order to determine the cellular basis for the apparent specific reconnection of the severed giant axons and the changing properties of through-conduction, we have examined longitudinal and transverse section of lesioned giant axons using light and electron microscopy. Severed giant axons in varying stages of recovery after lesioning (24h, 2d, 1wk, 2wk, 4wk, 8wk, 10wk, 3mo) were filled with horseradish peroxidase or lucifer yellow-CH by in situ intracellular iontophoresis in order to identify neuronal processes in the site of reconnection and the intercellular contacts made by these processes. We are correlating the appearance of various intercellular junctions with physiological records of giant fiber activity at the lesion site. Thus, we expect to provide a morphological description of junctions responsible for the initially labile one-way conduction through the lesion, two-way conduction, spike-rebounding, and the strengthening of conduction across the lesion which is seen in animals which have had longer recovery times. We expect to show that processes of medial (lateral) giant axons on one side of the lesion do not form electrical or chemical junctions with the processes of lateral (medial) giant axons on the other side of the lesion, explaining the electrophysiological evidence for specific reconnection.

(This research is supported by grant NIH NS 19764 to G.D.B.)

22.2 TARGET-REGULATED PROTEINS IN DORSAL ROOT GANGLION NEURONS. L. Baizer, M.C. Fishman. Neuroscience Group of the Howard Hughes Medical Institute, and the Department of Medicine, Harvard Medical School and Massachusetts General Hospital.

The set of macromolecules within presynaptic neurons whose level of expression is regulated by their interaction with target cells presumably includes those related to the transition between growth cone and nerve terminal. In order to identify such "target-regulated" proteins we have analyzed by two-dimensional electrophoresis the axonally transported proteins of rat dorsal root ganglion (DRG) neurons. DRG neurons were cultured in a compartmentalized system, with their somas restricted to the center chamber where they can be labelled metabolically, and their axons growing through to the side chambers where they can be exposed to particular target cells. Axonal proteins constituted a subset of total cellular proteins. They included a group of rapidly transported, glycosylated proteins, most prominent of which was a family of proteins with Mw about 68 kilodaltons and pI from 6.1 to 6.6. The next wave of transported proteins included actin, and the slowest, α and β tubulins.

The effect of contact between growing axons and target cells upon expression and transport of particular proteins was examined. Since such target cells were cultured outside of the labelling chamber, labelling was restricted to axonal proteins of the presynaptic (DRG) neurons. Contact with target tissue caused the decreased expression of several high molecular weight, rapidly transported proteins. One of the most prominent of these was a protein with Mw about 210 kilodaltons and an isoelectric point that ranged from 6.2 to 6.7. Non-neuronal cells when presented as targets did not affect expression of this protein.

The exact target-specificity and chemical identity of these target-regulated proteins are under investigation.

22.3 CONNECTIONS FORMED BY SENSORY NEURONS TRANSPLANTED TO A NOVEL ENVIRONMENT C.L. Smith and E. Frank, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

During development, sensory neurons form highly specific connections between structures in the periphery and neurons in the central nervous system. One hypothesis proposed to account for this specificity is that developing sensory neurons acquire chemical labels from their peripheral targets that cause their central axons to establish synaptic connections with the appropriate neurons. To test this hypothesis, we are examining the connections formed by sensory neurons transplanted to novel positions along the neuraxis in bullfrog tadpoles.

Dorsal root ganglia (DRGs) are transplanted in tadpoles at stages coinciding with the innervation of the forelimb (stages V-VIII). The DRG that would normally supply the forelimb is removed and replaced with two thoracic DRGs. These thoracic DRGs would normally innervate skin of the trunk. The tadpole is allowed to metamorphose and then both the peripheral and central projections from the transplanted sensory neurons are examined.

Sensory neurons in the transplanted DRGs innervate the arm. Anatomically, their central projection resembles that from the DRG that normally supplies the arm in that it consists of two distinct neuropils, one located in the dorsal horn and the second, more ventrally, in the vicinity of motoneurons. In normal frogs, thoracic sensory neurons project exclusively to the dorsal horn; the ventral neuropil, composed of muscle spindle afferents, is not present at thoracic levels. The finding that neurons in the transplanted ganglia form a ventral neuropil suggests they are muscle spindle afferents. Electrophysiological recordings from muscle nerves have confirmed that some of the transplanted sensory neurons are activated by stretching muscles. The patterns of connections between these muscle afferents and motoneurons have been examined by electrophysiological methods. Just as in normal frogs, sensory afferents from the medial triceps muscle have direct, monosynaptic connections with triceps motoneurons while projections to functionally unrelated motoneurons are much less strong.

The thoracic ganglia that were transplanted to the brachial level never innervate limbs in normal frogs. The finding that neurons in these ganglia will innervate the arm shows that sensory neurons can innervate novel peripheral targets. The observation that transplanted neurons form central connections appropriate to their novel peripheral targets supports the hypothesis that developing sensory neurons are influenced in their choices of central targets by their interactions with peripheral tissues. (Supported by the Muscular Dystrophy Association and NSF grant BNS-83178).

22.4 PROPERTIES AND DISTRIBUTION OF MUSCLE AFFERENT PROJECTIONS TO LUMBOSACRAL MOTONEURONS IN THE SPINAL CORD OF THE CHICK EMBRYO. M. Koebbe* and M.J. O'Donovan. (Spon: C. Gisolfi) Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242.

The synaptic connections of anterior thigh muscle afferents with lumbosacral motoneurons were examined electrophysiologically in St.36-39 chick embryos using an isolated spinal cord preparation. Motoneuron synaptic potentials were recorded electrotonically from cut ventral roots or individual muscle nerves in response to muscle afferent stimulation. Synaptic potentials recorded in this manner were small (10-100μV), slow and fatigued rapidly following a few impulses at 1 Hz. The amplitude of synaptic potentials recorded from individual muscle nerves could be significantly enhanced by superfusion of the parent ventral root with isotonic sucrose. A small triphasic presynaptic potential was observed to preceed the onset of the ventral root synaptic potential by approximately 5 msec. Synaptic potentials were completely abolished when the cord was bathed in a low calcium, high magnesium solution indicating that they were predominantly chemical at this developmental stage. The afferents from individual muscles projected widely to various motoneuron pools and to different segments of the spinal cord (see Fig. 1). The largest ventral root potentials were recorded from those segments containing the largest number of homonymous motoneurons. Activation of muscle afferents projecting to both synergist and antagonist motoneuron pools prouced excitatory synaptic potentials, although it is likely that such potentials are composed of both excitatory and inhibitory components.

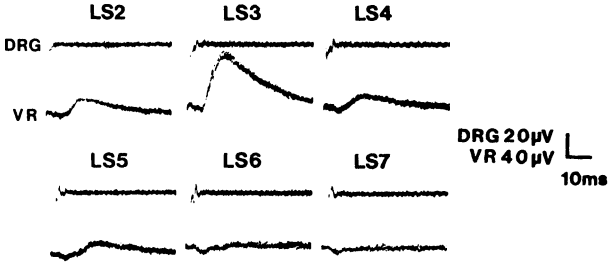

FIG. 1. Variation in the amplitude of potentials recorded from the lumbosacral ventral roots in response to a single supramaximal stimulus to the femorotibialis muscle nerve. The upper traces (DRG) show the afferent volley recorded in the dorsal root ganglion (LS1 for VR LS2 and LS2 for VR LS3-LS7).

22.5 ORGANIZATION OF MOTOR UNITS IN A SIMPLE MUSCLE. R.S. Wilkinson and J.W. Lichtman, Departments of Cell Biology & Physiology and Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The transversus abdominis of the garter snake is a thin (one fiber thick) segmental muscle containing ~80 muscle fibers. Three distinct muscle fiber types (faster twitch, slower twitch, and tonic) may be identified by morphological, electrophysiological, contractile, or biochemical criteria; these 3 fiber types are arranged within the flat muscle sheet as a repeating sequence. Because of its geometric simplicity and accessibility, innervation patterns in this muscle can be characterized precisely.

Motor unit "maps" were obtained by activating individual motor axons and identifying their targets using either activity-induced uptake of extracellular markers to label presynaptic terminals (J.W. Lichtman et al., Nature 314, 357-359, 1985) or systematic intracellular recording to identify activated muscle fibers. Each muscle contained 4-5 twitch and 3-4 tonic motor units. Fibers belonging to a given motor unit were strictly homogeneous in type, but were distributed randomly (among fibers of the proper type) throughout the muscle.

Twitch motor axons of either type innervated 5-15 muscle fibers and constituted the sole source of innervation to those fibers. In contrast, tonic axons innervated more fibers (20-40) but generally each fiber was multiply innervated. The larger size and overlap of tonic motor units evidentally stems from the ability of tonic muscle fibers to sustain polyneuronal innervation, both by providing multiple endplate sites (~7 endplates/fiber) and by allowing terminal boutons from different axons to co-innervate the same endplate.

In studies where several motor units were mapped in the same muscle, one axon's innervation was not only randomly distributed within the muscle as a whole, but also randomly distributed with respect to the innervation supplied by other axons. Thus all the motor units were intermingled throughout this small muscle. The random distribution of motor units argues 1) that the repeating fiber type pattern in this muscle is of intrinsic rather than neural origin, and consequently, 2) that highly selective recognition between axons and particular muscle fibers operates to generate homogeneous motor units. (Supported by the NIH, MDA, and McKnight Foundation).

22.6 TOPOGRAPHIC MAPPING OF MOTOR POOLS ONTO MUSCLES. M.B. Laskowski[1] and J.R. Sanes.[2] Depts. of Physiology, St. Louis Univ.[1] and Washington Univ.[2] Medical Centers, St. Louis, MO 63110

The selective reinnervation of muscles transplanted from different segmental levels to a common site has suggested the existence of positional labels that bias synapse formation between axons and muscles: rostral muscles are preferentially reinnervated by axons from rostral levels of the spinal cord, while caudal axons preferentially reinnervate caudal muscles (Wigston and Sanes, Nature 299, 464, 1982; J. Neurosci., 1985). If such a system operates during development, one might expect motor pools to "map" onto the rostrocaudal axis of normal adult muscles, much as the retina "maps" onto the tectum. To test this idea, we sought trunk muscles that (1) span several segments, but (2) have single fibers associated with individual segments; we also required that they (3) be innervated through a single nerve (4) by axons that arise in several spinal segments. Two muscles that meet these criteria are the serratus anterior and the diaphragm. The serratus anterior inserts on ribs 1-7 and is innervated through the long thoracic nerve from cervical (C) segments 6-8; the diaphragm inserts on ribs 7-13 and is innervated through the phrenic nerve from C3-6. For each muscle, we assessed the topography of innervation by recording intracellularly from muscle fibers while stimulating motor axons in individual bundles of ventral rootlets. In both muscles, we found a consistent and significant tendency for axons from rostral portions of the motor pool to innervate rostral portions of the muscle; progressively more caudally-derived axon groups innervated more caudally disposed muscle fibers. Histological confirmation of this pattern exploited the finding that N-CAM accumulates in denervated muscle fibers (Covault and Sanes, PNAS, 1985); transection of individual ventral roots led to topographically restricted partial denervation that was detected by immunofluorescent staining. Thus, the rostrocaudal axes of motor pools and muscles are matched; topographically ordered innervation also occurs in limb muscles (e.g., Bennett and Lavidis, J. Neurosci. 4, 2204, 1984), and may be a general feature of neuromuscular connectivity. The preparations we have used should be well suited for learning how this order arises during development and whether it can be re-established during regeneration.

22.7 MORPHOLOGICAL CORRELATES OF TRANSIENT RETINA-MUSCLE SYNAPSES. J.M. Thompson and S.W. Norby. Dept. of Anatomical Sciences and Dept. of Physiology, University of Illinois, Urbana, IL 61801.

Retinal neurons have been shown to form cholinergic synapses on myotubes in culture as detected by electrophysiological recording of muscle synaptic responses. These mismatched synapses form quickly, but are transient. The maximum number of innervated muscle cells occurs at 1 day of coculture with muscle. By 7 days of culture, retina-muscle synapses are no longer detected. (Puro et al., Proc. Natl. Acad. Sci. USA, 74:4977, 1977; Ruffolo et al., Proc. Natl. Acad. Sci. USA, 75:2281, 1978; Thompson et al., Int. J. Devel. Neurosci., 1:25, 1983.)

To determine the nature of the loss of connections, we have examined the retinal neuron - muscle contacts at the ultrastructural level. The hypothesis which we are testing is that retina-muscle synapses are lost due to competition between target cells (other retinal neurons versus muscle cells). This should be seen as a decrease in retina-muscle contacts with a concomitant increase in retina-retina contacts during the time of coculture. At one day after coculture, retinal neurons adhered to the myotubes, to each other to form small clumps, and to the substrate or underlying fibroblasts. Neural processes can be seen to run longitudinally along the myotube, thus, making contact on the muscle for the entire length of the neurite. At three days of coculture, the number and length of neurites increased greatly. Neurites reached over 100 microns in length, and most terminate on myotubes. The retinal neurons are still observed to often adhere to myotubes and send processes along their length. Many of the neural processes ramify into many fine processes which contact the myotube. At five days of coculture, little change is observed compared to three day cultures. Neurons often adhere to myotubes, and neurites, often very long, will terminate on myotubes. At seven days after coculture, the number of neurites contacting the myotubes was greatly reduced. In this later stage of coculture, although the retinal neurons still have extensive networks of processes, few processes make contact on the muscle cells. The neurites usually pass over or under the myotube without terminating. Most neurons associate only with other neurons either in small clusters or by process extension. These initial studies demonstrate that at early times of coculture, retinal neurons will contact myotubes directly or by extending their processes along the myotubes. At further times in culture, the number and length of neurites increases, as do the number of contacts on the myotubes. At later stages of culture, the number of contacts on myotubes decreases dramatically. Thus, there is an apparent decrease in retinal-muscle contacts with a maintenance of retina-retina contacts during the time of loss of retina-muscle synapses detected physiologically.

22.8 PROJECTION PATTERNS OF SYMPATHETIC PREGANGLIONIC AXONS IN CHICK EMBRYOS. Joseph W. Yip. Department of Physiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261.

The projection patterns of sympathetic preganglionic neurons arising from different spinal cord segments were studied in stages 24-35 (4½-9 days) chick embryos using orthograde and retrograde horseradish peroxidase (HRP) labelling techniques. An HRP solution was injected into individual spinal cord segments or sympathetic ganglia of an isolated spinal cord-sympathetic chain preparation. The tissues were fixed, serially sectioned and reacted according to a DAB-cobalt method.

The adult patterns of preganglionic axon projection are apparent after the first week of embryonic life. Preganglionic axons arising from individual spinal cord segments exit via the adjacent ventral root and consistently innervate the same ganglia of the sympathetic chain. These ganglia are contiguous. However, the number of ganglia innervated by preganglionic axons arising from different spinal cord levels is variable. For instance, T1 preganglionic axons innervate considerably more ganglia than T4 preganglionic axons. Individual preganglionic axons, moreover, will branch to innervate multiple ganglia. A striking feature in the projection patterns of preganglionic axons is the stereotyped rostro-caudal distribution of preganglionic axons arising from different levels of the spinal cord. For example, T1 preganglionic axons project predominantly in the rostral direction whereas T4 preganglionic axons project predominantly in the caudal direction. Axons from the T2 spinal level assume an intermediate type of distribution, with many axons projecting in both rostral and caudal directions.

The characteristic rostro-caudal distribution of preganglionic axons is evident as soon as they enter the sympathetic chain at stage 27 (5-5½ days). The pathways taken by preganglionic axons towards their targets are therefore correct from the onset.

These results indicate that the outgrowth of sympathetic preganglionic axons is precise and suggest that there are specific cues for the guidance of these axons.

Supported by BNS 82-10028 and by a Basil O'Connor Starter Research grant from the March of Dimes Birth Defects Foundation.

22.9 IDENTIFIED SUBCLASSES OF BULLFROG SYMPATHETIC NEURONS PROJECT TO DIFFERENT TYPES OF PERIPHERAL TARGETS. J.P. Horn, W.D. Stofer* and S. Fatherazi* Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

In the ninth and tenth paravertebral sympathetic ganglia of the bullfrog, three subclasses of neurons (fast B, slow B and C cells) can be identified on the basis of their axonal conduction velocities (Dodd & Horn, J. Physiol. 334, 255 (1983)). In addition, the two B cell groups are innervated selectively by preganglionic axons that enter the sympathetic chain via spinal nerves four to six while C cells receive their input via spinal nerves 7 and 8. However, the functional significance of multiple ganglionic cell types is obscure. One possibility is that subclasses of sympathetic neurons modulate different types of targets. To test this hypothesis we have measured the sympathetic fiber spectrum of nerves innervating visceral* smooth muscle, striated muscle and the skin.

All experiments were done on isolated preparations of sympathetic chain ganglia 7 through 10 with attached peripheral nerves. At the start of each dissection, after double pithing the animal, targets of interest (eg. the sartorius muscle) were identified. The nerve entering the target was located, marked, dissected back to the sciatic nerve and then removed in continuity with the sympathetic chain. Suction electrodes were placed on spinal nerves 7 and 8 for stimulating preganglionic C fibers and on the sympathetic chain rostral to ganglion seven for stimulating preganglionic B fibers. Two pairs of recording suction electrodes were used in each experiment. One pair was used to record a control response from the sciatic nerve. The sciatic nerve contains all three groups of sympathetic axons and enabled us to adjust the stimulating eletrodes for supermaximal responses while maintaining selective stimulation of the B and C inputs. The second pair of recording electrodes was applied to the identified target nerve.

By recording compound extracellular action potentials in this manner we have found that the visceral branch of the pelvic nerve innervating the bladder and motor nerves innervating the adductor magnus, gracilis, iliofibularis, sartorius, gastrocnemius and semitendinosis muscles all contain only C sympathetic axons. Even with signal averaging, no B inputs to these muscle were detected. By contrast, the cutaneous branch of the gracilis nerve and the cutaneous lateral and posterior crural nerves each contain fast B, slow B and C axons. Thus it appears that subclasses of sympathetic axons segregate as they project through peripheral nerves and that B axons selectively innervate targets in the skin. The precise cellular targets of the different sympathetic cell groups remain to be determined.

This work was supported by NIH grants NS21065 and MH30915.

22.10 **THE ONTOGENIC DEVELOPMENT OF SEROTONERGIC FIBERS IN THE RAT OLFACTORY BULB.** J.H. McLean, M. Lazoff, E.B. Sieloff, W.T. Nickell, and M.T. Shipley. (University of Cincinnati College of Medicine).

We have shown that serotonergic (5-HT) raphe neurons project heavily to the glomeruli of the rat main olfactory bulb (Schumacher et al., 1984). This suggests that raphe neurons may have a marked influence on olfactory information processing at the level of the first synaptic relay. Recently, it has been proposed that 5-HT fibers have trophic effects on target neurons during neural development (Lauder et al, 1982). Here we have examined the ontogenic development of serotonergic fibers in the main olfactory bulb (MOB) of the postnatal rat using immunocytochemistry and image analysis.

The distribution and density of 5-HT fibers in the MOB undergo a dramatic transition from the neonate to the adult rat. In the first week of life 5-HT fibers are located in all layers of the MOB including the proliferative ependymal zone. Beginning with day 8, there is a progressive redistribution of fibers to the glomerular and internal part of the external plexiform layer (epl). In addition, there is a steep overall increase in the number of 5-HT fibers in all layers. From day 14 - 37 the fibers become progressively concentrated in the glomerular layer; a sparce innervation remains in epl. With increasing age the density of serotonin fibers appeared to diminish slightly in the glomeruli. We have four possible explanations for this observation: 1) There may be an initial over proliferation of 5-HT fiber in the glomeruli followed by a modest reduction to adult levels; 2) the proliferation of glomerular 5-HT fibers may stop at ca. 6 weeks while the size of the glomeruli continues to increase. 3) The 5-HT in the mature glomeruli may be less accessible to the antisera. 4) 5-HT levels in glomeruli may undergo circadian fluctuations. We are currently investigating these possibilities.

The dramatic laminar redistribution of developing 5-HT fibers suggests that there is transition from non-specific to specific 5-HT fiber deployment during the first weeks of life. The mechanism of this redistribution is unknown. One possibility is that fibers die off in the deep layers. The initial presence of 5-HT fibers in the deep, proliferative layers of the bulb could mean that 5-HT plays a trophic role during neurogenesis and migration. Alternatively, early arriving 5-HT fibers may contact their definitive target cells from the outset; accordingly the redistribution of 5-HT terminal arbors would represent the co-migration of terminals and target neurons from proliferative zones to their definitive loci in the glomeruli.

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22.11 **ORGANIZATION OF TRANSIENT CEREBROCEREBELLAR PROJECTIONS FROM THE PRIMARY SOMATOSENSORY CORTEX.** T. Donnigan*, D.L. Tolbert*, and W.M. Panneton. (SPON: L.C. Massopust) Francis and Doris Murphy Neuroanatomy Res. Lab., Depts. of Anatomy and Surgery (Neurosurgery), St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

There are transient bilateral projections from the primary sensorimotor cortex to the cerebellum in neonatal cats. Most ipsilateral cerebrocerebellar projections course through the superior cerebellar peduncle and end in the deep nuclei on that side, whereas most contralateral cerebrocerebellar projections enter through the inferior cerebellar peduncle and end in the internal granule cell layer in the cortex. Previous findings suggested that cerebrocerebellar projections were somatotopically organized in the cerebellar cortex (J. Comp. Neurol. 221:216, 1983).

In the present study, the organization of cerebrocerebellar projections from forelimb, hindlimb, and face areas of the primary somatosensory cortex (S-I) was examined in two-week-old cats. Labeled cerebrocerebellar projections were in the folial white matter and internal granule cell layer in different areas of the cerebellar cortex after tritiated amino-acid injections into forelimb and hindlimb areas of S-I. Injections into the forelimb S-I area labeled mostly contralateral cerebrocerebellar projections to lobules IV and V in the anterior lobe and to the dorsal sublobule and dorsal folia of the intermediate sublobule of the paramedian lobule in the posterior lobe. Fewer labeled projections were to similar lobules and folia in the ipsilateral anterior lobe and paramedian lobule. After injections into the hindlimb area of S-I, most labeled projections to the contralateral cerebrocerebellar cortex were in lobules I-III in the anterior lobe and in the ventral sublobule of the paramedian lobule. Fewer hindlimb cerebrocerebellar projections were directed ipsilaterally to the rostral anterior lobe and to the ventral paramedian sublobule. Forelimb and hindlimb cerebrocerebellar projections to the anterior lobe were more dense in intermediate and lateral parasagittal zones than in medial zones. Ongoing studies are examining the distribution of cerebrocerebellar projections from the face area of S-I.

These findings indicate that the transient cerebrocerebellar pathway from S-I is somatotopically organized. Furthermore, the organization of forelimb and hindlimb S-I projections to the cerebellar anterior lobe and paramedian lobule is very similar to the topography of precerebellar afferent projections relaying fore- and hindlimb somatosensory input from the periphery. It is unlikely, however, that cerebrocerebellar projections organize other cerebellar afferent systems.

Supported by NIH grant 20227 and NIH Training Grant HL 7486.

22.12 AN ULTRASTRUCTURAL STUDY OF THE SPECIFICITY OF ABERRANT RUBRAL AFFERENTS FOLLOWING NEONATAL CEREBELLAR OR SENSORIMOTOR CORTICAL LESIONS IN THE RAT. C.C. Naus, A.W. Hrycyshyn* and B.A. Flumerfelt. Department of Anatomy, The University of Western Ontario, London, Canada.

The afferent projections to the rat red nucleus are topographically organized, with the nucleus interpositus and the nucleus lateralis projecting to the contralateral magnocellular and parvocellular areas, respectively, and the sensorimotor cortex projecting to the ipsilateral parvocellular region. To this topographic specificity is added a specificity of synaptic localization at the ultrastructural level. The interpositorubral projection synapses on somatic and proximal dendritic membrane of magnocellular neurons, while the projection from the nucleus lateralis synapses on small to medium-sized dendrites of parvocellular neurons. In addition, the afferents from the sensorimotor cortex synapse on distal dendrites of parvocellular neurons. Following neonatal hemicerebellectomy, an aberrant ipsilateral cerebellorubral projection develops which maintains the topographic specificity of the normal contralateral projection. Similarly, neonatal lesions of the sensorimotor cortex lead to the appearance of an aberrant contralateral corticorubral projection which mirrors the topographic specificity of the normal ipsilateral input. The specificity of synaptic localization in these aberrant projections was studied using ultrastructural visualization of anterogradely transported HRP-WGA. Neonatally lesioned adults received HRP-WGA injections in the unablated deep cerebellar nuclei or sensorimotor cortex. After 48 hours, animals were sacrificed and processed for ultrastructural localization of anterogradely transported HRP-WGA. In hemicerebellectomized animals, both the contralateral and ipsilateral interpositorubral projections synapsed on somatic and proximal dendritic membrane of magnocellular neurons. Some of these labeled synaptic terminals were located on somatic or dendritic spines. Following HRP-WGA injection into the unablated nucleus lateralis, anterogradely labeled synaptic terminals were located on small to medium-sized dendrites of parvocellular neurons bilaterally. Injection of HRP-WGA into the remaining sensorimotor cortex of animals which had undergone neonatal unilateral ablation of the sensorimotor cortex resulted in anterogradely labeled corticorubral terminals which synapsed on distal dendrites of ipsilateral and contralateral parvocellular neurons. These results demonstrate that, following neonatal deafferentation of the rat red nucleus, the topographic specificity of the aberrant rubral afferents is accompanied by a specificity of synaptic localization on discrete membrane areas of rubral neurons.

(Supported by the M.R.C. of Canada)

22.13 THALAMIC AFFERENTS TO FETAL CORTICAL TISSUE TRANSPLANTED TO THE CEREBRAL CORTEX OF NEONATAL RATS. N.Aa. Sunde*, A.J. Castro, J. Zimmer*, E.L. Bold and M.M. Molinari*. (SPON: D. Anderson) Instit. of Anat. B., Aarhus Univ., Aarhus, Denmark; Dept. of Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153 and Instit. of Neurol., Catholic Univ., Rome, Italy.

Several reports demonstrate that fetal cerebral cortical tissue will survive and grow when transplanted into the brain of newborn rats. In the present study, host thalamic projections to cortical grafts were examined using retrograde fluorescent tracers.

Neocortical tissue was dissected from rat fetuses (E 15-17) that were removed from pentobarbital-anesthetized dams and placed in sterile saline. Plates of fetal cortical tissue (2-3 mm) were layered using a small glass "shovel" into host (0-1 day old) sensorimotor cortical lesions made by gentle aspiration. The transplant was then sealed with a bone flap. At maturity (6-10 wks) and under pentobarbital anesthesia, the cranium was carefully reopened and the transplant identified using an operating microscope. Using Hamilton syringes mounted to a micromanipulator, separate injections (0.2-0.3 ul) of diamidino yellow (DY) and fast blue (FB) were placed into the transplant. Animals were sacrificed 5-8 days later by vascular perfusion with buffered formalin in sucrose. Brains were cryostat-sectioned at 30 um, mounted and coverslipped. Alternate sections were stained with thionin.

Analysis by epifluorescent microscopy demonstrated that at least one of the dyes appeared confined to the transplant in 6 of 7 animals, and in 2 of these cases both dyes appeared confined to the transplant and with little overlap between them. Numerous single-labeled DY and FB neurons were observed within several thalamic nuclei, including the ventral, intralaminar and midline groups. Furthermore, an apparent medial to lateral topography of labeled cells was observed particularly in the 2 cases with both injections confined to the transplant. Double-labeled cells were rarely found.

These findings demonstrate that host thalamic neurons can innervate cortical transplants. Additionally, the lack of double labeling and the topographic distribution of labeled cells suggests a selective growth of host fibers into the transplant. (Supported by funds from the Danish MRC and NIH Grant NS13230.)

22.14 THALAMIC AND CORTICAL PROJECTIONS ARISING FROM FETAL CORTEX TRANSPLANTED TO THE CEREBRAL CORTEX OF NEONATAL RATS. A.J. Castro, J.Zimmer*, N.Aa. Sunde* and E.L. Bold. Dept. of Anat., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153 and Instit. of Anat. B, Aarhus Univ., Aarhus, Denmark.

Several reports demonstrate that fetal cerebral cortical tissue will survive and grow when transplanted into the brain of newborn rats. In the present study, neural projections from transplant to host were were examined using retrograde fluorescent tracers.

Neocortical tissue was dissected from rat fetuses (E15-17) that were removed from pentobarbital anesthetized dams and placed in sterile saline. Grafts were placed at bregma in the cortex of 0-1 day old pups according to two basic protocols. In one group (n=7), plates of fetal cortical tissue (2-3mm) were layered using a small glass "shovel" into host sensorimotor cortical lesions made by gentle aspiration. The transplant was then sealed with a bone flap. In other animals, smaller blocks of donor tissue were aspirated into a glass cannula fitted to a Hamilton syringe and then injected into the host cortex of normal animals (n=6) or animals with small cortical lesions (n=6). At maturity, separate injections of 0.8 to 1.2 ul of diamidino yellow (DY) and fast blue (FB) were stereotaxically placed into the host thalamus ipsilateral to the transplant and into the host cortex on the side opposite the transplant. Animals were sacrificed 5-8 days later by vascular perfusion with buffered formalin in sucrose. Brains were cryostat-sectioned at 30 um, mounted and coverslipped. Alternate sections were stained with thionin.

Transplanted tissue consistently survived and grew within the host brain in all groups. Grafts were easily identified by characteristic whorls of cell bands separated by fibrous septae. Transplant to host neural connections were demonstrated by numerous single-labeled DY and FB cells observed within the transplant. No double-labeled neurons were found. In cases where areas of the transplant demonstrated apparent lamination, labelled cells showed a distribution pattern that resembled the normal pattern in which neurons projecting to the contralateral cerebral cortex are located superficial to those projecting to the thalamus. These findings suggest that fetal cortical transplants may establish normal connections with the newborn host brain. (Supported by funds from the Danish MRC and NIH Grant NS13230.)

22.15 SPECIFICITY OF INNERVATION OF VASOPRESSIN NEURONS IS MAINTAINED FOLLOWING TRANSPLANTATION INTO ADULT HOSTS. J.R. Sladek, Jr., T.J. Collier and P.F. Aravich. Department of Anatomy, University of Rochester, Rochester, NY 14642.

Norepinephrine (NE) neurons of the caudal brainstem heavily innervate magnocellular vasopressin (VP) neurons of the supraoptic nucleus (SON) of the hypothalamus. Another hypothalamic nucleus, the suprachiasmatic nucleus (SCN), also contains a population of vasopressin neurons. However, these parvicellular neurons receive a very light NE innervation. These different vasopressin target nuclei present a useful model for studying the ability of transplanted tissue to become "reinnervated" in a specific manner by adult host brain systems. In order to test this specificity, we have transplanted fetal hypothalamic tissue containing SON and SCN VP neurons into the region of the medial forebrain bundle (MFB), bilaterally, in adult male Sprague-Dawley rats. Animals were allowed to survive 1-2 months after implantation and were examined with the ALFA histochemical fluorescence technique coupled with immunohistochemistry for vasopressin-specific neurophysin. Adjacent section analysis was performed in a comparator bridge microscope which allowed direct superimposition of catecholamine (CA) histofluorescence patterns and neurophysin-positive perikarya. Clusters of transplanted parvicellular VP neurons were identified in the region of the MFB immediately adjacent to a dense plexus of catecholamine fibers. Evidence for reorganization of these fluorescent fibers included the presence of 1) swollen varicose profiles reminiscent of regenerative fibers, 2) dense islands of CA fluorescence adjacent ot the MFB, and 3) numerous fine-sized fluorescent fibers. In spite of the close proximity of SCN-like neurons, the NE fibers did not form axosomatic juxtapositions with these parivcellular VP neurons. However, an occasional linear fluorescent profile was seen within the outline of the SCN-like group. In contrast, transplanted magnocellular VP-positive neurons seen adjacent to the MFB were characterized by NE varicosities in close apposition to the outlines of their cell bodies. These NE-VP relationships are reminiscent of those found in the intact hypothalamus, suggesting the retention of a high order of specificity in the relationship of NE fibers to neurons. Thus, transplantation of VP neurons appears to stimulate the establishment of "anatomically appropriate" connections with the host NE system. These findings encourage the view that some degree of integration reminiscent of that seen in the intact brain may be possible in neural transplantation.

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22.16 ONTOGENY OF PEPTIDE-MONOAMINE INTERACTIONS IN THE BRATTLEBORO RAT: EFFECTS OF SYSTEMIC VASOPRESSIN REPLACEMENT. B.C. Blanchard*, P.F. Aravich* and J.R. Sladek, Jr. (SPON: I. Shoulson). Department of Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

There is an intimate and relatively specific morphometric relationship between vasopressin (VP) perikarya and afferent noradrenergic varicosities within the supraoptic nucleus (SON) of the hypothalamus. It has been suggested that the VP target neuron is necessary for the maintenance of this interaction. This hypothesis is based, in part, upon the observation that the catecholamine (CA) innervation of the SON of VP-deficient Brattleboro rats is relatively normal during the initial phases of postnatal development, but diminishes dramatically during later stages. The current investigation determined the effects of systemic VP replacement on the CA innervation of the SON of the developing Brattleboro rat. The V2 agonist, 1-deamino-8-D-arginine vasopressin (DDAVP), also was evaluated.

Male Brattleboro rats suffering from diabetes insipidus (DI) and normal Long-Evans rats served as subjects. Three postnatal ages were examined: 24, 42, and 90 days. Within each age group there were three groups of DI rats (Saline replaced, VP replaced, and DDAVP replaced) and one group of LE rats (Saline replaced). VP replacement (13 days) was achieved using a subcutaneous osmotic minipump, VP (322.58 ng/hr x kg), and DDAVP (6.45 ng/hr x kg).

VP and DDAVP replacement enhanced the SON CA histofluorescence of DI rats relative to saline-treated controls across all age groups, though the DDAVP effect was less than the VP effect. However, despite these drug-related increases, CA fluorescence patterns were not equivalent to those in LE rats. The enhancement effect of replacement also was age dependent: 24- and 42- day old animals were more affected than 90- day old animals. Both VP and DDAVP reduced water intake and urine output to near normal levels across groups, with the DDAVP effect slightly greater than the VP effect in the 24- and 90-day old DI rats.

Thus, VP replacement appears to exert an indirect maintenance effect on the CA innervation of the SON via a peripheral route of action. (The dose of VP employed in this study does not cross the blood-CSF barrier.) Systemic VP administration can affect hemodynamic, water balance, and metabolic factors. The substantial increase in SON CA fluorescence produced by systemic DDAVP treatment excludes the contribution of hemodynamic factors, but does not distinguish between water balance and metabolic factors. The greater susceptability of young DI rats to the replacement effects is consistent with our earlier observations indicating that, with age, the DI rat gradually loses CA terminals within the SON. Finally, the inability of systemic replacement to increase CA fluorescence to LE levels is consistent with the view that VP may also exert a direct CA maintenance action within the SON.

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22.17 AN ULTRASTRUCTURAL STUDY OF DOPAMINE (DA) NEURONS IN REAGGREGATE CELL CULTURE. L. Won*, S. Price*, B. Wainer, A. Heller, P. Hoffmann and J.P. Bolam[a] The University of Chicago, Chicago, IL 60637 and [a]The University Department of Pharmacology, Oxford, U.K.

DA neurons mature biochemically and morphologically when cocultured for 21 days with striatal target cells in reaggregate cell culture. In particular, the presence of target cells (striatum) but not nontarget cells (tectum) results in a dense proliferation of varicose dopamine-containing fibers suggestive of axons and boutons. In the present study immunocytochemical localization of tyrosine hydroxylase (TH) immunoreactivity was examined at the light and electron microscopic level to determine: 1) whether TH-positive neurons are morphologically similar to mesencephalic DA neurons in vivo; 2) if TH-immunoreactive neurons form and receive synaptic connections which resemble those observed in the nigrostriatal system. Dissociated fetal mesencephalic cells containing DA neurons were coaggregated with dissociated striatal cells in rotatory culture for 21 days (Kotake et al., J. Neurosci. 2:1307-1315, 1982). Perikarya positiyely labelled for TH were approximately 20 micrometers in diameter and either multipolar or fusiform in shape. The nucleus was slightly indented and eccentrically located within the neuron. The cytoplasm contained a moderate amount of organelles and appeared granular perhaps due to the peroxidase-anti-peroxidase reaction. These labelled neurons morphologically correspond with the description of DA neurons in the substantia nigra and ventral tegmental area. Unlabelled boutons containing pleomorphic vesicles synaptically contacted TH-immunoreactive cell bodies. Symmetric and asymmetric axosomatic synapses were identified. Several labelled perikarya closely abutted unlabelled cells within the aggregate; however, membrane specializations were rarely observed between the opposed plasma membranes. TH-positive dendrites generally had a smooth contour and were contacted by unlabelled boutons along their length. Both symmetric and asymmetric axodendritic synapses were observed. In adult animals symmetric synapses predominate. A few proximal dendrites projected between 1-3 spinous processes which were synaptically contacted by unlabelled boutons near their base. Spines are rarely observed on dopaminergic dendrites of adult animals but may occur during neuron development. TH immunoreactive boutons were usually found in a region away from positively labelled cell bodies. Label in these boutons was dark and granular which frequently obscured the shape of the synaptic vesicles. These bouton profiles were among the smaller of the bouton profiles within the reaggregates. Dendrites were the most common post-synaptic targets of TH-positive boutons although a few axosomatic synapses were evident. The majority of these synapses had symmetric membrane specializations which have been described in the adult corpus striatum. Thus, DA neurons in reaggregate cell culture form and receive synaptic connections which resemble those seen in vivo. (Supported by MH-28942 and L.W. was supported by GM-07151.)

22.18 SELECTIVE ASSOCIATION OF DOPAMINERGIC (DA) AXONS WITH THEIR STRIATAL TARGETS IN VITRO. A. Heller and L. Won* Department of Pharmacol. and Physiol. Sciences, The Univ. of Chicago, Chicago, Il 60637

Proliferation and/or maintenance of DA axons in aggregate cultures occurs only in the presence of target cells (Hemmendinger et al., PNAS 78(2):1264-1268, 1981). The purpose of the present study was to determine whether the outgrowth of such DA axons would be restricted to areas containing target cells in aggregates composed of both target and non-target cells. We examined the distribution of DA axons after simultaneously confronting DA cells with both target cells of the corpus striatum (CS) and with non-target cells of the tectum (T). Dissociated fetal cells of the rostral mesencephalic tegmentum (RMT) containing DA neurons and dissociated non-target T cells were exposed to wheat germ agglutinin conjugated to the fluorescent dye, rhodamine, prior to reaggregation in rotary culture in order to allow one to distinguish these cells from non-dyed CS cells in the resulting aggregates. After 9 days in culture the RMT-T-CS aggregates were exposed to 10^{-6} M DA and processed for DA histofluorescent development. Single aggregates were serially sectioned and color photomicrographs prepared from each section. It was found that non-target cells (red fluorescence) segregated from the undyed target cells forming discrete areas consisting of the 2 cell types. DA neurons and their processes could be distinguished by their green fluorescence. Since it is not possible to distinguish axonal from dendritic processes in a single section, the extent of the dendritic fields was estimated from aggregates prepared from cells of the RMT and non-target T cells, in which there is no extensive proliferation of DA axons and DA fluorescent processes are confined to the area near the cell body. DA neurons were identified in the photos of RMT-T-CS aggregates and the dendritic fields surrounding the cell bodies were subtracted. A 0.5 cm^2 transparent grid was placed over the photomicrographs and the squares containing either green (DA) or red (rhodamine) fluorescence marked on separate grids. By comparison of superimposed grids the extent of overlap of green DA fluorescence into areas of non-target cells (red rhodamine dye) could be determined. Following the exclusion of DA dendritic fluorescence it was found that 85.2% of the presumed axonal fibers were present in the non-dyed areas containing striatal target cells. This finding suggests that DA axons either do not invade, or do not persist, in non-target areas when given the opportunity afforded by the presence of adjacent target cells. Moreover, the target cell factor(s) responsible for the proliferation and/or maintenance of DA axons does not appear to be diffusable into adjacent non-target cell-containing areas. (Supported by MH-28942 and L.W. was supported by GM-07151.)

23.1 INCREASED BODY WEIGHT PRODUCED BY GESTATIONAL UNDERNUTRITION. A. P. Jones & L. S. Crnic, Depts. of Psychiatry & Pediatrics, Univ. Colorado School of Medicine., Denver, CO. 80262.

Previous work by Jones and colleagues demonstrated that male offspring of rats restricted to 50% of pre-pregnancy intake during the first 2 trimesters of pregnancy and then refed normally during the 3rd trimester develop hyperphagia and obesity as adults. This obesity is characterized by increased adipocyte size but not number, and is not a function of altered lipoprotein lipase activity, triglyceride levels nor locomotor activity. In the current study we assessed the status of several metabolic systems which are important in the etiology of obesity. Hepatic lipogenesis was assessed by measuring glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, and fatty acid synthetase. The function of brown adipose tissue (a major thermogenic organ) was determined by monitoring core temperature in a 4°C room for 24 hours. Intrascapular brown adipose tissue (IBAT) depots were weighed. Activity of hepatic cytochrome P-450 monooxygenase, (a pathway associated with the exothermic oxidation of NADPH) was determined. Body composition (lipid, protein, water and ash) was determined at birth.

Initial attempts to continue this work in Denver (1600m) were unsuccessful because litters were resorbed at levels of food restriction (50% of pre-pregnancy baseline) successfully used at sea level: 68% of baseline intake was the minimum requirement for the litter to be carried to parturition. Rats were either fed ad libitum throughout gestation or 68% of baseline intake for the first two weeks of pregnancy and then refed either ad libitum (DA), or a daily ration equivalent to the intake of control animals (DP). The latter group was included as a control for the hyperphagia which follows such a period of food restriction. No differences were found in birth weight or carcass composition however, male DA offspring weaned onto a high-fat diet were significantly overweight by 60 days of age ($p < .05$). No group differences were found in hepatic lipogenic enzyme activity nor were any differences detected in P-450 levels, core temperature, nor IBAT weight following 24 hour cold exposure.

In conclusion, increased body weight in rats deprived during early gestation does not appear to be due to changes in the synthesis or storage of lipids, nor in energy expenditure as measured by locomotor activity, core temperature, IBAT weight, and P-450 levels. Supported by NIMH MH09169, HD18378 and the Developmental Psychobiology Research Group.

23.2 AMINO ACIDS IN BRAIN REGIONS OF UNDERNOURISHED RATS. M.J. Druse-Manteuffel and W. E. Rathbun*. Department of Biochemistry and Biophysics, Loyola University Stritch School of Medicine, Maywood, Illinois 60153.

Sprague Dawley rat mothers were fed either a protein-calorie deficient (30% reduction) or control diet from the 5th to the 21st postnatal day. The concentrations of aspartate, glutamate, γ-aminobutyric acid (GABA), glycine, glutamine, serine and taurine were determined in the cortex, cerebellum, corpus striatum, hippocampus, hypothalamus, brain stem and midbrain of 17- and 35-day-old undernourished and control offspring. Amino acids were derivatized with o-pthaldialdehyde, and then separated and quantitated using reverse phase HPLC coupled with fluorometric detection.

At 17 days of age, taurine was the amino acid with the highest concentration, while at 35 days glutamate had the highest concentration. This change was due to the fact that the levels of taurine decreased significantly in all brain regions between 17 and 35 days, while those of glutamate remained high or increased somewhat in all brain regions except the hypothalamus and brain stem.

In comparison to control rats, the concentrations of glutamate and aspartate were significantly lower (decreased 16 to 34%) in the cerebellum, brain stem, cortex and midbrain of 17-day-old undernourished rats. Aspartate was also significantly decreased in the corpus striatum and hypothalamus in 17 day offspring. However, normal levels of aspartate and glutamate were found in all brain regions of 35-day-old undernourished rats. In contrast to the transient changes in aspartate and glutamate, the concentration of taurine was increased in the hypothalamus (31%) and hippocampus (12 to 33%) at both 17 and 35 days of age. Taurine was also increased in the midbrain (17%) of 17-day-old undernourished rats. Other transient abnormalities in the regional levels of amino acids were found in the undernourished offspring.

The results of these experiments suggest that undernutrition during lactation causes delayed CNS development which is manifested in altered concentrations of the neurotransmitters aspartate, glutamate and taurine.

This research was supported by a grant from the Potts Fund.

23.3 MAINTENANCE OF LONG TERM POTENTIATION OF THE EPSP IN THE DENTATE GYRUS OF PROTEIN MALNOURISHED RATS. K. Austin * and P.J. Morgane (SPON:O.Resnick). Worcester Foundation for Expt. Biol., Shrewsbury, MA 01545.

To determine the effects of protein malnutrition on brain development and function, we are studying hippocampal long-term potentiation (LTP) in the offspring of protein restricted dams. Field potentials evoked by stimulation of the perforant path were studied in the dentate gyrus of anesthetized adult rats. These animals were born to dams fed either a 6% or 25% casein diet initiated 5 weeks prior to mating. The prenatally malnourished group (designated 6/25) consisted of pups born to malnourished dams that were cross-fostered at birth to normal (25% casein diet) lactating dams and maintained on 25% casein after weaning. The chronically malnourished group (designated 6/6) were derived from malnourished dams and maintained on a 6% casein diet after weaning. Control animals were derived from dams fed the 25% casein diet and maintained on this diet after weaning. Dams subjected to the 6% casein diet yielded pups with significantly lower brain ($p < .05$) and body weights ($p < .001$) than the controls (Resnick et al., Neurosci. Biobehav. Rev., 6:55-75, 1982). After obtaining baseline field potentials, a brief burst of pulses was administered once every 5 seconds for 20 seconds (5 bursts total). Test pulses were administered periodically for 4 hours after conditioning to track the maintenance of the established potentiation. Following this paradigm, the control group showed potentiation of the EPSP slope of 39%. Potentiation was maintained through the 4 hours after conditioning. In contrast, both experimental groups lost the EPSP potentiation they had initially gained. Prenatally protein malnourished rats (6/25) had EPSP slopes that potentiated 22% but returned to baseline after 3 hours. The chronically malnourished animals (6/6) potentiated the EPSP by 9% which returned to baseline after 2 hours. These results suggest that a purely prenatal dietary protein restriction has an effect on synaptic plasticity in otherwise normally reared offspring when tested in adulthood. This prenatal influence when combined with chronic postnatal malnutrition leads to a further deficit in establishing and maintaining LTP. This data supports our previous findings showing that chronically malnourished (6/6) animals potentiate the EPSP significantly less than controls when conditioning stimulation was delivered during the still, alert state of waking. The ability to modify synaptic activity for the long-term is a prerequisite for neuronal information storage and retrieval. Although the behavioral and functional consequences of a deficit in developing and maintaining LTP are unknown, it can be speculated that some memory processes are affected which may be associated with long-term learning deficits (Supported by NIH Grant HD-06364).

23.4 BEHAVIORAL EFFECTS OF SEVERE AND MODERATE EARLY MALNUTRITION. C. Wolf*, C. R. Almli, S. Finger, S. Ryan*, and P. J. Morgane. Dept. Psychol., Washington Univ., St. Louis, MO 63130 and Worcester Found. Exper. Biol., Shrewsbury, MA 01545.

Two recent studies on radial arm maze performance in previously undernourished rats have presented contradictory results. Jorden et al. (Dev. Psychobiol., 1981) reported that early undernourished rat offspring (50% food restriction to the dam during pregnancy and lactation) performed poorly relative to well-fed rats, whereas, Hall (Dev. Psychobiol., 1983) found no group differences in a study involving offspring of rats fed low protein diets (8% casein) during pregnancy and lactation. For this reason, radial arm maze performance was reexamined in the present study, but under 2 undernutrition conditions and in conjunction with other behavioral measures.

Dams were prenatally and postnatally undernourished (6 or 8% casein diets) or well-fed (25% casein). All female offspring received 25% protein diets after day 21, and approximately 100 days later were tested in an open field and in an 8-arm radial maze. The 6 and 8% rats weighed less than the 25% rats at testing, but did not differ from each other.

Although the 6% rats seemed hyperactive, the groups only showed a trend toward differences in activity and rearing up in the open field. Inspection and further analysis of the data showed that the failure to achieve statistical significance was due to one deviant (hypoactive) 6% rat. In the radial arm maze, the 6% rats again appeared to be hyperactive and ANOVAs revealed significant group x test day interactions. The undernourished rats (6% and 8%) made more entry errors as well as correct entries than the 25% group during the first 10 days of testing. After this, the undernourished group scores more closely approximated those of the well-fed rats. The scores of the 6% rats deviated more than those of the 8% rats on these measures.

These results show that rats subjected to severe, early undernutrition (6% casein) display greater performance deficits than those experiencing milder undernutrition (8% casein). Further, the data suggest that severe, early nutritional deficiencies may result in hyperactivity and an inability to inhibit responding. The radial arm maze and open field performance profiles of the 6% rats may be comparable to descriptions of hyperactive children. Further experiments are underway to evaluate the possible relationship between early nutritional deficiencies and hyperactivity. (Supported by BRSG-7054 & HD-06364 Grants).

23.5 DIFFERENCES IN PASSIVE AVOIDANCE BEHAVIOR DUE TO MILD PERINATAL ZINC DEFICIENCY AND/OR UNDERNUTRITION. E.S. Halas, T.P. Tinius* and B.E. Beckwith. USDA, ARS, Grand Forks Human Nutrition Research Center and University of North Dakota, Department of Psychology, Grand Forks, ND 58202.

Hesse, Hesse & Catalanotto (1979) attempted to determine if young adult rats, who were chronically zinc deficient, behaved like hippocampal lesioned rats in a passive avoidance paradigm. The Hesse et al. (1979) results showed that chronic zinc deficient rats (ZD) had slower entry times (latency) than undernourished (PF) rats and concluded that the abnormal behaviors associated with zinc deficiency are opposite from those observed in hippocampal lesioned animals. The present experiment tested the hypothesis that adult male rats who were zinc deprived or undernourished during gestation and lactation would have slower test latencies in a passive avoidance paradigm than animals fed a normal diet (AL). Thirty-nine male Long Evans rats were used for this study. The test apparatus was a passive avoidance chamber. Beginning on the day of conception, one group of dams was fed ad libitum a mildly zinc deficient (ZD) diet (10.00 µg Zn/g) and maintained on the diet until their pups were weaned. Since zinc deficiency causes anorexia, a second group of dams, pair-fed (PF), was fed the same quantity of the diet as consumed by their ZD mates. A third group of dams was fed the diet ad libitum (AL). After weaning at 23 days of age, all litters were fed Purina Chow ad libitum. On the first day of training, each animal was placed in the dark chamber for 2 min. This was immediately followed by a single trial in which the animal was placed on the runway facing away from the door. The door was opened immediately and the animal was allowed to enter the dark chamber. Ten sec later the animal was removed and placed in its home cage. On the second day of training, all animals received 3 more trials to enter the dark chamber, with a inter-trial interval of 2 min. On the third trial, each animal received a 2 sec unavoidable foot-shock (0.5 mA supplied by a shock scrambler) through the grid floor immediately after entering the chamber with all four feet. The animal was removed from the chamber 10 sec after the completion of the shock and placed in its home cage. Test trials for each animal were conducted at 24, 48, 72, and 96 hr intervals after the learning trial. The present study found that only the AL rats showed a decrease in test latency ($p < .05$) over a 4 day period and illustrates why it is necessary to use both the PF and AL groups when studying zinc deficiency. The ZD rats had faster latencies ($p < .05$) than the PF rats only on day 3 and both the PF (246 sec) and ZD (209 sec) rats had longer latencies ($p < .05$) than the AL (163 sec) rats on days 2 to 4. The PF group is not an adequate substitute for the AL group.

23.6 DEVELOPMENTAL MALNUTRITION IMPAIRS REVERSAL LEARNING OF A SUCCESSIVE CONDITIONAL DISCRIMINATION. C.R. Goodlett and M.L. Valentino. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

An increasing number of studies indicate that the hippocampal formation may be vulnurable to permanent structural and physiological alterations following maternal dietary protein restriction. Behaviorally, we have observed that chronically malnourished rats exhibit perseverative deficits on delayed spatial alternation learning, but are not impaired in simple spatial location learning in the Morris maze. To further characterize the behavioral effects of developmental malnutrition on learning tasks thought to involve hippocampal processing, we examined the acquisition and reversal of a successive conditional discrimination.

Virgin female Sprague-Dawley rats were provided either a 6%, 8% or 25% casein diet for five weeks prior to mating. The dams were maintained on their respective diets throughout gestation and lactation, and at weaning all pups were given the 25% casein diet. At 4 months of age, female rats from each maternal dietary treatment were tested on the successive conditional maze discrimination. The maze contained a start box, four discrimination units (each having two alleys) and a goal box. Over a three week period all rats were adapted to a water deprivation schedule and trained to traverse the maze. For the successive conditional discrimination training, all rats were given 4 training trials per day, encountering four choices on each trial. During training, the correct alley (allowing passage through the unit) was contingent on the illumination of the unit.Thus, when both alleys were illuminated the right alley was the correct choice; when neither alley was illuminated the left alley was correct. The order of the correct choices across units within each trial and across trials within each unit was randomized each day. Choices at each unit were recorded and retracing of units was prevented. All rats were given 17 days of acquisition training followed by 12 days of training on the reversal problem.

All three groups acquired the original successive conditional discrimination, and no statistically significant effects of dietary history were seen for errors during acquisition. However, on the reversal problem, the rats with a history of malnutrition committed significantly more errors than controls ($p<.001$ for both 6% and 8% groups). The rats from the 6% group also committed more errors than those of the 8% group ($p<.05$). Continuing studies are examining the relative importance of gestational versus lactational protein restriction in producing the learning deficits in mature rats. (Research supported by NIH grant HD 06364).

23.7 ALTERED BEHAVIORAL RESPONSE TO KINDLING FOLLOWING PRENATAL PROTEIN MALNUTRITION. R. J. Austin-La France,* J. D. Bronzino, E. Muik,* J. Senaldi,* P. J. Morgane (SPON:D. Jones).Trinity College Hartford, Ct. 06106 and The Worcester Foundation For Experimental Biology, Shrewsbury, Ma. 01545.

Previous studies have indicated that animals subjected to various forms of malnutrition are more susceptible to seizures than animals reared on normal diets (Taber et al., Experientia, 36: 69-70, 1982). The present studies were designed to evaluate the impact of a specific form of malnutrition - prenatal protein malnutrition - on the electrophysiological and behavioral correlates of the kindling process. In order to evaluate these effects, rats born to dams maintained on either a 25% or 6% casein diet were bilaterally implanted in homologous regions of hippocampal field CA1 and in the frontal cortex with chronic electrodes at 30 days of age. Kindling was induced by daily electrical stimulation of the CA1 field of the hippocampus. We found that animals raised on the 6% diet had a significantly lower ($p < 0.01$) mean threshold intensity to afterdischarge (AD) and a significantly longer ($p < 0.01$) mean AD duration compared to the 25% diet group. The spread of AD activity to the frontal cortex was markedly enhanced in the 6% diet group, appearing on day 1 of stimulation as opposed to an average of day 15 in the 25% diet group. The behavioral manifestations of the kindling process were also considerably different in the 6% group. While the 25% diet group progressed through four behavioral stages as described by Araki et al., (Jap. J. Pharmacol., 33: 57-64, 1983), 6% diet animals rarely followed this normal progression. For example, they seldom showed rearing as a stage 2 behavior and instead would progress from stage 1-type behaviors to a behavior characterized by freezing with rhythmic waves of myotonic contractions moving from neck to tail. Finally, all of the 25% diet animals reached the convulsive stage after an average of 22 daily stimulations while 80% of the animals in the 6% group failed to reach this stage after 40 stimulations. These findings suggest that prenatal protein malnutrition may retard or alter the development and functional maturation of neuronal mechanisms involved in local excitability, generalized spread and the behavioral correlates of epileptogenic activity within the limbic system.(Supported by NIH Grant HD 06364).

23.8 AUTORADIOGRAPHIC CHANGES IN DOPAMINE AND MET-ENKEPHALIN RECEPTOR BINDING IN RATS PRENATALLY EXPOSED TO ETHANOL. D.E. Hand and S.F. Hoff, Dept. of Pharmacology, University of Health Sciences/ The Chicago Medical School, North Chicago, IL 60064.

Cognitive and behavioral deficits found in animals exposed to alcohol in utero may result from underlying aberrations in the ontogeny of central neurotransmitter systems. We examined the distribution of dopamine and enkephalin receptor binding sites using in vitro autoradiography to localize and quantitate [^{3}H]-spiroperidol and [^{3}H]-Met-enkephalin binding in the CNS of 15 day old rats.

Pregnant rats were fed a liquid diet (BioServ) containing 35% ethanol derived calories and pairfed controls received the caloric equivalent in liquid diet without alcohol. Alcohol exposure was terminated on the day of birth as the litters were culled to eight pups and cross-fostered to mothers that consumed normal rat chow diets throughout pregnancy. On day 15, animals were sacrificed by decapitation, and the brains were quickly removed and frozen in isopentane at -25°C. Serial sections six microns in thickness were collected in the coronal plane, mounted onto slides, and incubated in buffers containing radioligands for total and nonspecific binding analysis. The sections were rapidly dried and apposed to Ultrofilm alongside brain paste standards for varying lengths of time. The film was developed and analyzed densitometrically for changes in binding between the two treatment groups.

Decreased dopamine binding in ethanol vs. pairfed animals occured in the frontal cortex, olfactory tubercle, nucleus accumbens septi, and caudate-putamen, while and increase in [^{3}H]-spiroperidol binding was observed in the olfactory bulb. Decreased [^{3}H]-Met-enkephalin binding in ethanol animals was observed in regions of the cerebral cortex, hippocampal formation, and thalamic nuclei while an increase in binding was observed in the caudate-putamen. Many of these regions receive dopaminergic innervation from the mesolimbic, mesocortical, and nigrostriatal systems. Alterations in these systems have been correlated with disorders of behavior and movement and may underly similar neurologic deficits associated with fetal alcohol exposure. (Supported by NIAAA AA06156.)

23.9 A BIOCHEMICAL EVALUATION OF BRAIN DEVELOPMENT IN OFFSPRING OF RATS FED WITH ETHANOL DURING GESTATION. F. E. Lancaster, J. -R. Yu* and L. L. Hsu. Dept. of Biology, Texas Woman's Univ. Houston, TX, and Dept. of Psychiatry and Behav. Sci., UTMB, Galveston, TX 77550.

Our previous work has shown that postnatal exposure to ethanol through lactation altered the specific activities of ^{3}H-spiperone (SPD) binding, glutamic acid decarboxylase (GAD) and choline acetyltranferase in the female rat brain striatum at 30 days old. We have further examined the possible effects of gestational exposure to ethanol on the neurochemistry of the developing brain of the offspring. Timed pregnant Long-Evans rats were received on day 4 of gestation, and housed separately in a temperature controlled room with a light, dark cycle of 12 hours. On days 4-19 of gestation, one group of dams (ET) had ad libitum access to a liquid diet containing 30% of calories as ethanol. A second group of dams (PF) had ad libitum access to control liquid diet on day 4 of gestation. Beginning on day 5 of gestation and through day 19 of gestation, the PF group received control liquid diet in amounts equal to the average consumption of the ET animals on the previous day. A third group (CT) had ad libitum access to control diet on days 4-19 of gestation. Following day 19 of gestation, all dams received ad libitum access to laboratory chow and water. On day 1 (day 0 = day of birth) litters were culled to 8 pups (4 males and 4 females) and toe clipped for identification. On days 14 and 28, pups (n = 15 litters per dietary group) were sacrificed by decapi-tation and brains were rapidly removed. Striata were dissected immediately from the brains over ice, frozen on dry ice and stored at -60 ^{o}C until used. Striatal tissues were homogenized in 5 vol. of K-phosphate buffer, pH 7.2, and aliquots were used for GAD and AChE assays. The remaining portions were used to repare the plasma membranes (Pm) for ^{3}H-SPD binding and adenylate cyclase (AC) assays. Results indicated that at 14 days old, the specific AC activity in the striatum was significantly increased in the ET group compared to the PF or CT group (20% and 130% respectively, $p < .005$), and the specific GAD activity was moderately but not significantly increased in the ET group compared to either the PF (29%) or CT (7%) group. At 28 days old, the striatal AC activity was slightly but not significantly decreased in the ET group compared to the PF group, and the GAD activity was not altered in the ET group compared to either PF or CT group. Moreover, no changes were observed in the striatal ^{3}H-SPD binding or AChE activities in either age group after the gestational ethanol exposure. Whether the increase of AC activity also exists in other brain regions after gestational ethanol exposure remains to determined.

23.10 A DOSE-RESPONSE STUDY OF THE EFFECTS OF ETHANOL ON CORPUS CALLOSUM DEVELOPMENT IN BALB/c AND C57BL/6 MICE. S.B. Cassells*[1], P. Wainwright[2] and K. Blom*[1]. [1]Dept. of Psychology, and [2]Dept. of Health Studies, Univ. of Waterloo, Waterloo, Ont. Canada N2L 3G1

The intent of this study was to further explore the interaction between the effects of heredity and ethanol on brain development in mice. Animals of the BALB/c inbred strain are prone to defective growth of the corpus callosum (CC) where this forebrain commissural tract is either absent at midline or greatly reduced in size (Wahlsten, D. Brain Res., 68: 1-18, 1974). Previous work has shown that ethanol affects the growth of the CC in BALB/c but not in C57BL/6 mice (Wainwright, P. and Gagnon, M. Exp. Neurol. 88: 84-94, 1985). The present experimental design was that of a dose-response study in each of the two strains, with food intake adjusted to control for strain differences in alcohol preference. Animals in all groups were fed 0.6 Kcal/g/day of a Sustacal-based liquid diet, with the ethanol content being 0, 15, 17.5 and 25% of the total calories. Maltose-dextrin was substituted isocalorically for ethanol so that all groups received 25% of their calories from non-nutritive sources. Preliminary work had indicated that BALB/c animals would not produce litters at an ethanol dosage level of 25% and therefore this group was not included in the study. Fetal development was assessed on day 18 of gestation. The dependent variables included the cross-sectional area in a midsagittal section of both the CC and anterior commissure (CA), as well as fetal brain and body weight. In addition, measures were taken of maternal blood alcohol concentrations. The results indicated an effect of ethanol on body and brain growth, with the effect on brain weight being independent of that on body weight. C57's had heavier bodies and brains than the BALB's and there was evidence of a strain by ethanol interaction on body weight, with the C57's showing a larger reduction in body weight at the higher doses. Preliminary analyses of the data indicated an effect on the growth of the CC, independent of an effect on brain weight, only in the BALB's. In contrast, there did not appear to be such an effect on the CA in either strain. Maternal blood alcohol concentrations reflected the ethanol content of the diet and there were no significant strain differences at any dose. These results suggest that hereditary factors may be an important consideration when predicting the effects of ethanol on specific aspects of fetal brain growth.

This research was supported in part by NSERC grant #A7617 to P. Wainwright.

23.11 MICROENCEPHALY INDUCED DURING THIRD TRIMESTER EQUIVALENT IS DEPENDENT ON ALCOHOL EXPOSURE PATTERN. D.R. Pierce and J.R. West. Dept. of Anatomy, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

Using a rat model system to study fetal alcohol effects (FAE) during a period of rapid brain development equivalent to the human third trimester requires exposing rats to alcohol postnatally. An artificial rearing procedure was implemented in which chronic gastric cannulas were surgically implanted in rat pups on postnatal day 4 (West, Hamre, and Pierce, Alcohol, 1:213, 1984). Pups were fed a milk formula in twelve 20 minute fractions daily.

Three groups of 7 pups each were analyzed on postnatal day 10. The groups were gastrostomized controls (G/C) fed the control formula, gastrostomized alcohol group with a uniform alcohol exposure (G/UAE) of 2.5% EtOH added to the diet for each feeding, and a gastrostomized alcohol group with a condensed alcohol exposure (G/CAE) of 5.0% EtOH added to the diet each day for 6 feedings (12 hours) followed by 6 feedings of alcohol-free diet. Both alcohol groups received an identical dose of 6.57 g/kg for each 24 hour period. Blood alcohol concentrations (BACs) taken on day 6 revealed BACs of 55.2 ± 9.9 mg/dl for the G/UAE group. BACs peaked at 268.7 ± 12.1 mg/dl in the G/CAE animals and dropped to 11.0 ± 7.8 mg/dl by the end of the alcohol free period.

There were no significant differences at day 10 in body, liver, or adrenal weights between any of the groups. However, significant microencephaly ($p<0.05$) was observed in the G/CAE animals. Brain weight and the brain weight/body weight ratio were reduced in the G/CAE group by 8.3% and 13.6%, respectively, compared to the G/C group. The G/C and G/UAE groups were not statistically different for these measurements.

Microencephaly was induced in the G/CAE animals using the same daily alcohol dose as the G/UAE animals by varying the alcohol exposure pattern. The different alcohol exposure patterns resulted in markedly different BACs. These data indicate that the blood alcohol concentration is more important than the dose for inducing microencephaly during the third trimester equivalent in the rat. This study was supported by NIAAA grant AA05523 to J.R.W.

23.12 **NICOTINE ADMINISTRATION DURING PREGNANCY AND ITS EFFECT ON STRIATAL DEVELOPMENT.** **L.C. Murrin, J.R. Ferrer* and W. Zeng* Dept. of Pharmacology, Univ. Nebraska Med. Ctr., Omaha, NE 68105**

Studies indicate a strong relationship between exposure to nicotine *in utero* via maternal smoking and increased incidence of CNS developmental problems, particularly learning and behavioral disorders such as minimal brain dysfunction (MBD). Other studies suggest an involvement of the nigro-striatal dopaminergic pathway in MBD and nicotine has been shown to affect dopaminergic neuronal activity in adults and neonates. The objectives of this study were to develop a reliable means of delivering nicotine to female rats during pregnancy and to study the effects of prenatal nicotine exposure on striatal receptors postnatally. Initial studies administered nicotine in drinking water, a procedure reported successful in the literature. Female rats displayed an apparent aversion to nicotine in drinking water. At 0.02% nicotine, which delivered approx. 2 mg/kg-day (eqivalent to a human smoking 1-2 packs of cigarettes/day), there was short term weight loss and a long term 50% decrease in water consumption. At higher doses water and food consumption were depressed further. At 0.4% nicotine water consumption was not sufficient for survival. Adding sucrose or adjusting pH to 7 did not improve water consumption. Because of the limitations on a dose response curve and the probability that water intake was probably insufficient for normal fetal development, we administered nicotine via Alza osmotic mini-pumps implanted on day 3 of pregnancy. With this method nicotine up to 4.5 mg/kg-day did not produce any significant effect on weight gain, water or food consumption in dams beyond those ascribable to surgery. Plasma nicotine and cotinine levels (J. Chronic Dis. 36:439, 1983) correlated directly (r^2=0.998) with dose delivered. Nicotine treatment of dams up to 4.5 mg/kg-day did not affect litter size, pup birth weight or weight gain for two weeks postnatally. Pups were cross-fostered at birth.

Striatal dopamine D2, cholinergic muscarinic and opiate mu receptors were examined at 14 days of age using membrane binding techniques and ^{3}H-spiperone (0.5nM:SP), ^{3}H-N-methyl-scopolamine (1.5nM:NMS) and ^{3}H-diprenorphine (1nM:DPN) as ligands, respectively.

Ligand	Control (vehicle)	Low nicotine (1.5mg/kg-day)	High nicotine (4.5mg/kg-day)
^{3}H-SP	18.2 ± 1.3	30.7 ± 2.4a	17.1 ± 2.1b
^{3}H-NMS	575.0 ± 56.0	741.0 ± 53.0	401.0 ± 33.0a,b
^{3}H-DPN	71.6 ± 13.8	97.8 ± 10.1	43.2 ± 4.6b

Data are specific fmol/mg protein ± SEM, n = 8-14.
a - $p < .05$, significantly different from control, Peritz' F test.
b - $p < .05$, significantly different from low dose, Peritz' F test.

It appears that prenatal exposure to nicotine alters normal striatal development, as seen by receptor binding at 14 days of age, and receptors are affected differentially. **Supported by the State of Nebraska Department of Health (#85-44).**

23.13 DOCOSAHEXAENOIC ACID DEPLETION IN RHESUS MONKEYS: PERSISTENT EFFECT ON RECOVERY TIME OF THE ELECTRORETINOGRAM. M. Neuringer*, W.E. Connor*, and S.L. Luck* (SPON: D. Rhodes). Oregon Health Sciences University, Portland 97201, and Oregon Regional Primate Research Center, Beaverton 97006.

Neural and photoreceptor outer segment membranes are unique in their very high content of docosahexaenoic acid (22:6ω3), a fatty acid of the ω3 series. 22:6ω3 must be obtained directly from the diet or by biosynthesis from other dietary ω3 fatty acids, primarily linolenic acid (18:3ω3).

We are examining the effects of dietary deprivation of ω3 fatty acids in rhesus monkeys during prenatal and postnatal development. Adult females were fed a semipurified diet containing very low levels of ω3 fatty acids (≤0.3% of total fatty acids) and their infants received a similar diet from birth. A control group of mothers and infants received semipurified diets providing ample linolenic acid (8% of total fatty acids).

At birth, levels of 22:6ω3 in phosphatidyl ethanolamine and phosphatidyl serine were reduced by 50% in the retina and by 75% in the cerebral cortex of deficient animals, relative to control values. Postnally, 22:6ω3 levels in control tissues doubled, while those in deficient tissues failed to increase. At two years, 22:6ω3 was reduced by 80-85% in the phosphatidyl ethanolamine of both retina and cerebral cortex of deficient animals. 22:6ω3 was almost entirely replaced by 22:5ω6, an ω6 fatty acid.

Electroretinograms were recorded at 7, 14, and 21 months of age. Following a saturating flash, the recovery of the dark-adapted ERG was significantly delayed in deficient animals. The time required for recovery to half maximal amplitude was increased by 80% and time for complete recovery by 70%.

The reversibility of these biochemical and ERG abnormalities was examined in four deficient monkeys, two at ten months and two at two years of age, after their diets were supplemented with high levels of 22:6ω3 and 20:5ω3. Biopsies of frontal cortex were obtained during the deficient state and at 8-12 week intervals after supplementation. Levels of 22:6ω3 in cortical phospholipids increased rapidly and achieved control levels within 12 weeks. Thus, even after the period of rapid brain growth, the fatty acid composition of neural tissues can be altered by changes in the diet. The reversibility of retinal 22:6ω3 depletion will be confirmed after functional tests are completed. ERG recordings, repeated at 12 and 24 weeks of supplementation, showed no improvement in recovery time. Delayed recovery of the ERG therefore appears to be a persistent effect ω3 fatty acid deficiency.

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23.14 DEPRESSED GLUCOSE METABOLISM BY CORTICAL BRAIN SLICES FROM BRINDLED MICE (Mo^{br}). J.E.Goldman and C.Peterson, (SPON: W.T.Norton), Department of Pathology (Neuropathology), Albert Einstein College of Medicine, Bronx, N.Y. 10461.

The brindled mouse (Mo^{br}) is an X-linked murine mutant with a defect in copper transport. Previous studies demonstrate neuronal degeneration in the cerebral cortex that is characterized by swollen dendrites, enlarged mitochondria and cytoskeletal abnormalities (Acta Neuropathol. 45:17 (1978)). Since copper deficiency may interfere with carbohydrate metabolism, glucose oxidation and its incorporation into the acid insoluble precipitate were examined in vitro with normal and brindled mice. Brain slices (0.3 x 0.3 mm) were prepared from the forebrains of 15 day old brindled mice and normal male littermates. The slices were incubated for 1 hr at 37°C in bicarbonate buffer with high potassium (31 mM) that contained 5 mM- [U-^{14}C] glucose. The incubation was terminated by acidification. Glucose incorporation into $^{14}CO_2$ and the acid insoluble precipitate were determined by liquid scintillation. Each condition was examined in triplicate and protein was assayed in the acid insoluble precipitate.

	Normal	Brindled
$^{14}CO_2$	19.36±0.64	4.49±0.14*
acid-insoluble	2.48±0.15	0.74±0.08*
number of mice	(6)	(6)

Values are nmol/mg protein per hr.
*Denotes difference ($P<0.05$)

Thus, deficits in glucose metabolism due to copper deficiency may contribute to neuronal degeneration. Supported in part by grant AG05386.

23.15 VULNERABILITY AND RECOVERY FROM TRAUMA DURING FAST BRAIN GROWTH. E. Murowchick, E. Moore* and J. Diaz. Dept. of Psych., Univ. of Washington, Seattle, WA 98195.

The procedure of rearing rat pups away from their mothers using a chronic intragastric cannula and a replacement formula has been found to result in 7-10% whole brain weight deficits compared to mother reared siblings, despite comparable whole body weights. This procedure is typically applied during the rat's brain growth spurt (BGS). Dobbing and Sands (1971) have hypothesized that a period of accelerated growth may also be a period of heightened vulnerability to insults. The purpose of this experiment is twofold: 1) to determine whether the insult of artificial rearing will affect brain growth differentially at different phases of the BGS and 2) to determine the efficiency of short term recovery from these insults depending on the phase of the BGS in which the trauma and subsequent recovery phase occurs.

Four day old Long-Evans rats were randomly assigned to either the gastrotomy reared (AR) or mother reared (NR) condition. Those animals assigned to the AR condition were then assigned to one of the three BGS phases (early-days 4 to 8, peak-days 8 to 12, late-days 12 to 16). Within each phase, each animal was assigned either to a recovery group (with an 8 day recovery period) or a non-recovery period. On the first day of the assigned phase, each AR animal was lightly anesthetized and implanted with an intragastric cannula. At the end of the four days of artificial rearing the animals were either sacrificed immediately (no recovery) or returned to a lactating dam and sacrificed 8 days later. NR siblings of the AR animals were sacrificed as age matched controls. At sacrifice, each animal's brain and visceral organs were removed and weighed.

Preliminary analysis of the data suggest that the magnitude of the effect of artificial rearing varies depending on when, during the BGS, the insult is sustained.

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24.1 ARM MOVEMENT CONTROL STRATEGIES: HAND TRAJECTORY VS. JOINT PLANNING. T. Kaminski*.& A.M. Gentile. Teachers College, Columbia University, New York, NY 10027.

Two control strategies could underlie rapid pointing movements: (1) planning hand trajectory or (2) planning joint movement parameters. If a strategy based on hand trajectory planning is used, then certain hand movement parameters should remain constant regardless of whether a single or multijoint movement is required. If a joint planning strategy is used, then certain joint movement parameters should remain constant.

Velocity profiles of the hand, shoulder and elbow were analyzed for 8 subjects who performed rapid pointing movements to a variety of target locations. A two degree of freedom manipulandum permitted shoulder and elbow motion in the horizontal plane. Joint displacements were equivalent during both single and multijoint movements. Single joint movements required either elbow or shoulder movement to three different target locations.

Velocity profiles of the hand differed during single and multijoint movements. The hand always had a higher peak velocity, shorter rise time and movement time during single joint movements, given the same required magnitude of displacement. The only consistency observed in the profile across conditions was its shape: smooth and bell-shaped. In contrast, the velocity profiles of the shoulder remained the same during both single and multijoint movements, while that of the elbow joint changed.

The results do not support a strategy based on hand trajectory planning because neither hand path nor velocity profile were the same for single and multijoint movements. At best, the consistent shape of the profile indicates a strategy that economizes effort by minimizing jerk of the hand. The consistency of the shoulder velocity profile indicates that arm movements can be planned in terms of joint parameters. Maintaining the same shoulder parameters for a variety of movements provides a stable base upon which additional components can be placed.

24.2 CHARACTERISTICS OF VELOCITY PROFILES OF HUMAN ARM MOVEMENTS AND SPEECH. D.J. Ostry*, J.D. Cooke and S.H. Brown. McGill University, Montreal, Quebec and University of Western Ontario, London, Ontario.

In recent years there has been interest in the idea that the geometric form of the velocity profile of voluntary movements is invariant under transformations of movement amplitude and rate. We have explored this problem by examining cyclical and discrete elbow and tongue movements. The form of the velocity profile of movements can be described by $V_{max}/A = c/T$, where V_{max} is the maximum velocity, A is amplitude, T is duration and c is a parameter that is characteristic of the shape of the velocity profile.

We examined both cyclical and discrete movements because the form of their velocity profiles has been shown to differ even if the movements are similar in duration and amplitude. For example, if movements are made so as to minimize jerk, c is equal to 1.56 for cyclical movements (Nelson, 1983) and 1.88 for discrete movements (Hogan, 1984).

For limb movements, our findings to date indicate that the numerical value of the velocity profile parameter ranges from 1.5 to 1.6 for cyclical movements. This holds for both flexion and extension movements of different amplitudes and durations. These estimates are consistent with predicted values for cyclical movements. In contrast, estimates of the velocity profile parameter for discrete movements vary systematically with movement duration, ranging from about 2.4 for short duration movements to approximately 1.8 for long duration movements. This suggests that in discrete arm movements velocity profiles do not form a single family of geometrically equivalent curves.

Values for the velocity profile parameter in speech movements are similar to those observed for elbow flexion and extension. Parameter estimates are higher for discrete than for cyclical movements and as in limb movements, parameter estimates for discrete movements vary systematically with movement duration.

The assumption of equivalence of the geometric form of velocity profiles thus appears reasonable in the case of repetitive or cyclical movements but appears untenable in the case of discrete movements. The findings point to an underlying similarity in the control of speech and limb movements.

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24.3 TRAJECTORY COMPENSATIONS IN PRACTICED MOVEMENTS. W.G. Darling and J.D. Cooke. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

In recent studies we have shown that although movement variability decreases during practice variability in the EMGs of movement related muscles may increase. Increased EMG variability was associated with increases in movement speed. Since the EMG reflects the neural activity controlling the muscle this suggests that fast movements are made with variable neural commands to agonist and antagonist muscles. Movement trajectory (velocity versus position during movement) could be maintained relatively constant under these conditions if, for example, variations in the first agonist burst were compensated by appropriate variations in the antagonist burst. This could be viewed as a form of trajectory compensation in which departures from a learned trajectory due to EMG variations are corrected to return the limb to the intended trajectory.

The interrelationships between movement trajectories and EMGs of movement-related muscles were studied in 20 normal human subjects performing 10^o and 30^o amplitude elbow flexion and extension movements in a visual step tracking task. Subjects were instructed to make accurate movements and to attempt to increase movement speed during practice while maintaining accuracy. Arm position and velocity were recorded along with surface EMGs from the biceps and triceps (lateral head) muscles. Data were sampled on-line at 500 Hz.

Averaged data from practiced movements provided an indication of the intended trajectory of the learned movements. The trajectories of individual movements deviated from the intended trajectory due to variations in the first agonist burst of EMG activity. These deviations could be compensated and the limb returned toward the intended trajectory by appropriate modifications in the timing and/or amplitude of the antagonist burst. The data indicates an ongoing control of the precise movement trajectory even in movements about a single joint. Compensatory modifications for errors in the initial trajectory are achieved through the antagonist burst and may occur through peripheral feedback influences, segmental mechanisms or through efference copy of the burst of neural activity initiating agonist contraction.

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24.4 CHANGES IN AGONIST AND ANTAGONIST EMG PATTERNS AS A FUNCTION OF DISTANCE, TARGET SIZE AND VELOCITY. D.M. Corcos, G.L. Gottlieb, G.C. Agarwal and R.D. Penn. Departments of Neurosurgery and Physiology, Rush Medical College, Chicago, Illinois 60612

This study investigated the EMG pattern associated with accurate, rapid elbow flexion and extension movements. Six individuals made movements over 18, 36 and 72 degrees to targets 3, 6, 9 and 12 degrees wide. They also made 72 degree movements to a 24 degree target.

Movement time increased as a function of increasing target distance and decreasing target size. The time to peak velocity occurred later with respect to increasing distance and earlier with respect to increasing target size.

Even though different strategies affect the agonist EMG, duration and area increased as: 1) distance moved increased: 2) target size increased. Both these factors are explained by the relationship between agonist EMG and velocity which increases as distance and target size increase. This relationship can be described by the equations:

$IAEMG = -27.0 + 1.65\ A + 3.7\ W\ R^2 = .93,\ F(2,9) = 61.2\ p < .05$

$IAEMG = -26.1 + .42\ V\ r^2 = .95,\ F(1,10) = 172.6\ p < .05$

where IAEMG is the integrated agonist EMG corresponding to the movement as determined from the onset of the EMG until the velocity of the movement falls below 5% of peak velocity, A=movement amplitude, W=target width and V=peak velocity of the movement.

The antagonist EMG duration and area under the EMG envelope do not appear to follow any clear scaling properties similar to those of the agonist EMG.

With reference to the onset of the agonist, the longer the distance moved, the later the antagonist was activated. When distance is held constant, higher movement velocities were associated with earlier antagonist activation.

The agonist was often activated before observable movement onset and, as such, can not be reflexively initiated. However, in some subjects another burst of antagonist activity occurred later which may be compatible with reflexively initiated EMG.

Early antagonist activation suggests that its function is not only to halt the movement but also to increase the impedance of the joint and thus stabilize it during and after movement. As a consequence, the EMG signal can only be understood in combination with information about the load, the direction of movement, a subject's specific strategy and the forces involved.

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24.5 HUMAN TRACKING PERFORMANCE: PARALLEL SPECIFICATION OF AMPLITUDE AND DIRECTION. M. Favilla*, W. Hening, & C. Ghez. Center for Neurobiology & Behavior, Columbia Univ., NYS Psychiatric Inst., New York, NY 10032.

This study examines whether the amplitude and direction of targeted motor responses are specified by serial or by parallel processes. We have used a paradigm which allows us to independently control the processes responsible for trajectory specification and those responsible for response initiation (Soc. Neurosci. Abstr., 10:801, 1984). Four human subjects were trained to initiate isometric force impulses (elbow flexion and extension) in synchrony with the last of a series of predictable tones; after reliable performance was achieved, subjects were instructed to also match both the amplitude and direction of their force impulse to those of a visual target. The interval between target presentation and the response-synchronizing tone was randomly varied from 0 to 500 ms to control stimulus-response (SR) interval; targets were unpredictable in direction and amplitude (three force amplitudes in both flexion and extension).

At short SR intervals, before the target could have an effect, subjects produced "default" responses whose amplitudes were clustered around the average force required, but which were of random direction (50% directional errors). An influence of the preceding target on response amplitude was first detectable at SR intervals of about 150 ms. Specification was complete at about 350 ms when response amplitudes correctly matched the targets. Directional specification occurred more abruptly, starting later (at SR intervals of about 200 ms) and finishing sooner, so that no directional errors were present after 250 ms. Thus, at SR intervals greater than 150 ms, the amplitudes of responses in the wrong direction correctly reflected the target force (correlations of the absolute values of peak force to the target force gradually increased to values between 0.6 to 0.9). In no session did directional specification precede amplitude specification.

Contrary to previous suggestions that response features must be specified sequentially, we conclude that subjects can utilize parallel processes to specify the amplitude and direction of motor responses. Specification of either response feature can begin without complete specification of the other.

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24.6 EFFECTS OF INITIAL POSTURE ON POSTURAL ADJUSTMENTS DURING RAPID AND SLOW VOLUNTARY ARM MOVEMENTS. W.A. Lee and S.H. Tang*. Northwestern University and Rehabilitation Institute of Chicago, Chicago, IL 60611.

The speed of disturbances to balance has been shown to influence EMG patterns of postural adjustments (PAs) in standing humans (Diener, Exp. Br. Res.,52:423, 1983; Horak, Neurol., Neurosurg. & Psychiat.,47:1020, 1984). For slow as compared with rapid voluntary arm movements, variability in the timing of PAs increases almost 10-fold (Lee, W. Neuros Abs:54.6, 1983). Some variability in postural EMG onsets for slow movements could reflect bias from initial posture accompanying sway. For rapid arm movements, initial posture might influence PAs less but still account for some variability reported in the order of postural and task muscle onsets (e.g., Belenkii, Biophys., 12:135, 1967; Lee, W., Jnl Mot Behav 12:185, 1980).

This study examined interactive effects of initial posture and arm velocity on EMG and mechanical correlates of PAs associated with voluntary arm flexions. Nine adult male subjects performed 120 bilateral arm flexions to 90 degree while standing. Twenty movements were performed at two speeds (as fast as possible; 2 sec duration) from three initial postures (neutral, leaning slightly forward or backward). EMG activity (anterior deltoid: AD; hamstring: HM; medial gastrocnemius: GS) and body motion (ankle and arm angles, hip and trunk sagittal displacements) were measured. EMG signals were normalized to maximal isometric contractions. The sagittal projection of the center of mass (CM) was estimated computationally.

HM and GS onsets were significantly delayed (post-arm movement) for slow as compared with rapid movements, which is consistent with previous studies. For rapid flexions, HM and AD onset latencies did not differ significantly and were followed by GS. Initial posture significantly influenced HM and GS latencies only for slow, not rapid flexions: earlier onsets occured with forward-leaning postures, as predicted by a peripheral bias model. Initial posture influenced EMG amplitudes for both slow and rapid arm movements (higher amplitudes for more forward postures). Results on mechanical correlates of PAs during rapid flexions showed that: (1) changes in ankle, hip and trunk typically lagged initiation of arm movement; (2) CM moved forward during arm flexion and was not fully compensated for by posterior trunk and hip movements; and (3) hip and trunk but not CM displacements were constant over the range of postures studied. These data imply that PAs were not modified to minimize changes in the location of the CM during arm movement.

24.7 TRANSITION FROM 'SLOW' TO 'FAST' MOVEMENTS: SUPERPOSITION OF MOTOR PROGRAMS. J.D. Cooke and S.H. Brown (SPON: J.D. Brown). Dept. of Physiology, Univ. of Western Ontario, London, Canada.

The division of movements into two functional types, fast and slow, has often been made on the basis of their patterns of muscle activation. Fast movements are initiated by a phasic burst of activity in the agonist muscle which may be followed by a burst in the antagonist and a second agonist burst. This is the triphasic pattern which is seen in movements of a wide range of speed and amplitude. In contrast, slow movements are made without phasic muscle activation, the tonic EMG levels in one or both muscles being changed. Tonic EMG levels in the opposing muscle groups have also been shown to determine the end-position of movement. The present study addressed the transition from slow to fast movements. The specific question asked was whether, as movement speed is increased, the phasic EMG activity characteristic of fast movements is simply superimposed on the tonic EMG activity characteristic of slow movements.

Experiments were performed on normal human subjects making flexion-extension movements about the elbow. The subjects moved a manipulandum arm which was pivoted at one end and which could be rotated horizontally about the pivot. Subjects tracked a target which moved at constant velocity between two positions. The target was presented as an audio signal whose pitch varied with the target position. Handle position (taken from a precision potentiometer) was also used to modulate the target tone. Thus the subject was to reproduce the target tone by moving the manipulandum. Ramp movements of different durations and amplitudes were studied. Handle position and velocity were recorded as were surface EMGs from the biceps muscle and the lateral head of the triceps.

The slower movements studied were produced without phasic EMG activity. Tonic agonist activity gradually increased throughout the movement to its final value when the movement end position was attained. As movement speed increased, the final tonic EMG level was reached progressively earlier in the movement. At the limit of these 'slow' movements, the tonic EMG changed in a step-like manner. With further increases in movement speed phasic EMG activity appeared. Movements were initiated with a brief phasic burst which was superimposed on the step-like tonic activity. The initial phasic burst increased in amplitude with further speed increases, remaining superimposed on the tonic activity.

The data indicate that the transition from 'slow' to 'fast' movements is accomplished by the superposition of phasic muscle activation on the tonic activation and that movements of different speeds form part of a continuum.

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24.8 SIMULATED BALLISTIC MOVEMENTS. M.M. Wierzbicka*, A.W. Wiegner, B.T. Shahani. Clinical Neurophysiology Lab., Mass. General Hospital, Boston, MA 02114.

Fast goal-directed voluntary movements are known to be associated with three distinct bursts of EMG activity in antagonistic muscles. The role of each burst (AG1, ANT, AG2) in controlling limb motion is not fully understood. Overall limb response is a complex function of the entire sequence of bursts recorded during experimental trials. In voluntary movements it is impossible to control bursts individually to determine the contribution of each burst to the overall motion.

The objective of the present study was to use simulation techniques to investigate relationships between activation patterns of antagonistic muscles acting at elbow and kinematic responses of the forearm. Two methods have been used. First, a computer-simulated mathematical model of antagonistic muscles was developed to calculate movement trajectories (position, velocity) for a given sequence of input torques. Secondly, in an experimental approach, agonist and antagonist muscles of normal subjects were stimulated with surface electrodes at their motor points and resulting trajectories were recorded. Both methods were shown to reproduce very closely the results of voluntary movement experiments in man.

A parametric study was performed for different amplitudes, durations, and relative timings of the input signals. Our results show that distance of fast movements is primarily controlled by the agonist muscle while time to reach peak displacement is a function of antagonist muscle activity. To move to a target in a minimum time, decelerative torque has to be of the same magnitude or larger than accelerative torque. Minimum movement time is independent of the distance traveled but depends on the duration of the torque pulses. The role of the antagonist in controlling the motion is not only to decelerate the arm, as has been suggested previously, but also to minimize time of the movement. Imbalance of accelerative and decelerative forces (including passive restoring forces), which tends to move the arm away from the target position, is corrected instantaneously by the third burst.

It can be concluded that the nervous system has a certain flexibility in choosing patterns of muscle activation to produce fast movements of similar distance, velocity and accuracy. The present techniques, in which any of the signals in the triphasic pattern can be independently changed, offer a convenient way to study the control of limb motion.

23.9 MUSCLE RESPONSES TO STOPPING LIMB MOVEMENTS OF RHESUS MONKEYS
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Deviations from an intended movement trajectory evoke changes in muscle activity that reflect an error between the actual and intended displacement. This error signal was studied by stopping wrist movements in three operantly conditioned monkeys. A visual tracking paradigm was used in which monkeys, with their hand secured to a handle, performed wrist flexion-extension movements between two hold zones of 1.5° separated by 15° of wrist arc. These two zones ("start" and "target") were shifted together over a 15° range so that constant amplitude movements were made in up to five different angular regions. During randomly chosen trials, movements were stopped for 200 ms by a servo-controlled torque motor. In one task (A), stops always occurred at the same angular position (the "stop position"). Thus, shifts of the start and target zones relative to this position altered the distance between the stop position and the target zone (the "remaining distance"). In a second task (B), the remaining distance was kept constant (at 12°) by shifting the stop position along with the start and target zones. Two effects of stop were examined: (1) the change in muscle (EMG) activity in comparison to unstopped movements and (2) a sustained build-up of torque applied by the monkey against the handle. These stop effects were analyzed in relation to the magnitude of the remaining distance (task A) and the stop position (task B).

Stops caused increases in agonist EMG activity at a latency of 15 ms to 45 ms. In task A, both the magnitude of the EMG stop response and the magnitude of the sustained torque build-up were proportional to the magnitude of the remaining distance. The EMG stop response increased by about 7%/deg (in comparison to the maximum response) as the remaining distance increased from 1.5° to 13.5°. The torque build-up increased by approximately 0.05 Nm/deg. At the maximum remaining distance tested (13.5°), the magnitude of the EMG stop response was similar to the magnitude of the pre-movement burst of EMG activity. In task B, the magnitude of the EMG stop response increased by approximately 3%/deg as the stop position was shifted towards joint angles at which the muscle was shorter. In contrast, the sustained torque build-up was independent of the stop position.

These results show that the magnitude of the agonist EMG response and associated build-up of torque during the stop of an active limb movement depend critically on the distance between where the stop occurred and the intended terminal position. This suggests that the torque during such an interruption is regulated via a mechanism that adjusts the EMG response according to the muscle length at the time of stop so that the torque build-up depends primarily on the angular distance remaining to be moved.

23.10 KINEMATICS OF CONSTRAINED MOVEMENTS.
T.E. Milner* and M.M. Ijaz*. (SPON: W.A. Richards). Dept. of Psychol. and Dept. of Mech. Eng., Massachusetts Institute of Technology, Cambridge, MA 02139.

Recently, Flash and Hogan (J. Neurosci., in press) have shown that the kinematics of horizontal point-to-point arm movements are very similar to those obtained by minimizing the third derivative of motion (jerk). We have examined the ability of jerk-minimization to predict hand trajectories in two tasks with kinematic constraints. Motion of the hand, forearm and upper arm were studied using a Selspot-based tracking system (TRACK: MIT Biomech. Lab.).

In the first task, subjects were required to toss a tennis ball (using an underhand motion) to targets placed on the floor. The hand followed an arc restricted primarily to the saggital plane. All subjects began the swing from a point behind them and released the ball as the hand was moving upward in front of them. The height at which the ball was released appeared to be independent of target distance. As target distance increased, the swing became longer and higher peak tangential velocities were achieved. The ball was always released near the point of peak tangential velocity. The release angle corresponded closely to the angle that minimized the kinetic energy of the ball needed to reach the target. The trajectory obtained by minimizing jerk and applying endpoint and interior point constraints fit the observed hand paths well.

The second task involved horizontal positioning movements of 20 cm in which a 3/8" peg was placed in target holes 7/16, 11/16, 1 or 2 inches in diameter. Subjects were instructed to move as quickly as possible. As the accuracy requirement became more stringent, peak velocity along the principal movement direction (X) decreased and movements became increasingly asymmetric (deceleration longer than acceleration). Movements made to smaller holes appeared to consist of several segments, as judged from inflections in the X-velocity profiles. Sometimes these inflections were correlated with abrupt changes of the hand path in the frontal plane, although deviations from a straight-line path to the hole were generally less than 1 cm. The wrist was relatively rigid in the saggital plane, but often rotated about its vertical axis. Peak velocity was reduced when the amplitude of movements to the smallest target hole were halved. However, the basic asymmetry of the movements was preserved. In particular, the ratio of peak velocity to average velocity did not change. Furthermore, the characteristic asymmetry of these movements was not replicated when subjects were asked to voluntarily slow the movements to the largest hole. We conclude that a constraint on the endpoint accuracy of a movement is dealt with by adopting a control strategy that generates a characteristic movement asymmetry, critically dependent on the severity of the constraint. A model that incorporates a penalty function related to positional error has been proposed to explain this asymmetry. (Supported by the AHFMR).

23.11 THE REGULATION OF MULTI-JOINT ARM POSTURE.
F.A. Mussa-Ivaldi*, N. Hogan* and E. Bizzi. Whitaker College, MIT, Cambridge, MA 02139.

The purpose of these experiments was to investigate the way in which the CNS regulates the postural response of the hand to external perturbations by tuning the elastic properties of arm muscles.

Recent investigations of multi-joint arm posture have revealed that hand posture is completely described by an ellipse which expresses the directional properties of hand stiffness (Mussa-Ivaldi et al., 1984). This stiffness ellipse is characterized by three parameters: size (area), orientation (direction of maximum stiffness) and shape (ratio of maximum and minimum axes).

In the present study we applied perturbations to the hand aimed at changing parameters of the ellipses. To this end, we asked our subjects to generate a voluntary imbalance in muscle activity or to adapt to external perturbations with simple and constant directional properties. Subjects were asked to maintain hand posture in the horizontal plane while holding the handle of a two-joint manipulandum. The stiffness was measured by applying small servo-controlled displacements to the hand along several directions. Three different tasks were considered at each work-space location: (1) Voluntary Co-contraction. While maintaining hand posture (the hand under a visual target), the subject was asked to co-contract selectively the muscles acting about one joint (either the shoulder or the elbow). To facilitate this task, EMG recorded by surface electrodes from the biceps and the pectoralis were simultaneously presented to the subjects. (2) Constant Bias Force. The subject was asked to maintain steady posture while a constant load (6-8 N) was applied to the hand. (3) Oscillating Bias. The subject was asked to maintain posture against a load characterized by constant direction and by a sinusoidally varying amplitude (0.5 Hz and 3 Hz).

Our results indicated that a significant (> 100%) increase of stiffness size occurs during co-contraction and constant and oscillating forces when compared with normal posture. In contrast, only minor changes in shape and orientation were detected. However, the changes in shape and orientation resulted in a predominant increase of the stiffness along the direction of the disturbing force, a trend that was more evident with the oscillatory bias. The increase in stiffness size suggests that the CNS has only a limited control over the other stiffness parameters and that the preferred strategy in face of external perturbation is a synergistic activation of arm muscles which preserves the pattern of shape and the orientation of the postural fields in the work space. This pattern, which consists in the polar orientation of the stiffness ellipses with respect to the shoulder (Flash and Mussa-Ivaldi, 1984) may play an important role in simplifying the control of posture and movement when the tasks are specified in terms of hand-related variables.

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23.12 MISREACHING FOLLOWING DAMAGE TO POSTERIOR PARIETAL CORTEX.
Deborah Claman and Thomas Zeffiro.Neurology Service, Massachusetts General Hospital, Boston MA 02114 and Dept. Psychology, M.I.T., Cambridge MA 02139.

Deficits in visually guided reaching have been described following damage to the posterior parietal cortex. The specific nature of the misreaching errors, including their spatial distribution has not been well described in the human literature. We have investigated the changes in reaching accuracy by measuring constant and variable error in control subjects and in patients with focal cortical damage under varying conditions of visual guidance.

Fifteen patients with unilateral damage to the posterior parietal cortex and eighteen control subjects performed a step tracking task on a digitizing tablet. In a condition without visual feedback, subjects sat in the dark and moved a stylus to targets that were only illuminated until the subject commenced movement. In a condition that permitted visual feedback, the targets remained illuminated throughout the entire trial. Each subject performed a total of 576 movements in each condition, alternating hands every 48 trials.

The magnitude and direction of the reaching errors were determined for each hand and each spatial hemifield separately. We define the constant error as a vector originating at each target position and terminating at the final hand position and the variable error as the standard deviation of these vectors. Since error direction is heavily dependent on movement direction, the error vectors were rotated by the direction of movement and thereby transferred into a reference frame where errors are represented normal and perpendicular to the direction of movement.

Patients with unilateral posterior parietal lobe lesions show two significant differences from the controls in the condition without visual feedback: 1) An increase in variable error, most pronounced in the hand contralateral to the lesion. 2) An exaggeration of the normal directional bias for reaching to the right of target locations with the left hand and to the left of target locations with the right hand. There was no effect on constant or variable error for either controls or patients when the movements were analyzed by spatial hemifield. With visual feedback, the errors were greatly reduced for both populations.

The finding that the misreaching deficit is most severe with the contralateral limb is consistent with results seen following similar lesions in subhuman primates. Damage to the posterior parietal cortex interferes with the execution of goal directed arm movements and results in final position errors.

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24.13 THE EFFECTS OF PRACTICE WITH INERTIAL LOADS UPON PREVIOUSLY-LEARNED BALLISTIC MOVEMENT PATTERNS. M.W. Cornwall* and G. Kamen (SPON:K.Klueber). Motor Control Laboratory, Indiana University, Bloomington, IN 47405.

The purpose of this study was to assess the effect of inertial loading on changes in EMG patterns following a previously-learned ballistic movement. Thirty-two subjects between the ages of 18 and 35 were randomly assigned to one of two practice conditions. Each subject performed a series of horizontal elbow flexion movements with the right arm over a 5 day period. The movement was a 100 degree arc and subjects were asked to move as rapidly as possible and stop within a 5 degree target. Subjects in the unloaded group (U) initially practiced the movement without additional weight on the limb. The loaded group (L) practiced with an inertial load of 4 times the moment of inertia of their forearm and hand. Following 4 days of practice (40 trials per day) in their respective conditions, subjects performed 40 trials on each of 2 days in the opposite condition. At the conclusion of this practice period, 40 trials were again performed under the original load condition. Surface EMG was recorded from the biceps and triceps brachii muscles using Ag-AgCl bipolar electrodes. The EMG signal was amplified and sampled at a frequency of 2 kHz. The kinematic, amplitude and frequency properties of the displacement and EMG signals were analyzed for any transfer of learning from one condition to the other. For subjects who initially practiced in condition U, the number of zero crossings (NZC) increased after practice with a load. Movement time showed a 13 msec improvement, but was not statistically significant. An increase in the NZC of the antagonist muscle as well as a delay in reaching its peak activity was also seen. Little or no change was noted in the kinematic variables of those subjects who first practiced with the load. A significant decrease in the number of turning points (NTP) of the agonist EMG was seen, however. It appears that practicing under different loads can alter the surface EMG characteristics of both agonists and antagonists with little or no change in movement time or other kinematic parameters.

	Unloaded		Loaded	
(* p (.02)	Practice Days	Test Day	Practice Days	Test Day
Movement Time (msec)	210.0	197.0	288.0	291.0
First Agonist Duration (% MVT)	92.9	87.9	67.9	68.7
Time to Peak Antagonist (% MVT)	55.5	62.1*	60.8	60.9
IEMG of Agonist (% MVC)	85.2	108.0	78.2	79.3
NTP of Agonist (No./sec)	256.6	265.9	280.3	268.2*
NZC of Agonist (No./sec)	123.1	130.8*	145.9	142.5
IEMG of Antagonist (% MVC)	49.0	56.7	51.9	56.5
NZC of Antagonist (NO./sec)	85.7	107.7*	113.3	125.3

24.14 INTER-LIMB RESPONSES TO MUSCLE-TENDON VIBRATION IN HUMANS. D.A. Al-Senawi and J.D. Cooke. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Mechanical vibration of muscle tendon affects the sense of position, probably through activation of spindle afferents. It has also been shown that tendon vibration during movement affects the end position reached; vibration of the lengthening muscle results in an undershoot of the movement. Previously, we have shown that normal humans tend to closely match movements performed by the two arms. In the present study we report disruption of this matching by unilateral vibration during performance of simultaneous, bilateral arm movements.

During experiments subjects held handles on the end of two manipulanda, which were pivoted on the other ends and could be rotated horizontally. Subjects performed alternate flexion/extension movements about the elbow with visual guidance. After a period of practice, the subjects were able to make symmetrical movements of matched amplitudes without visual guidance. Mechanical vibration (120 Hz) of the biceps or triceps tendon of one arm was applied during arm movement. In some experiments subjects held one arm stationary while moving the other and vibration was applied continuously (15 sec) to the stationary arm.

During simultaneous, bilateral arm movements, vibration of the tendon of the lengthening muscle of one arm produced an overshoot of the intended movement of the contralateral arm. This effect was seen whether the lengthening muscle vibrated was the biceps or the triceps. When the subject was asked to hold one arm stationary and make alternate flexion/extension movements with the other, vibration of the tendon of the stretched muscle on the stationary arm produced an overshoot of the movements of the contralateral arm. This overshoot occurred in flexions when the contralateral triceps was vibrated and in extensions when the contralateral biceps was vibrated.

The data show that vibration of one arm affects movements being made at the same time with the other arm. The changes in movements of the non-vibrated arm are opposite to those which occur in the vibrated arm. The data suggest a reciprocally organized linkage between the limbs which can be activated by proprioceptive inputs during movement.

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24.15 ROLE OF THE UPPER MOTOR NEURON IN INCREASING THE EFFECTS OF MUSCLE WEAKNESS ON POSTURAL CONTROL L.D. Lehmkuhl, B.L. Bowser,* M.R. Dimitrijevic, M. Gregoric* and I.S. Solis*. Dept. Clin. Neurophysiology, The Institute for Rehabilitation & Research and Dept. Rehabil., Baylor Coll. Med., Houston, TX 77030.

We hypothesize that deficiencies in postural control of lower extremity muscles in patients with muscular weaknesses due to progressive neuromuscular diseases such as Duchenne muscular dystrophy and spinal muscular atrophy are due not only to the biomechanical consequence of weak muscles but also to the neurophysiological consequence of the sensorimotor reaction to such weaknesses. We propose that the neurocontrol response to muscle weakness paradoxically interferes with the utilization of relatively strong muscle groups. In order to test this hypothesis, we studied volitional efforts to lean forward and backward in five neurologically healthy adult subjects. Position of the subject was indicated by the displacement of the center of body mass as recorded on a force platform, together with electromyographic recording of motor unit activity from quadriceps, hamstrings, tibial anterior and triceps surae muscles of both legs. As expected, leaning forward from a neutral standing position induced a characteristic activation of triceps surae, hamstrings and paraspinal muscles near the peak of forward excursion, and leaning backward activated the tibialis anterior, quadriceps and abdominal muscles.

The deep branch of the peroneal nerve or tibial nerve was injected with a 2% solution of Xylocaine to block conduction, thereby causing complete motor paralysis and loss of sensation. The deficits were temporary and were followed by slow recovery of motor and sensory functions. During this procedure we noticed that, regardless of the degree of motor deficit, the subject was "locked" into the neutral position while attempting to lean in a direction requiring use of the affected muscle. We interpreted this to mean that the central neurocontrol of the sequential activation of leg and trunk muscle groups was blocked due to a lack of information from muscle groups which are essential to the movement (ankle flexor or extensor). We shall compare the features of temporary weakness and altered sensory function induced by peripheral nerve block with the characteristic features of neurocontrol in patients with muscle weakness, and discuss our hypothesis in light of these findings.

24.16 NEURONAL ACTIVITY IN MONKEY POSTARCUATE PREMOTOR CORTEX BEFORE VISUALLY-CUED ARM MOVEMENTS. M. Godschalk[x] and R.N. Lemon[x]. Dept. of Anatomy, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands. (SPON: European Neuroscience Association).

Macaque postarcuate premotor area neurones receive information from cortical and subcortical areas and project somatotopically into the precentral motor cortex (Godschalk, M., Lemon, R.N., Kuypers, H.G.J.M., & Ronday, H.K., Exp. Brain Res. 56: 410-424, 1984). Some premotor neurones modulate their firing frequency in relation to motor tasks which require visual information. We previously reported the behaviour of premotor neurones during execution of a detour reaching task in which the movement phase was separated in time from the phase in which the monkey received a visual cue for the movement required to retrieve a food reward (Godschalk, M., Lemon, R.N., Nijs, H.G.T., & Kuypers, H.G.J.M., Exp. Brain Res. 44: 113-116, 1981). A large proportion of task-related neurones (75%) were modulated during this 'visual' phase, in which no task-related movements were made. This modulation was associated with changes in the position of the food reward, which served as the visual cue.

In the present experiments, carried out in monkeys prepared for chronic recording under barbiturate anaesthesia, several variations of the task were used in order to test whether the 'visual'-related neuronal modulation could be involved in preparation of the upcoming movement. This modulation is unlikely to be related to any eye or arm movements occurring during the visual phase or to environmental illumination. Neither can it be related to the presence of the visual cue in a particular part of the visual field, since the pattern of neuronal modulation was similar when a cue with a fixed position was used. This modulation was, however, contingent upon the occurrence of food retrieval during the subsequent 'movement phase', since it was abolished or diminished during presentation of a 'food-reward' which the monkey did not retrieve.

For several neurones, modulation pattern during the visual phase depended on whether the food reward was to be retrieved with a gross hand movement or with fractionated finger movements.

It is likely, therefore, that neurones in the postarcuate premotor cortex are involved in preparation of arm movements with the help of visual cues.

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24.17 MECHANISMS CONTRIBUTING TO ACCURACY IN AIMED FORCE IMPULSES: RISE TIME REGULATION AND CORRECTIVE ADJUSTMENTS. J. Gordon* & C. Ghez. Dept. Mov't. Sci. Teachers Coll. & Center for Neurobiology & Behavior, Columbia Univ., NYS Psych. Inst., New York, NY 10032.

When human subjects produce rapid isometric force impulses of different amplitudes, peak force is proportional to the peaks of the first and second time derivatives, while force rise time is largely independent of peak force. The trajectory must be substantially pre-programmed because early features of the trajectory are highly predictive of the final force achieved; rise time invariance allows for proportional control of response amplitude. We now examine: 1) whether the apparent regulation of force rise time promotes accuracy or merely reflects constraints related to short rise times; 2) whether the trajectory of these brief force impulses also reflects ongoing error correction. Six human subjects were trained to produce isometric elbow flexion pulses and to match their peak force to visual targets of random amplitudes. Subjects were to refrain from amending their responses once initiated; reaction time was left unconstrained. They were given two alternative instruction sets: 1) Fast, i.e. produce the briefest possible force rise time; 2) Accurate, i.e. be as accurate as possible, and select the optimal force rise time.

Accurate responses showed less variable error than fast responses; average absolute errors and overall scaling to different targets were similar in the two conditions. Rise time increased (mean was 80ms in fast, 100ms in accurate). In fast responses, 5 of 6 subjects showed a small but statistically significant dependence of peak force on force rise time. In accurate responses, this dependence disappeared. All responses were produced with sequential agonist and antagonist bursts. While the strong relation of agonist EMG to force was similar in both conditions, the antagonist burst was markedly reduced and occurred later in the accurate condition.

We used multiple regression analysis to determine whether the peak force and its rise time incorporated corrections for initial trajectory errors (i.e. errors in peak d^2F/dt^2). All subjects showed compensatory adjustments (e.g. initial positive errors in d^2F/dt^2 were compensated by shortening the rise time thus reducing peak force eventually achieved).Corrections were implemented by modulation of both the antagonist and the latter half of the agonist burst. The degree of compensation varied among subjects (15-76% of the peak force variance unexplained by the peak d^2F/dt^2); in some subjects accurate performance was achieved by improved scaling of initial trajectory parameters (i.e. improved programming), in others by enhanced error correction. These results indicate that 1) regulation of rise time is greatest when subjects seek to maximize accuracy; 2) error corrections, presumably utilizing internal feedback, can be implemented shortly after response initiation.

24.18 SENSE OF EFFORT IN DEAFFERENTED HUMANS. J. N. Sanes. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205.

The sense of muscular effort is thought to be mediated by internal recognition of central nervous system signals generating muscular activity. A potential implication of central effort sense is that maintenance of muscular steady states, requiring continuous or periodic evaluation, can be achieved simply by monitoring output from motor brain areas. Nevertheless patients with pathological loss of large-fiber sensory afferents have considerable difficulty in setting the muscular activity necessary to sustain constant postures or to perform accurate movements. These results suggest anew that proprioceptive afferent inputs are integral for effort sense.

Six patients with large-fiber sensory neuropathy were examined. These patients had no position or vibration sense or deep tendon jerks, and had severe impairment of cutaneous sense. Muscular strength was normal or near normal. Effort sense was evaluated with three methods all using the wrist joint. First patients were required to position the wrist at 0^o while torques were changed every 4-6 s to 1 of 6 pre-determined levels (0-0.8 Nm). In the second task patients were pushed against a stop by one of the torques (45^o extension)and were required to move to 0^o. For both tasks the patients had to compare torque on the current trial with torque on the previous trial. In the third task, a reference torque (0.16-0.8 Nm opposing flexion) was applied continuously to the left hand (reference) while a load applied to the right hand (matching) varied (0.08 Nm steps). A tracking procedure based on the Method of Limits was used in which patients noted the hand with the greater torque. Ascending and descending thresholds were obtained for 5-10 reversals of torque.

Deafferented patients were impaired in their ability to detect changes in torque in all tasks. For the unimanual tasks errors when torques actually changed ranged in the posture task from 13%-71% (normals-12%) and in the movement task from 25%-55% (normals-12%). When no torque change occurred the patients incorrectly responded that torque had changed more so than normals--posture task, 32%-70% (normals-13%); movement task 34%-73% (normals-33%). Both patients and normals made most of their errors when shifts in torque were smaller than 0.5 Nm, though patients made more of these errors. For the bimanual task, the ascending and descending thresholds of patients deviated from unity (i.e. perceived equality between matching and reference torques).

These results suggest that proprioceptive information is important in perception of muscular effort. It was noted, however, that the deafferented patients successfully recognized large changes in torque. It is concluded that static or active muscular sense relies extensively on proprioceptive afferent information, though for relatively high levels of torque shifts a central sense of effort, or corollary discharge, prevails.

24.19 CHARACTERISTICS OF MUSCLE ACTIVITY PATTERNS DURING CYCLING UNDER VARIOUS SPEED AND LOAD CONDITIONS. J.A. Johnson* and A.E. Patla (SPON: R. Beauchamp). Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

EMG signals were recorded from seven lower limb muscles during cycling under two conditions: 1 kp load at constant speeds of 30, 50, 70, 90 and 110 rpm (N = 5), and 70 rpm speed with constant loads of 1, 2.5 and 4 kp (N = 4). Sixteen seconds of processed EMG and pedal position signals for each trial were ensemble averaged.

Average EMG for each muscle was normalized to its average value during the 70 rpm, 1 kp trial. EMG gain increase with increased load was greatest for rectus femoris (RF), vastus lateralis (VL) and biceps femoris (BF), while the increase in tibialis anterior (TA), medial gastroc nemius (MG) and gluteus maximus (GM) were lowest in all cases. Increase for the soleus (SO) though lower than RF and BF, was more variable. There was more inter-subject variability with increased speed; the greatest increases were seen in GM for 3 subjects, MG and SO for one subject each. Gain increases at a power output of 110 W (110 rpm, 1 kp) were greater than increases at 175 W (70 rpm, 2.5 kp). This may reflect both greater accelerations and decelerations of the limbs during high speed cycling, and reduced need for muscles to stabilize joints when resistance is higher.

The phasic component of the EMG patterns were analysed using the Karhunen-Loeve expansion. This analysis gives common features among muscle patterns, while residual error (unaccounted variance) when a few features are selected to approximate the muscle pattern, gives some insights into how the patterns are modulated. Across condition analyses for each muscle revealed some common features; this suggests that EMG patterns for different conditions do not have to be generated independently. GM showed the highest residual error in both the speed and load condition. The GM pattern during a cycle changed from a series of poorly defined peaks at 30 rpm, 1 kp to distinct peaks at higher speeds and loads. The proximal muscles (RF,BF,VL) showed more complex changes (higher residual error) in phasic activity than distal muscles (MG,SO,TA) when the speed was increased. This greater modulation of the proximal muscle activity patterns across speeds is similar to the results obtained during walking at different speeds. These results are discussed in terms of possible control strategies adopted by the nervous system during cycling.

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24.20 MODULATION OF THE RESPONSE TO PERTURBATION DURING CYCLING. A.E. Patla and M. Belanger, Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

The response of the ipsilateral limb to perturbation (20 ms train, 10 - 1ms pulses, 4 x 5 threshold) applied to the foot at four phases during bicycling were analysed. Tibialis anterior (TA), soleus (SO), medial gastrocnemius (MG), vastus lateralis (VL), biceps femoris (BF), and rectus femoris (RF) were monitored. The subjects (N = 6) wore no toe clips. The difference in the area under the curve (calculated over 100ms starting at 20ms after stimulus) between a perturbed and a normal cycle gave the magnitude and the sign of the response. Subtraction of averaged (5 trials) EMG patterns of perturbed from the normal cycle provided the reflex response from which the latencies were determined.

When the stimulus was applied to the limb in the top pedal position, flexor response was observed in the ankle joint (TA-82ms) while an extensor response was seen at the knee (VL-128ms,RF-118ms) and hip (BF-112ms) joint. This enhanced extensor response resulted in a shorter cycle duration ($p<.06$). In the pedal position at 90 deg. going down, the stimulus elicited a flexor response in the ankle joint (TA-57ms), and enhanced BF(114ms) and RF(121ms) response. This could be interpreted as a flexor response at the knee and hip joint or as a stabilizing response at the two joints. No significant changes in the cycle duration were observed. Similar responses were observed in the pedal position at 90 deg. going up (TA-56ms,SO-111ms,RF-111ms,BF-78ms), the only difference being the facilitation of SO. When the stimulus was applied to the limb in the bottom pedal position, a flexor response was observed in the ankle joint (TA-59ms), while an extensor response was observed in the knee and the hip joint (VL-123ms,RF-117ms,BF-138ms). No significant changes in the cycle duration were seen.

Unlike responses to perturbation during walking, these responses show a relatively simpler organization. This could be due to the fact that there is no need to actively control postural stability during stationary cycling. One major difference was the uncoupling of the MG response from the SO.

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24.21 BEHAVIORAL CONTEXT ALTERS TRIGEMINAL SENSORIMOTOR FUNCTION. K.C. Berridge* and J.C. Fentress. Dept. Psychology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada.

Simple actions (e.g., rhythmic tongue protrusions, forelimb facial strokes, and forelimb flails) are emitted by rats both during taste-elicited ingestion/aversion and during postprandial grooming. This study combined peripheral trigeminal deafferentation with a detailed analysis of action form to examine the use of cutaneous reafference from the face in the production of these actions.

Male Sprague-Dawley rats were anesthetized and implanted with chronic oral cannulae, to allow the infusion of taste solutions (sucrose, HCL, or quinine). Responses to taste solutions were videotaped and subsequently scored in slow motion by an observer who keyed the class, amplitude, and laterality of each action into a computer. Spontaneously occurring grooming was similarly videotaped and scored. Rats were then subjected either to hemilateral (n=6) or bilateral (n=11) trigeminal deafferentation or to a control procedure (n=4). Deafferentation (peripheral transection of the lingual, inferior alveolar, and auriculotemporal nerves of the mandibular branch, and of the anterior superior alveolar and infraorbital nerves of the maxillary branch) eliminates pain and somatosensation from the face and mouth while sparing gustation and trigeminal motor function. After deafferentation, the ingestive/aversive and grooming actions of the rats were again videotaped and scored.

Changes in action form after deafferentation were found to be context dependent: deformations characterized rhythmic tongue protrusions when they occurred in ingestive but not in grooming contexts. The opposite was true for alterations in forelimb actions: the amplitude distribution of facial forelimb strokes was distorted during postprandial grooming but not during aversive face washing. Similarly, forelimb flails intruded and disrupted postprandial grooming but not aversive face washing. Further, postprandial grooming as a whole was found to comprise distinct sequentially-defined phases. Actions occurring in one highly stereotyped sequence phase were protected from deafferentation effects, although the same actions occurring outside of this phase were not. These results suggest that behavioral context (motivational state and sequence phase) can shift the integration of sensory guided and endogenous mechanisms that pattern simple actions.

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24.22 PROGRAMMING OF SERIAL MULTIARTICULATE MOVEMENTS: DATA FROM SPEECH MOVEMENT SEQUENCES. Vincent L. Gracco & James H. Abbs. Speech Motor Control Labs. Waisman Center, University of Wisconsin, Madison, WI 53705-2280.

Numerous investigations have focused on the sensorimotor control of single unidirectional movements. The control of sequential movements has not been addressed to the same degree, although serial movements characterize many purposive motor behaviors. It is not known, for example, whether the control parameters for an entire movement sequence are specified in advance, or whether each movement element is specified individually. In the present study we investigated this issue by examining the sensorimotor control of sequential multiarticulate speech movements.

Subjects produced the word 'sapapple' which requires two successive lip and jaw opening/closing movements separated by the vowel 'a'. Movements of the upper lip, lower lip and jaw were transduced and EMG was recorded from multiple lip muscles and one jaw closing muscle. Unanticipated perturbations (40 gms; 15 ms rise time) were applied to the lower lip prior to EMG onset associated with the first 'p' in 'sapapple', under two conditions. In the load-sustained condition, loads remained on for the duration of the movement sequence. In the load-terminated condition, the load was removed prior to the onset of EMG activity for the second 'p' movement. EMG magnitude, timing and movement changes were analyzed for the control and the two load conditions.

For the load-sustained condition, statistically significant EMG and movement increases were observed. For the load-terminated condition, EMG activity associated with the second lip closing was found to approximate the control (no load) condition. Close inspection of the EMG and kinematic adjustments revealed that the magnitude of the adjustments systematically varied with load-offset timing. For the load-off condition it was also observed that the EMG onset for the second 'p' (relative to the first EMG onset) was significantly delayed.

Overall, the results of the present study augment those previously reported for single multiarticulate speech movements (Gracco & Abbs, J. Neurophysiol., 1985). It appears that speech movement sequences may be composed of strings of single movements, each parameterized individually prior to movement execution. Such sensorimotor updating suggests a continuous sampling of the peripheral conditions and eliminates the need for separate representations of both simple and compound actions. (Supported by NIH; NS-20668, NS-13274, and HD-03352).

24.23 SENSORIMOTOR CONTRIBUTIONS TO ORAL-LARYNGEAL COORDINATION FOR SPEECH. Susan Shaiman*, James H. Abbs, & Vincent L. Gracco, (SPON: F. Graham). Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison WI 53705-2280

Previously we demonstrated that oral movement coordination for speech (Abbs & Gracco, J. Neurophys. 1984) depends in part upon nonautogenic sensorimotor actions (i.e., lower lip perturbations yield corrections in upper lip movements). Perhaps the most studied pattern of speech motor coordination is between oral and vocal fold movements. The timing between movements in these remote muscle groups is critical to generate linguistically significant acoustic distinctions between different consonants (e.g., "p" vs. "b", "k" vs. "g"). For example, when the lips close for a "p" (as in "apa"), the vocal folds are abducted actively and their vibration is terminated, giving the "p" its distinct voiceless character and contrasting it to the voiced "b". The tolerance for timing errors between oral and laryngeal movements for these consonants is very narrow, clearly not exceeding +/- 15 msec. Predictably, individuals with nervous system impairment (e.g., with lesions of premotor cortex and Broca's area, cerebellar patients) manifest problems in oral-laryngeal coordination for these consonant contrasts.

In this context, the purpose of the present study was to determine if ascending afferent signals from lip movements for these consonants might be utilized to control the critical timing of parallel vocal fold movements. On 13% of the trials, unanticipated lower lip perturbations were introduced prior to and during the lip closing for the first "p" in the word "sapapple", using a dc brushless torque motor. Five subjects, naive as to the experimental purposes and motor control research, were studied. As reported previously, these lip perturbations yield a finite increase in the duration of the compensatory lip closing movement, essentially delaying the time of lip closure for "p". As hypothesized, in response to the lip perturbations, the timing of vocal fold movements, transduced with an impedance technique, was adjusted correspondingly. That is, with perturbation-induced delays in lip closure, there were: (1) increased durations of vocal fold vibration for first "a" in "sapapple", i.e., delayed abduction, (2) reduced durations of the nonvibrating (abduction) interval associated with the "p" voiceless segment, and (3) reduced durations of the combined vibratory ("a") plus voiceless segment ("p") interval.

These data suggest that the critical timing between oral and vocal fold movements is not wholly pre-specified, but rather adjusted on-line, via nonautogenic sensorimotor processes, in accord with movement variations. Such sensorimotor linkages are significant particularly inasmuch as the lips and larynx are innervated by different cranial nerves and are quite independent mechanically. (Supported by NIH; NS-13274 & HD-03352).

24.24 TASK-DEPENDENT SENSORIMOTOR ACTIONS ARE INHERENT IN SPEECH MOTOR PROGRAMS. James H. Abbs, Susan Shaiman*, Vincent L. Gracco, & Kelly J. Cole. Speech Motor Control Labs. Waisman Center, University of Wisconsin, Madison WI 53706-2280

Recent studies indicate that certain sensorimotor actions are gated dynamically with variations in actual or perceived postural support, subject intentions, or task-inherent mechanical coupling. These data support the supposition that neural networks underlying a given class of voluntary motor acts are configured and reconfigured, flexibly, by subtle changes in intended goals (cf. Abbs et al., J. Motor Behav.,1985). Based on the need to perform strings of similiar, but subtly different motor acts such as are involved in grooming, food gathering, sign language, speech, tool making/using, etc.,such neural variations are of considerable interest. Most previous studies addressing this issue have, by necessity, involved variations in voluntary motor behaviors that were not inherent in the organism's repertoire.

The present experiments were aimed at exploring such task-dependent sensorimotor variations in the motor control of speech. The value of examining speech is that this motor behavior is learned naturally and of considerable ecological significance. The paradigm involved perturbations of the lower lip during different speech sounds that required subtle, but linguistically-significant contrasts in lower and upper lip movements. It thus was possible to manipulate the intended motor goals in a natural manner,i.e., reading of different syllables.

One speech contrast studied was the generation of two vowels ("ah and "oo") which vary in their lip positions for generation of appropriate acoustic patterns; i.e., for "ah", labial positioning is of minimal significance while for "oo" the lip positions are critical for its normal generation. When the lower lip was perturbed for these two vowels, distinct differences were seen in the sensorimotor compensations; Viz., minimal or no compensations were observed to perturbations introduced during "ah", while consistent responses were observed to loads introduced during "oo". In a second speech motor contrast, lower lip loads were applied during "p" which requires movements of both the upper and lower lips and during "f" which only requires movement of the lower lip. Predictably, while there were upper lip (and lower lip) responses to lower lip loads during "p", there were no discernible upper lip movement responses to loads introduced during "f".

These data suggest that one element of motor programs for voluntary acts is the prescription of those sensorimotor pathways to be enabled and those to be inactive, based upon subtle, task-dependent features. As such these and like data appear to render moot the classical debates regarding afferent-independent, motor "preprogramming". (Supported by NIH; NS-13274 and HD-03352).

25.1 PATHWAYS MEDIATING HORIZONTAL OPTOKINETIC NYSTAGMUS IN THE RAT: NUCLEUS OF THE OPTIC TRACT PROJECTIONS TO THE NUCLEUS RETICULARIS TEGMENTI PONTIS AND PRAEPOSITUS HYPOGLOSSI. B.G. Korp*, R.H.I. Blanks and Y. Torigoe* (SPON: S.E. Fraser) Depts. of Anatomy and Surgery, Coll. of Med., Univ. of Calif. Irvine, Irvine, CA 92717.

Electrolytic lesions of the n. reticularis tegmenti pontis (NRTP) in rat abolish the slow and fast components of horizontal optokinetic nystagmus. However, it is not certain whether it is the neurons of the NRTP and/or currently unknown fiber bundles which pass through the NRTP that mediate visual information from the n. of the optic tract (NOT) to the praepositus hypoglossi (ph) and vestibular nuclei. In order to examine the connections between the NOT, NRTP and ph, injections of tritiated leucine were placed into the NOT (4 rats) or NRTP (8 rats) and the tissue processed autoradiographically. The topography and projections of these nuclei were examined using the retrograde horseradish peroxidase (HRP) technique.

Autoradiographic injections into the NOT identify a strong projection to the medial parts of the rostral two-thirds of the NRTP and a smaller projection to the ph. Both are entirely ipsilateral. The fibers destined for the ph travel with the NOT-NRTP-bundle, pass through the NRTP and are distributed to the rostral one-half of the ph with the strongest labelling being in the supragenual part. Injections into the NRTP in the region of the NOT-NRTP terminal fields, demonstrate an ipsilateral bundle of axons which descend in a paramedian position to terminate similarly within the supragenual part of the praepositus nucleus. Retrograde studies in which HRP was injected into the ph or NRTP confirm these pathways and further show that the NOT projections to the NRTP and ph arise largely from the superficial portions of the NOT.

Both the direct NOT-ph and indirect NOT-NRTP-ph connections provide the anatomical basis for visual-vestibular interaction within the vestibular nuclei given the abundance of reciprocal connections between the ph and vestibular nuclei. However, the relative importance of the two pathways is currently unknown given that the earlier electrolytic lesion studies destroyed the neurons of the NRTP and the fibers en passage of the NOT-ph pathway. (Supported by grants EY03018 and EY000160 to RHIB. YT is a recipient of NASA Research Associate Award #MAGW-70.)

25.2 FURTHER EVIDENCE FOR OPTOKINETIC VELOCITY STORAGE IN DIRECT AND INDIRECT VISUOMOTOR PATHWAYS. S.Lafortune*, D.J.Ireland* and R.M.Jell. Depts. of Physiology and Otolaryngology, University of Manitoba, Winnipeg, Canada.

The dependence of human optokinetic afternystagmus (OKAN) velocity storage and optokinetic nystagmus (OKN) characteristics on optokinetic stimulus velocity and exposure time was investigated using the two component double exponential model for human OKAN decay: A exp(-Bt) + C exp(-Dt), where coefficients A and B define the fast component of OKAN decay and coefficients C and D define the slow component (Jell,R.M., Ireland,D.J., Proden,O., Soc Neurosci Abstr 9:866,1983). Optokinetic drum velocities were varied from 10 deg/sec to 70 deg/sec at a constant exposure time of 60 seconds. Optokinetic exposure times were varied from 5 seconds to 60 seconds at a constant drum velocity of 40 deg/sec, that which produces maximal OKAN as measured by cumulative eye displacement. Coefficients A and C of the OKAN decay were found to be velocity dependent and exhibited velocity saturation characteristics above 40 deg/sec. OKN and OKAN cumulative eye displacements demonstrated a velocity sensitivity such that two distinct levels of response were apparent: a low level response at drum velocities in the pursuit range (10,20,30 deg/sec) and a high level response in the non-pursuit range (40,60,70 deg/sec). These results provide further support for our postulate that the fast and slow components of OKAN represent direct (pursuit) and indirect (non-pursuit) visuomotor pathways respectively. Both the fast component time constant (1/B) and the slow component time constant (1/D) appeared to be independent of both velocity and exposure time, while coefficient C of the slow component of OKAN was clearly shown to be dependent upon stimulus exposure time. There was some indication of direction sensitivity in the latter response. Results are compatible with the concept of two velocity storage integrators involved in OKAN, which may be direction sensitive: one responsible for the short time constant decay (mediated by direct pathways) and the other for the long time constant decay (mediated by indirect pathways).

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25.3 THE EFFECTS OF STIMULUS DURATION ON OPTOKINETIC AFTERNYSTAGMUS AND SECONDARY OPTOKINETIC AFTERNYSTAGMUS IN RABBITS. B. J. Winterson. University of New England College of Osteopathic Medicine, Biddeford, ME 04005.

The rabbit, subjected to moderately high velocity (5°-30°/sec) continuous optokinetic stimulation, shows a slow rise in the velocity of the slow phase of optokinetic nystagmus (OKN) during stimulation. When the stimulation is removed, the nystagmus continues, showing a slow fall in the velocity of the slow phase of OKN until the nystagmus disappears. This is called optokinetic afternystagmus (OKAN). These phenomena suggest neural circuitry which functions as a leaky storage mechanism for slow phase eye velocity.

In order to further explore the properties of the rabbit's eye velocity storage mechanisms, Dutch belted rabbits were exposed to continuous optokinetic stimulation for various durations: 20 sec, 1, 3, 10, 30, and 90 min. The optokinetic drum was 29.5 cm in diameter, subtended 45° superior and inferior to the horizontal plane passing through the interocular axis. The drum was lined with a black and white checkerboard pattern whose elements subtended 1.4°. Drum velocity during stimulation was 26°/sec. Stimulation was monocular for the left eye and always in the nasal direction. Eye movements of the left eye were recorded with the search coil technique.

OKAN observed after 20 sec, 1 min, and 3 min stimulation was not markedly different than that observed in prior studies. However, after 10 min and 30 min stimulation there was an increase in the duration of optokinetic afternystagmus and the appearance of secondary OKAN in which the slow phase was in the opposite direction. Once secondary OKAN appeared during an experimental session, which were typically about 4 hours long, it did not disappear from the responses of subsequent testing. This implies that the storage mechanism responsible for secondary OKAN is more sluggish than for OKAN.

After 90 min of stimulation, some animals showed a further increase in the duration of OKAN but still showed secondary afternystagmus. Other animals showed a substantial increase in OKAN duration (17 - 20 min) which was then followed by no secondary OKAN.

Continuous optokinetic stimulation is clearly artificial, but such continuous unidirectional visual input could occur if neuronal cell losses caused the vestibulo-ocular reflex (VOR) to become imbalanced. The changes observed in OKAN suggest that restoring the balance of the VOR with visual input can begin at one and one half hours in the rabbit.

25.4 EFFECTS OF SPATIAL FREQUENCY ON OPTOKINETIC NYSTAGMUS AND AFTERNYSTAGMUS IN THE CAT. W. B. Thoreson* and J. H. Anderson. (Spon: R. Purple) Depts. of Otolaryngol. and Physiol., Univ. of Minn., Minneapolis, MN 55455.

Changing the pattern of an optokinetic stimulus will influence the response of the neural elements which generate optokinetic nystagmus (OKN) and the optokinetic after-nystagmus (OKAN). To examine this quantitatively we measured the OKN and the OKAN due to various visual patterns rotated horizontally around restrained, alert cats. The stimulus patterns used were black and white vertical bars with spatial frequencies of 0.07, 0.18, and 0.64 c/deg; a two-dimensionally varying pattern of irregular black blots; and a number of "offset" patterns created by shifting the bars of the 0.18 c/deg vertical bar pattern one-half cycle in parts of the visual field. The stimulus velocity ranged from 10 to 80 deg/sec. Both monocular and binocular stimulation were used. The results show that the gain of the slow phase eye velocity (SPV) during OKN is greater for a vertical bar pattern with a spatial frequency of 0.18 c/deg than when the spatial frequency is higher or lower. Also, two-dimensionally varying patterns produce a greater gain than one-dimensional patterns. The SPV during the OKAN was fitted by the function $A(1-e^{-t/\tau}\sin(\omega t+\alpha)/\sin\alpha)+B$, describing a bounded, underdamped, second-order system, where B is the eye velocity at time zero, the onset of OKAN, and A+B is the final velocity. The variability of the time course of the OKAN seems to be strongly correlated with the amplitude of the OKAN (A) and not with the type of pattern; when A is greater than 17 to 20 deg/sec, the mean of tau is 10.3 (±4.0) sec; the mean of omega is 0.05 (±0.001) rad/sec; and the mean of alpha is -0.41 (±0.084) rad. The variability of the parameters during nasotemporal monocular stimulation is similar to that during binocular stimulation, when the gains are comparable. These and results from others suggest that visual cortical mechanism affect the dynamics of the OKAN only indirectly by influencing the gain during OKN.

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25.5 VERTICAL COMPONENT OF QUICK PHASES DURING OPTOKINETIC AND VESTIBULAR NYSTAGMUS. M. LeTaillanter* and J. H. Anderson. Depts. of Otolaryngol. and Physiol., Univ. of Minn., Mpls, MN 55455.

The asymmetry of the vertical component of slow phase eye movements during vestibular and optokinetic stimulation has been described. It has been shown that the upward slow phase attains higher velocities than downward slow phases during rotation in the dark and during optokinetic after-nystagmus (OKAN). It is also known that there is some coupling of slow phase eye movements to the generation of the quick phases (QP). The present experiments were aimed at quantitating the parameters of the vertical component of QP and to correlate the QP profile with that of burst neurons recorded in the midbrain.

The magnetic search coil was used to record the vertical and horizontal components of eye movements in 8 cats. They were positioned on their sides and either rotated in darkness or viewed the inside of a drum which rotated around them. After 45 to 60 seconds of optokinetic stimulation, the light was turned off and the after-nystagmus recorded. 7,000 quick phases were identified and the amplitude vs. duration (A-D) and maximum eye velocity vs. amplitude (E-A) were plotted. 3,800 quick phases were averaged over one-half degree increments in amplitude, giving an average profile for the QP of different amplitudes and under different stimulus conditions. During optokinetic nystagmus (OKN), the slope of the regression line for A-D shows considerable variability. The slopes for each cat for QP up ranged from 14 to 70; for QP down, the slopes ranged from 14 to 171. For down QP, the mean of the slopes for A-D was 67 deg/sec and was significantly higher than the mean of the slopes during OKAN, which was 32 deg/sec. The linear regression of E-A is not different for OKN compared to OKAN. These results indicate that the profile and time course of the down QP during OKN is different than that during OKAN. During vestibular stimulation in the dark, the down QP had a significantly steeper A-D curve than the up QP.

The differences in the vertical component's A-D during OKAN in the dark may be due to oblique eye movements with a large horizontal component. If this were also the case for vestibular nystagmus in the dark, then there would be a preferred direction for the oblique movements in order to account for the up-down asymmetry.

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25.6 **Visual and Oculomotor Response Properties of Single Units in the Pretectum of the Behaving Rhesus Macaque.**

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The pretectum of lower mammals has been shown to play a role in visually elicited eye movements. In order to assess whether the primate pretectum also plays a role in eye movement generation we recorded the activity of one-hundred units histologically verified to be within the borders of the pretectum in four rhesus monkeys. The monkeys were trained to make eye movements in response to a target moving either against a dark or a textured visual background. While the monkeys fixated a stationary target spot, the visual sensitivity of units was tested with both flashing and moving full field or small stimuli.

Based on their discharge during smooth pursuit and/or their response to visual stimulation, the units were divided into three main types. 1) VISUAL-ONLY units (21 %) responded to either flashed full field background stimulation and/or to moving visual stimuli during fixation. 2) EYE-MOVEMENT-ONLY units (51%) discharged during smooth pursuit tracking but not during saccades. These units showed no evidence for any visual sensitivity when tested with either flashed or moving stimuli. In a few eye movement units (N=8) we tested the effect of extinguishing the target briefly (400 msec) during smooth pursuit (during which time the monkey continued tracking) and found that the unit response continued throughout the period when the target was off. 3) EYE-MOVEMENT-AND-VISUAL units (28%) showed both a visual response and an eye movement related discharge. Many of these units demonstrated a response to smooth pursuit of a target as well as a response to full field background movement during fixation. The eye movement and visual direction selectivities of these units could be either the same or opposite. The majority (79%) of pretectal units encountered in our study had discharges that were modulated during smooth pursuit of a small target spot against a dark background over the full range of velocities tested (6.3-63 deg/sec).

Our results demonstrate that the primate pretectum has units that discharge in relation to smooth pursuit eye movements. Therefore, in addition to its possible role in vision and control of the pupillary light relex, at least parts of the primate pretectum appear to be involved in eye movements.

25.7 RASHBASS REVISITED: ACTIVE AND PASSIVE RESPONSES TO STEP-RAMP TARGET MOTION. H.J. Wyatt and J. Pola, Institute for Vision Research, SUNY State College of Optometry, NY, NY 10010.

In the classic Rashbass experiment (1961), subjects tracked a target that made a step in one direction followed by an opposite ramp. The initial response was often a smooth eye movement away from target position but in the direction of target velocity; the stimulus for such a response must presumably be target velocity.

Based on work with periodic open-loop stimuli, we suggested earlier that the component of smooth pursuit that is specifically a response to target velocity might sometimes be largely non-attentional, resembling passive optokinesis. If this also held for step-ramp closed-loop targets, it would imply that the initial response might similarly be largely non-attentional.

We have tested the roles of attention and velocity-sensitivity in pursuit by asking subjects to view step-ramp motion of small targets and extended targets (with vertical flanking stripes). Subjects remained passive or actively tracked the target. Step size was 2 deg; ramp velocity was 4 or 8 deg/sec.

We found that:

(1) A substantial passive response occurred; it had the same latency as the active response and, for some subjects, a comparable velocity. (This was especially true for the extended target and the lower stimulus velocity.) This argues for a significant role for a non-attentional velocity mechanism.

(2) The earliest response was often a small, brief, smooth eye movement in the step direction. This appears to arise from a movement-sensitive mechanism responding to the brief stimulus generated by the step.

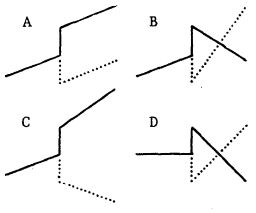
(3) Velocity overshoots (eye velocity > target velocity) were common in the active responses; these sometimes occurred while the eye was leading the target. This does not fit with the view that such overshoots are based entirely on target position error.

(4) Extensive oscillations (~3 Hz) often occurred during active pursuit of the 8 deg/sec extended target.

Findings (3) and (4) seem to depend on a high-gain velocity mechanism with a gain modulated by attention and augmented by the stripes. We have modeled our results with considerable success using a simple model of the pursuit system which has a short dead-time, and position- and velocity-sensitivity. The model also predicts values for pursuit of open-loop sinusoidal target motion that are in reasonable agreement with experiment.

25.8 HUMAN SMOOTH PURSUIT: THE RESPONSE TO CONFLICTING VELOCITY AND POSITION STIMULI J.R. Carl and R.S. Gellman, Laboratory of Sensorimotor Research, National Eye Inst., NIH, Bethesda, MD 20205

In a companion presentation we note that the response to a ramp differs from that to a ramp accompanied by an oppositely directed step, again raising the question of the role of position errors in smooth pursuit. We evaluated the pre-saccadic pursuit in response to tracked targets that stepped onward or backward and either continued at the same velocity (A) or changed velocity so that the retinal slip was towards the fovea (B). The search coil was used to record the responses of 7 subjects. They tracked a $1/10^{o}$ spot of light moving at either 5 or $10^{o}/s$ before one of 12 or more stimulus conditions was presented in pseudo-random order.



For steps up to 4^{o}, with no change in velocity (A), all subjects responded with a latency of 100 ms and an acceleration in the direction of the step that lasted about 40 ms and then tapered off. There was a pronounced asymmetry with a greater acceleration to backward steps (dotted line in A) than to onward steps ($35^{o}/s^{2}$ vs $8^{o}/s^{2}$). This asymmetry occurred for horizontal and vertical stimuli, but not when the steps were combined with retinal slip away from the fovea (C).

When a step was combined with an oppositely directed velocity stimulus so that the retinal slip was towards the fovea (B), the initial 40 ms of response was identical to that for a step alone (A). The initial acceleration was always in the direction of the step and only after 60 ms did the acceleration reverse to the direction of the target velocity. The amplitude of the initial acceleration in the direction of the step was as asymmetric as when a step alone was used.

If the subjects were not tracking but viewed a fixation spot before a step-ramp (D), they responded with a much smaller initial acceleration, but still in the direction of the step, and with the same latency. Comparison of this response with an open-loop version of (D) showed at 200 ms an identical deceleration of the eye to match the original target velocity, suggesting that the system was responding to an internal representation of the target motion ("prediction" or "feed forward").

In summary, (i) the latency of the response to position errors is 100 ms, the same as to velocity errors, (ii) the acceleration to position errors is dependent on the direction of the step relative to the tracking, (iii) when position and velocity errors are in opposite directions, the response to position predominates for the first 60 ms, (iv) predictive mechanisms become apparent at about 200 ms.

25.9 HUMAN SMOOTH PURSUIT: EARLY RESPONSES TO SUDDEN CHANGES IN TARGET VELOCITY. R.S. Gellman and J.R. Carl, Laboratory of Sensorimotor Research, National Eye Inst., NIH, Bethesda, MD 20205.

Studies of the initiation of pursuit have generally used a target which steps away from the fixation point, then ramps back. This delays the pursuit response and eliminates early saccades, but raises a question: Is the response merely delayed pursuit or does it reflect a different mechanism? We have used ramps and evaluated only the pre-saccadic elements of the response. In particular, we have addressed three issues: (i) Are the dynamics of pursuit initiation determined by target velocity? (ii) What is the influence of retinal target position? and (iii) Is the response different when the subject is already tracking? The responses to steps alone and to retinal slip towards the fovea are examined in a companion presentation.

We used the search coil method to record the eye movements of 7 normal adults. The target, a $1/10^{o}$, bright spot, underwent velocity changes in one of three ways: (i) it moved away (ramp); (ii) it stepped to an eccentric retinal location (up to 20^{o}) and moved away at a constant velocity (step-ramp); (iii) it changed velocity while the subject was tracking it (ramp-ramp). The timing and the sequence of the different stimuli were varied pseudo-randomly to minimize anticipatory and predictive responses.

The objectively determined latency of the pursuit movement in the ramp condition varied little from the mean of 100 ms ±4 (SD) over the stimulus velocity range $10\text{-}40^{o}/s$, but was about 7 ms longer at $5^{o}/s$. In the step-ramp condition the latency increased slightly as the eccentricity increased. The latency in the ramp-ramp condition was indistinguishable from that in the ramp condition, regardless of the direction and magnitude of the final velocity, including the case where the final velocity was $0^{o}/s$.

The acceleration for the first 40 ms of the response, which was invariably pre-saccadic, was low, fairly constant, and weakly dependent on target velocity. Over an 8-fold change in target velocity ($5\text{-}40^{o}/s$) the acceleration varied only between $42^{o}/s^{2}$ and $53^{o}/s^{2}$ (±13). In the step-ramp experiments the acceleration declined rapidly for steps $>2^{o}$, plateauing at $14^{o}/s^{2}$ by 10^{o}. As with the latency, the accelerations in the ramp-ramp condition were the same as those in the ramp condition, regardless of the initial and final velocities of the target.

In summary, we note that: (i) The latency, 100 ms, is about 30 ms shorter than that commonly reported; (ii) The initial acceleration shows little sensitivity to the magnitude of the velocity, but is determined almost entirely by the direction of the stimulus. This contrasts with the strong dependence on velocity found in studies using a "Rashbass" step-ramp; (iii) The initial acceleration is strongly dependent on the eccentricity of the target.

25.10 A COMPUTER MODEL THAT PREDICTS MONKEY SMOOTH PURSUIT EYE MOVEMENTS ON A MILLISECOND TIMESCALE. E. J. Morris* and S. G. Lisberger (SPON: M. Merzenich). Dept. Physiol., Div. Neurobiol., UCSF, San Francisco, CA 94143.

The aim of our study was to predict the time course of monkey smooth pursuit (SP) eye movements in single behavioral trials. Our model was based on data showing that SP eye velocity is sustained in the absence of visual error signals and that three error signals are commands for eye acceleration: retinal position error (RPE = target position - eye position), retinal velocity error (RVE = target velocity - eye velocity), and retinal acceleration error (RAE = target acceleration - eye acceleration).

We determined the functions relating eye acceleration to each error signal in two ways: i) Presentation of "open-loop" retinal errors, where we prevented the monkey from correcting an error by driving target motion with a signal composed of the output from the eye position monitor plus the desired error. We measured average eye acceleration in response to imposed RPEs, RVEs, and RAEs of various amplitudes. ii) Cross-correlation of transient retinal errors with eye acceleration during normal "closed-loop" pursuit. The two methods gave very similar results. Acceleration increased approximately linearly as a function of RPE, RVE, or RAE amplitude and acceleration commands due to the three different error signals added approximately linearly with one another. The response latency to these visual inputs was 90 msec.

We then constructed a computer model for each monkey in which eye velocity at each msec was computed as eye velocity at the previous msec plus the integrated eye accelerations due to RPE, RVE, and RAE 90 msec earlier. The model accurately predicted each monkey's SP eye movements in single behavioral trials for at least 500 msec and often more than 1000 msec. But the relative sensitivity to RPE, RVE, or RAE varied greatly from monkey to monkey, so that the optimum acceleration functions for any one monkey predicted his own SP much better than they predicted SP of other monkeys.

All of our monkeys exhibited spontaneous oscillations of the eye about the target during SP of constant-velocity targets. The period of oscillations was fairly constant for each monkey but varied from 180 to 390 msec among monkeys. Both the presence of oscillations and the different periods for different monkeys are predicted by the feedback and time-delay properties of our model. If a monkey's SP system is insensitive to RPE and RAE, our model predicts that the period of oscillation must satisfy the equation: cos(2 x pi x latency/period) = 0, implying period = 360 msec. This agrees with our experimental finding that a monkey insensitive to RPE and RAE had SP oscillations with a period of 390 msec. Our model also predicts that sensitivity to RAE will decrease the period, and we found that the monkeys with faster oscillations did have greater sensitivity to RAE. (Supported by NIH Grant EY03878.)

25.11 MONKEY SMOOTH PURSUIT EYE MOVEMENTS TO SINE-WAVE AND SQUARE-WAVE TARGET MOTION UNDER OPEN-LOOP CONDITIONS. C. Neary*, J. Pola and H.J. Wyatt. (SPON: V. Sutija). Institute for Vision Research, SUNY College of Optometry, New York, N.Y. 10010

We have studied monkey smooth pursuit eye movements to sine-wave and square-wave target motion under open-loop conditions (target motion stabilized on retina). The purpose was (a) to determine the relationship between open-loop and closed-loop responses during steady state pursuit, and (b) to examine the role of target position (offset from the fovea) in the control of smooth pursuit eye movements. Monkeys were trained to look at and track a small target light (detection of a brief dimming of the light was rewarded). The monkey's task during all experimental trials was to track the horizontal motion of the target. Open-loop trials began with closed-loop target motion (target motion fixed in space); after a number of cycles, the target motion was stabilized at the monkey's fovea and tracking continued for 20-40 secs. Eye position was monitored by the search-coil technique.

Relationship between open- and closed-loop: Monkeys tracked sinusoidal target motion (3 deg peak-to-peak) in the open-loop condition (sine-wave motion stabilized at the fovea) over a range of frequencies (0.75-3.0 Hz). The same stimuli were also used in the closed-loop condition. Gain and phase lag of the smooth eye movement response were determined as a function of frequency for both the open- and closed-loop conditions. The open-loop sine-wave stimulus produced a vigorous pursuit response, i.e. a high open-loop gain. As frequency increased, gain decreased and phase lag increased. Using simple linear assumptions, the open-loop data were found to accurately predict the closed-loop data.

Sensitivity to target position: Monkeys tracked square-wave target motion in the open-loop condition (square-wave motion stabilized at the fovea). As target velocity on the retina is zero, except for the brief jump, this open-loop square-wave was considered to be a pure position stimulus. Over a range of frequencies (0.5-1.5 Hz) the square-wave stimuli produced slow sinusoid-like eye movements interspersed with saccades. Smooth response velocity increased with target offset from the fovea up to 2 deg; the response fell off for larger offsets. These results demonstrate that target position can be a stimulus for the monkey smooth pursuit system and that it is most potent for targets close to the fovea. Furthermore, the response magnitude appeared to be related to the monkey's level of attention, suggesting that the pursuit response is not purely stimulus-bound.

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25.12 CHANGES IN EYE VELOCITY DURING SMOOTH PURSUIT TRACKING INDUCED BY MICROSTIMULATION IN THE DORSOLATERAL PONTINE NUCLEUS OF THE MACAQUE. J.G. May, E.L. Keller and W.F. Crandall*. Smith-Kettlewell Inst. of Vis. Sci., San Francisco, CA 94115 and Dept. Elect. Eng., University of California, Berkeley, CA 94720.

The dorsolateral pontine nucleus (DLPN) has been implicated in the control of smooth pursuit eye movements by virtue of its anatomic connections, the electrophysiological response properties of DLPN units and by the effects of chemical lesions in this area on smooth pursuit tracking. In this study we report that microstimulation within the DLPN produces short-latency alterations in slow eye velocity, but only when the stimulus train is delivered during the initiation or steady state maintenance of smooth pursuit eye movement. While electrical stimulation is known to produce saccadic eye movements from a variety of different brain structures, there have been few reports of stimulation induced smooth eye movements.

Animals were trained to fixate and track a visual target which appeared briefly at primary position and then ramped away at a constant velocity. The DLPN was located by monitoring neural activity during pursuit tracking through 0.5-0.8 mohm microelectrodes. Electrical stimuli (pulse train width - 40-200 msec, 0.5 msec cathodal pulses, frequency -300 Hz) were delivered through the same recording electrode during 1) fixation, 2) the initial eye acceleration phase of pursuit, or 3) the following phase of steady-state pursuit tracking. Current levels were restricted to below 80 μ amps. Thresholds for smooth changes in eye velocity during pursuit ranged from 25-70 μ amps. Current levels above 70 μ amps usually elicited rapid saccadic type eye movements.

Eye velocity was augmented when the stimulation occurred during the initial phase of pursuit such that eye velocity often exceeded target velocity. This was followed in most cases by a smooth deceleration at the end of stimulation to a level either matching or somewhat below that of target velocity. Stimulation during the initial fixation period failed to elicit smooth eye movements. Latencies for these effects on eye velocity were as short as 18 msec. These stimulation effects were directional in that stimulation induced velocity perturbations were only seen during pursuit tracking in specific directions. We feel that current spread was reasonably small since stimulations on penetrations only 250 microns apart often elicited effects for different directions.

These results provide additional evidence that the DLPN forms part of a pathway involved in the control of smooth pursuit tracking in the macaque.

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25.13 EYE-POSITION NEURONS IN THE PARAMEDIAN PONTINE RETICULAR FORMATION (PPRF) OF ALERT CATS. R.S. Remmel and R.D. Skinner. Biomedical Engineering Dept., Boston Univ., Boston, MA 02215 and Anatomy Dept. Univ. of Ark. for Medical Sciences, Little Rock, Ark. 72205.

A central oculomotor problem is understanding the behavior of brainstem neurons in relation to those of motorneurons. We thus recorded from abducens motorneurons and from 25 PPRF eye-position neurons (located immediately rostral to the abducens nucleus and lateral to burst neurons).

Cats were prepared surgically through the implantation of a head mount and recording chamber. A 6th-nerve stimulating electrode was used to antidromically identify motorneurons. Eye movements were recorded with a scleral coil (Remmel, IEEE Trans. Biomed. Eng., BME-31 (1984) 388). A turntable produced vestibular stimulation and moving stripes produced optokinetic stimulation. Units were recorded extracellularly with micropipettes. Spike trains and eye and turntable positions were recorded on magnetic tape. A PDP11/23 computer performed pattern recognition on spikes (Remmel, EEG Clin. Neurophysiol. 56 (1983) 528).

For modeling we started with Robinson's model of the plant (J. Physiol. 174 (1964) 245), which includes 2 viscoelastic elements for the passive orbital tissues. We assumed that the force $f_0(t)$ of the contractile elements is proportional to the ensemble average of the motorneuronal firing rates. The model was solved numerically. For motorneurons it well predicts firing rates.

The prediction for PPRF eye-position neurons was never adequate. The model has therefore been extended as follows.

$$F_0(s) = [a + b/(1 + \tau s)] X(s). \qquad (1)$$

Here $F_0(s)$ is the Laplace transform of the contractile force $f_0(t)$ from Robinson's model; the plant dynamics are thus accounted for. $X(s)$ is the Laplace transform of the firing rate $x(t)$ of a PPRF eye-position neuron. The "a" is the proportional term, i.e., the neuron carries an eye-position signal. The b-term describes a leaky integrator with time constant τ. We calculated $x(t)$ from $f_0(t)$ numerically, with the parameters, a, b, τ and the threshold of firing varied for best fit. (Eq. 1 is not a transfer function.)

The fits to PPRF eye-position neurons were well described by eq. 1 with τ of the order of 0.5 sec. In general the "a" term was somewhat larger than the "b" term. Eq. 1 applied during saccadic, vestibulo-ocular and optokinetic movements. The $b/(1+\tau s)$ leaky-integrator term is suggestive that PPRF eye-position neurons may be involved in the oculomotor integrator.

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25.14 PATTERNS OF EYE AND HEAD MOVEMENTS PRODUCED BY MICROSTIMULATION OF THE MIDBRAIN RETICULAR FORMATION (MRF). M. Gerez and D. Sparks. University of Alabama at Birmingham, Birmingham, AL 35294

The purpose of the present series of experiments was to compare, in head-free and head-fixed conditions, eye and head movements produced by stimulation of the midbrain reticular formation (MRF).

Search coils attached to the eye and to the head were used to obtain measurements of gaze, eye and head position. With the head free, the eye coil provides a direct measure of gaze; records of eye position were obtained by subtracting head position from gaze position. The most commonly used stimulation parameters were: train duration - 200 msec; pulse duration - 0.2 msec; pulse frequency - 500 Hz; and current - 30 μA.

Different patterns of eye and head movements were obtained depending on the site and depth of stimulation. The contribution of head or eye movements to the total gaze shift varied from pure eye movements to predominantly head movements. Stimulation at some sites produced constant trajectory eye movements regardless of the initial eye position, in both, head-fixed and head-free conditions. In the head-free condition, the gaze shift was the same as the eye movement shift since no head movement occurred.

Stimulation at other sites produced "goal-directed" eye movements in the head-fixed condition. With the head free, stimulation at some of these sites produced changes in gaze that were clearly goal-directed. In this condition, the eye and head moved in the same direction until the goal region was obtained. Continued movement of the head was compensated for by the VOR. Stimulation at other sites produced large changes in gaze, and "goal-areas", if present, were difficult to detect. For these large gaze shifts, the head and eye moved in the same direction and the VOR was not active.

The MRF appears to be involved in the generation of both eye and head movements. Very little is known about the signals carried by neurons in this region. Is information about changes in gaze encoded spatially or by discharge frequencies proportional to gaze error? How are head and eye motor error signals extracted from signals of gaze motor error and proportionally channeled to the appropriate subsystem?

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25.15 INTERACTIONS BETWEEN VESTIBULAR NYSTAGMUS AND EYE MOVEMENTS EVOKED BY ELECTRICAL STIMULATION OF THE SUPERIOR COLLICULUS OF THE CAT. Robert M. Douglas, Aerospace Medical Research Unit, Dept. of Physiology, McGill University, Montreal, Quebec, Canada.

Stimulation of the caudal superior colliculus (SC) in cat evokes saccadic eye movements that take the eye to a constant orbital position (Guitton et al, Exp Brain Res 39:1980). Besides these "goal-directed" saccades, head movements are also evoked (Roucoux et al, Exp Brain Res 39: 1980), and thus it is of some interest to see how the evoked eye movements interact with the vestibular signals produced by head motion. In the present study this was done in a controlled manner by keeping the head fixed and passively moving the whole animal while occasionally stimulating the SC with 333 Hz trains lasting from 300 to 5000 ms. The interactions observed were very similar to those we have seen during voluntary eye-head movements (Guitton et al, J Neurophysiol 52: 1984).

Slow phases. When the head was stationary and the SC stimulated, the eyes did not hold a constant gaze direction after the evoked saccade. Rather they continued to move outwards with a velocity which gradually declined from about 20 deg/s. Both horizontal and vertical drifts were observed at different SC stimulation sites, with the direction being similar to that of the preceding saccade. When the animal was rotated while stimulating, the drift added to the slow phase velocity, either increasing or decreasing the net ocular gain. While the summation was almost linear for low head velocities, there was a suggestion of relatively more suppression with velocities above 40 deg/s. The feline SC thus may use pursuit eye movements as well as saccades and head movement to correct errors in gaze.

Quick Phases. Long stimulation trains did not block any ongoing vestibular nystagmus. However when the evoked saccade was in the same direction as the quick phases, the amplitude of the quick phases was reduced and the rate increased. When the evoked saccade was in the opposite direction to the quick phases, the subsequent slow phase took the eyes to a more eccentric position near the limits of the oculomotor range where the eyes then rapidly oscillated until the stimulation was turned off.

The direction of vestibular nystagmus had only a slight effect on the "goal" position of electrically-evoked saccades. Moreover both the collicular saccades and vestibular quick phases typically took the eyes to a position about 10-12 deg from center. They thus may share a common saturation element.

25.16 THE SYNAPTOLOGY OF LARGE EFFERENT NEURONS IN THE CAT SUPERIOR COLLICULUS. M. Behan, P.P. Appell*, M.J. Graper* and C.R. Norris*. School of Veterinary Medicine and Neurosciences Training Program, University of Wisconsin, Madison, Wis. 53706.

Multimodal cells have been identified in the intermediate and deep layers of the cat superior colliculus which respond to visual, auditory and somatosensory stimuli. These cells in turn, relay integrated multisensory information in descending efferent projections, to areas which control movements of the eyes, head and pinnae (Merideth & Stein, Sci., 227:657, '85). These multimodal cells are included in the population of large efferent neurons (>30 μm diameter) located beneath the stratum opticum. We have examined the distribution of large neurons in the superior colliculus, and can demonstrate consistent variations rostrocaudally, mediolaterally and dorsoventrally.

Many of these large neurons can be retrogradely labeled from either the crossed medial efferent (predorsal) bundle or the uncrossed lateral efferent bundle. We have examined at the light and electron microscopic level those neurons whose axons project in the predorsal bundle, by retrogradely labeling them with HRP. Eight cells from three animals were examined in detail. Their location, size, and dendritic arborization pattern was measured. In addition, we used the classification system of Norita (J.C.N., 190:29, '80) to identify synaptic terminals contacting cell bodies, proximal and distal dendrites, and the axon hillock region. The data are based on analysis of single sections and serial reconstruction of selected regions of these cells.

The cells are located for the most part in the stratum griseum intermediale, in the lateral half of the colliculus. Soma diameters range from 35 to 60 μm. The mean dendritic field diameter is 1.3 mm, and dendrites of some cells extend into the lower part of the stratum opticum. Cells are multipolar, with from 5-10 primary dendrites extending to at least eighth, and in some cases twelfth order dendrites. Over 80% of the surface of somata and dendrites are contacted by synaptic terminals, while the axon hillock region has fewer synaptic contacts. Very few spines are present on the somata and dendrites of these cells. Significant differences are apparent between the distribution of specific terminal types on different regions of the cells: for example, between the soma and axon hillock region, between proximal and distal dendrites, and between apical and basal dendrites.

It is hoped that such detailed study of the synaptology of these large multimodal efferent cells in the superior colliculus may yield information as to how they integrate convergent sensory information.

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25.17 EYE-POSITION-RELATED UNITS IN THE SUPERIOR COLLICULUS OF THE CAT. C. K. Peck, School of Optometry, University of Missouri-St. Louis, MO 63121

Many recent observations of oculomotor function appear to require a neural signal of the position of the eye in orbit. Current models of the saccadic oculomotor system assume that neural signals representing eye position are added to retinal error signals in order to produce an internal representation of target position in space. In cat, the visual responses of some neurons in the superior colliculus (SC) are contingent on direction of gaze, and saccadic eye movements produced by electrical stimulation in the same region are also dependent on the position of the eye in the orbit. Moreover, in monkey, the discharge patterns of both auditory and saccade-related SC neurons shift with changes in eye position, suggesting that SC is organized in motor ccordinates. An unresolved issue is whether sensory signals are translated into motor commands within the SC.

Single unit activity was recorded in the SC of two alert cats using standard recording procedures. The cats were trained on fixation and saccade tasks, eye position was monitored with the scleral search coil technique, and the head was fixed.

Of 67 units which were isolated from the intermediate-deep layers and studied extensively in the absence of visual input, 6 discharged regularly during periods of steady eye position, and their rate of discharge increased with eye position in one direction. Discharge rates were nearly identical in light and during fixation of visual targets. For most units, a linear regression line provided a roughly satisfactory fit to the rate-position plots. Units varied in preferred direction (contralateral, up, or down, direction being dependent on location within the collicular "map" of motor error), in slope (from about 2 spikes/sec./deg. to about 11 spikes/sec./deg.), and in the threshold for steady discharge (usually near primary position).

Another group of units showed tonic activation (N=14) or suppression (N=5) during fixation of small (1^0) spots of light but not during periods of steady gaze in the absence of visual stimuli.

These findings suggest: 1. that an internal representation of either eye position (which was monitored) or head position (which may be closely correlated with eye position) is available within the SC even in total darkness, and 2. that only a fraction of SC neurons which discharge tonically during fixation of a visual target are also active during steady gaze in the absence of visual stimuli. The eye (or head) position signal may arise from proprioception or from corollary discharge. Experiments are currently being conducted in cats free to move their heads in order to determine if this signal is better related to eye position or to head position.

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25.18 **SHORT TERM SACCADIC ADAPTATION DOES NOT AFFECT THE SACCADES EVOKED BY ELECTRICAL STIMULATION OF THE MONKEY SUPERIOR COLLICULUS** **Edmond J. FitzGibbon*, Michael E. Goldberg, and Mark A. Segraves Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, Maryland 20205.**

Man and monkey can adapt the gain of the saccadic system when extraocular muscle activity is inappropriate for moving the eye accurately to a visual target. This inaccurate muscle activity can be mimicked by requiring a subject to make a saccade to a target that moves consistently during the saccade. This target movement is inapparent to the subject. After the saccade it is clear that the eye undershot when the target moved in the same direction of the the saccade (lengthening paradigm) or overshot when the target moved back towards the original fixation point (shortening paradigm). To acquire the target the subject must make a saccade either greater or smaller than that dictated by the target's original position.

Two rhesus monkeys were trained to make saccades between two spots of light. The saccade target was moved consistently during the eye movement so that at the end of the saccade the monkey had over- or undershot the target by 25% to 50%. Within 400-800 trials the monkeys had learned to adjust the gain of their saccade so that they now made a longer or shorter saccade in response to the original target. When the target first began to move during the saccade, the monkey would make a saccade to the first target and then a corrective. In the lengthening paradigm the latency and amplitude of the corrective saccade progressively decreased over multiple trials until no corrective was necessary. The velocity profile of the now larger saccade revealed a biphasic waveform, suggesting the rapid initiation of two succcessive saccades. In the shortening paradigm, 50-200 trials were required to achieve adaptation and no such biphasic velocity waveform was apparent.

In two awake behaving monkeys prepared for chronic neurophysiological recording we electrically stimulated the superior colliculus to evoke saccades. The amplitude and direction of these saccades were consistent with neuronal activity recorded at the stimulation site. The monkeys' saccadic gain was then altered along the vector of the stimulated eye movement using either lengthening or shortening paradigms. The superior colliculus was again stimulated and we noted no change in the amplitude of the electrically evoked saccade after adaptation. Alternating electrical stimulation trials and visually guided trials resulted in adapted saccades to visual stimuli and unadapted saccades to electrical stimuli. These results imply either that the superior colliculus plays no role in the generation of adapted saccades or that the adaptation occurs before the superior colliculus is activated.

25.19 THE MORPHOLOGY AND CONNECTIONS OF PREDORSAL BUNDLE CELLS IN THE RAT'S SUPERIOR COLLICULUS. M. E. Bickford,* P. D. Levine,* C.-S. Lin, C. Owen,* and W. C. Hall, Dept. of Anatomy, Duke Univ., Durham, N.C. 27710

Determining the afferent connections of predorsal bundle cells is of importance for understanding the mechanisms of sensorimotor interactions in the superior colliculus. As a step toward determining the afferent connections of the rat's predorsal bundle cells, retrograde transport and intracellular injections of HRP were used to study their morphology and distribution. HRP was injected into the decussation of the predorsal bundle to retrogradely fill the cells. For the intracellular injections, stimulating electrodes were placed in the decussation and predorsal bundle cells were identified by their antidromic responses.

The majority of the predorsal bundle cell somas are located either within the deep part of or just beneath stratum griseum intermediale (SGI). They are most common in the lateral parts of SGI. Their dendrites branch radially in the intermediate grey layer and also often extend superficially into stratum opticum and ventrally into stratum profundum. In the lateral parts of the SGI, dendrites may extend to the pial surface, but in this area there is no stratum griseum superficiale (SGS) overlying the stratum opticum (SO).

In one set of experiments, the nigrotectal tract was anterogradely labelled with WGA - HRP to compare the distribution of its terminations with the location of the predorsal bundle cells. The nigrotectal terminals form a band within the intermediate and deep layers which corresponds closely to the distribution of the predorsal bundle cells. In particular, the band is widest and densest in the lateral portions of SGI, where predorsal bundle cells are most common.

In other experiments, following intraocular injections of HRP, the retinal terminals in the superior colliculus were anterogradely labeled. Retinal fibers terminate in the SGS and the upper SO. Overlap of these terminal distributions with the dendrites of predorsal bundle cells was found only at the lateral edge of the superior colliculus. This is the area in which no SGS overlies the SO. The zone of SGI that lies beneath this lateral edge receives heavy projections from both the substantia nigra and the trigeminal nucleus (Huerta et al., J. Comp. Neurol. '83).(SupportedbyNIH#EY04060, #EY05490 and NSF #BNS 8109794).

25.20 EFFECTS OF LESIONS OF THE SUPERIOR COLLICULUS, STRIATE CORTEX AND ANTEROMEDIAL CORTEX ON INSTRUMENTAL HEAD MOVEMENTS IN THE RAT. E. Garcia*, C. Charman*, J. Sotsky*, H.M. Sinnamon (Spon: D.B. Adams). Neuropsych. Laboratory, Wesleyan Univ., Middletown, CT 06457

To study the neural systems that control head position, we made brain lesions in water-deprived rats that were highly trained to make various head movements. The test apparatus presented a 3X3 array of holes each of which contained a water-baited dipper. A subject were required to approach the array, orient its head, sample each dipper once and withdraw to a waiting chamber so that the dippers could be baited again. The insertion of the snout in the hole was detected by infrared sensors and a computer recorded for each dipper the order in which it was sampled when baited (preference), the number of times it was not sampled (neglect), and the number of times it was sampled when dry (perseveration). After proficiency was achieved (approximately 20 sessions), radio-frequency lesions were made and following 2-3 days recovery, testing resummed for five sessions.

Unilateral lesions of the superior colliculus produced a severe neglect of dippers contralateral to the lesion and an increase in perseverative errors to the dippers ipsilateral to the lesion. In the most severe cases, the center dippers were also neglected. The neglect showed a pronounced recovery in subsequent sessions but contralateral dippers continued to show a decreased preference in the sampling sequence. Bilateral lesions of the superior colliculus produced a bilateral neglect that was less severe than would be expected from the addition of two unilateral neglects.

Unilateral lesions of the anteromedial cortex which damaged the medial precentral region, the anterior cinglulate and prelimbic cortex did not produce a neglect and only slight effects on the preference of sampling of the dippers. However, these lesions markedly increased the number of perseverative errors. Bilateral lesions produced more errors. The perseverative errors were not lateralized and showed significant recovery.

Unilateral or bilateral lesions of the striate cortex produced no apparent effects on performance.

25.21 ELECTROPHYSIOLOGY OF THE TECTOFACIAL PATHWAY. P.-P. Vidal*, P.J. May and R. Baker. Dept. Physiol. & Biophysics, New York Univ. Med. Ctr., New York, NY 10016.

In addition to a role in the control of eye and head movements, the superior colliculus also coordinates the activity of numerous facial muscles to orient the whiskers and pinna. In fact, the facial nucleus offers a unique site for studying the manner in which the spatially organized tectum plays on motoneurons to initiate orienting movements. Consequently, we studied the physiologic attributes of the tectofacial pathway in the acute cat by electrically stimulating the superior colliculus and recording intracellularly from facial motoneurons identified antidromically following stimulation of dissected facial nerve branches. In contrast to the situation in extraocular and neck motoneurons, small (<1mV), graded, monosynaptic EPSPs with latencies ranging from 0.7-1.1 msec were produced in nearly all facial motoneurons by supramaximal stimulation of the contralateral superior colliculus. This potential was followed by either a larger (<4mV) disynaptic EPSP with a latency of 1.2-1.8 msec or a (<7mV) disynaptic IPSP with a latency of 1.5-1.9 msec in motoneurons of the temporal, auriculoposterior and ventral divisions of the facial nucleus. Furthermore, tectal inputs were not limited to motoneurons involved in orienting the ears and whiskers, for those neurons supplying the platysma and orbicularis oculi muscles displayed tectally elicited monosynaptic potentials despite the fact they move the skin and eyelid. Thus all the facial muscles may be influenced directly by the tectum. Surprisingly, stimulation of the ipsilateral superior colliculus produced essentially the same spectrum of synaptic potentials and latencies in facial motoneurons. The monosynaptic EPSP elicited from the contralateral superior colliculus was eliminated by cutting the predorsal bundle at the level of the rostral pons, but the disynaptic potentials were not affected. In another series of experiments the effect of collicular topography on the synaptic potentials elicited in pinna motoneurons was tested by placing an array of 3-6 stimulating electrodes 2mm deep in the superior colliculus. Each motoneuron exhibited topographic differences in the presence and amplitude of the mono- and disynaptic EPSPs and disynaptic IPSPs elicited from points in either superior colliculus. As predicted from the spatial map in the tectum, rostral sites produced EPSPs in the temporozygomatic division while caudal ones produced EPSPs in the auriculoposterior division. Larger IPSPs were generally elicited by stimulation of the caudal tectum. We conclude, that these topographic differences provide a structural substrate for the spatiotemporal transformation by which the superior colliculus initiates and sustains orienting movements of the pinna and whiskers.

25.22 ELECTROPHYSIOLOGY OF THE PARALEMNISCAL-FACIAL PATHWAY. P.J. May, P.-P Vidal* and R. Baker. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

The previous abstract reported the presence of disynaptic inhibitory and excitatory potentials in facial motoneurons following electrical stimulation of the contralateral superior colliculus. Henkel and Edwards (1978) suggested that the paralemniscal nucleus in the midbrain provides the relay for a disynaptic tectofacial pathway controlling pinna movements. Therefore, we studied the electrophysiological attributes of the paralemniscal-facial projection in the acute cat by stimulating the contralateral paralemniscal nucleus with concentric bipolar electrodes and recording in antidromically identified facial motoneurons. Within the auriculoposterior and temporal divisions of the facial nucleus, which supply the pinna muscles, motoneurons displayed monosynaptic EPSPs (<3mV) with latencies ranging from 0.6-0.9 msec. Other motoneurons exhibited monosynaptic IPSPs (<3mV) with latencies of 0.8-1.2 msec or, more frequently, combinations of EPSP and IPSP. These monosynaptic potentials were often followed by longer latency synaptic potentials. In contrast, monosynaptic potentials were not seen in motoneurons supplying either the inferior buccal nerve or platysma muscle, although longer latency synaptic potentials (>1.2 msec) were sometimes present. While these results support the contention that the paralemniscal nucleus subserves pinna movements, further examination showed that those motoneurons in the zygomatic division that supply the whiskers also exhibit mono- and disynaptic potentials following stimulation of the paralemniscal nucleus. Sagittal cuts extending the length of the fourth ventricle floor did not alter the potentials evoked in facial motoneurons following either tectal or paralemniscal stimulation, demonstrating that the pathways from these structures cross in the midbrain. Indeed, following HRP injections in the facial nucleus, axons from retrogradely labelled cells in the contralateral paralemniscal nucleus, pass ventral to the brachium conjunctivum, cross the midline just posterior to the trochlear nucleus, and then turn to run caudally in the central tegmentum. When this pathway was cut in the pons, electrical stimulation of the contralateral superior colliculus only elicited monosynaptic EPSPs in facial motoneurons. We conclude that the paralemniscal nucleus is a critical part of the system by which the superior colliculus controls the orientation of both pinna and whiskers. Furthermore, we suggest that both the size and topographically determined differences in amplitude of the disynaptic tectofacial potentials mediated by the paralemniscal nucleus indicate its importance for altering motoneuron activity. Thus, the paralemniscal nucleus may be a critical site in which to investigate spatiotemporal transformation.

25.23 EYE FIXATION UNITS IN THE SUPPLEMENTARY EYE FIELD OF MONKEY. J. Schlag and M. Schlag-Rey. Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Single-unit recordings were made at the dorsomedial edge of the frontal lobe in 4 trained monkeys (Macaca nemestrina). Just rostral to the classical supplementary motor area and about 10 mm medial to the frontal eye field a small region has been previously described which contains neurons active with eye movements and yields contraversive saccades upon low-threshold (e.g. less than 20 μA) microstimulation.

The 2 most frequent firing patterns observed in this region are: 1) gradual and long (e.g. 400 msec) build-ups of activity preceding saccades, and 2) changes in discharge frequency accompanying fixation of a visual stimulus. We studied 85 neurons showing this fixation pattern in order to clarify its significance. Besides the usual mode of presenting stimuli by turning them on and leaving them to be observed for several sec (paradigm #1), we let them appear progressively from subthreshold to suprathreshold brightness until they were discovered by the animal (paradigm #2), and we also used 50 msec flashes (paradigm #3). In all cases including paradigm #3 reinforcement was made contingent on fixation of the target for 1-4 sec.

Two-thirds of the units were tonically activated during fixation, one-third was silenced. In either case, the latency of the fixation pattern in paradigm #1 and #3 was usually longer than the delay for targeting. The pattern persisted for the whole fixation time and beyond stimulus offset, but it was interrupted before and during eventual glances away. The frequency of firing changed slightly after small corrective saccades. The fixation pattern was not due: 1) to photic stimulation because it was present for several sec after flashes when fixation was maintained (paradigm #3), 2) to eye position because it never appeared at any position in absence of a target, or 3) to eye immobility because it was evoked as readily by slowly moving stimuli as by stationary ones. Three types of unit behavior were seen with periodically moving stimuli: 1) constant fixation pattern any time during pursuit in 80% of the cases, 2) gradual acceleration as stimulus-and-eye position became more eccentric in a preferred direction, and deceleration in the off-direction, 3) gradual acceleration in a preferred direction of movement and silence in the opposite direction. Most position-dependent or movement-dependent fixation units also had the property of discharging before saccades in the same preferred direction. Microstimulation at these sites evoked saccades in this direction. The results indicate that fixation units of the supplementary eye field must be involved in the maintenance or signalling of active fixation.

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25.24 PRESACCADIC UNITS IN THE SUPPLEMENTARY EYE FIELD OF MONKEY, HEAD FIXED AND FREE. M. Schlag-Rey and J. Schlag. Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Single units discharging before spontaneous saccades were recorded in the supplementary eye field (dorso-medial edge of the frontal cortex) of 4 monkeys (Macaca nemestrina) trained to acquire and fixate visual targets with head fixed or entirely free. Eye and head positions were recorded by search coils attached to one eye and to the head. All facial movements were continuously observed through an infra-red video-monitor.

1. In the head fixed condition, 61 units were found increasing their firing rate before spontaneous saccades in a preferred direction. To determine how presaccadic preludes were related to saccade parameters, quantitative analyses were made on units at which site microstimulation induced saccades with minimum currents (6μA-30μA, 10-40 pulses, 0.2ms duration, 250 Hz). None of these units appeared to encode saccade direction only, i.e. they discharged most vigorously when saccades in a preferred direction had a preferred amplitude. For the majority of units, presaccadic preludes also depended on initial or end position in orbit. For instance, among units prefering rightward vectors, some discharged more if the saccades terminated at eccentric right positions; others discharged more if the saccades originated at eccentric left positions. These observations applied to units having different amplitudes of preferred saccade vector (15-30°).
2. Experiments with head free (19 units) showed that most presaccadic activities were related to eye, possibly to gaze, but not to head movement because (a) all units active before saccades, with head fixed, also discharged before saccades in absence of voluntary head movement; (b) microstimulation with head free induced eye saccades only. At more caudal recording sites, simultaneous head and eye saccades were obtained with low thresholds (e.g. 12μA) but head movement was never induced alone.
3. Fast (e.g. 200°/s) and repeated 50-60° horizontal head movements within ± 30° from center were induced by requiring the monkey to get the reward (following a successfull visual fixation) at one of two spouts, 25° left and right. Gaze strategies varied depending on whether voluntary head movements were made to get the reward or to look at the target. Up to now, units studied during comparable head rotations were seen to fire with eye saccades or quick phases but not compensatory movements in their preferred direction; their discharges were also affected by vertical eye components in gaze shifts involving pure horizontal head rotation. These results suggest that presaccadic units in the supplementary eye field participate in the initiation of voluntary saccades.

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25.25 ELECTROMYOGRAPHIC ACTIVITY IN NECK MUSCLES DURING HEAD MOVEMENT IN THE ALERT, UNRESTRAINED CAT. F.J.R. Richmond, G.E. Loeb and D. Reesor*. Department of Physiology, Queen's University, Kingston, Canada K7L 3N6 and Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205

The cat neck contains more than twenty muscles that can participate in head movement, but the varying internal architectures and fiber-type compositions of these muscles suggest that they may play specialized roles. Theoretical models have been developed recently to predict the contributions of neck muscles to head position on the basis of their anatomically defined actions. However, missing from the experimental literature is a systematic description of neck muscle activity during normal head movements in the alert, freely moving cat. In the present work, chronic electromyographic (EMG) recordings were made simultaneously from up to 16 neck muscles or muscle compartments in each of three untrained cats. Cats were allowed to move their heads freely as they executed routine eating, grooming, orienting and locomotor behaviors. Head movements were videotaped from two directions. Changes in vertical head position were additionally monitored using a length transducer implanted between the skull and the axis. Patterns of EMG activity were analyzed both while the cat held its head stationary in different positions and as it moved the head in different directions and at different speeds.

In most stationary postures, moderate EMG activity was recorded in only a few muscles (eg., biventer cervicis, occipitoscapularis, semispinalis cervicis). Activity levels in these muscles were modulated in relation to head position, but were reduced to near zero levels in only a few, extreme postures. In contrast, other neck muscles (eg., splenius, complexus, clavotrapezius, obliquus capitis caudalis) showed little or no EMG activity when the head was held stationary in most positions. During active head movements, each neck muscle showed a characteristic pattern of EMG activity that was consistent with its anatomy and fast:slow fiber ratio. Again, most normal movements of the head were associated with moderate levels of EMG activity in only a few muscles. Vigorous EMG activity was observed in these same muscles during fast, forceful movements, such as head shakes. Patterns of recruitment in most muscles were influenced greatly by the initial posture of the head and neck and the specific intervertebral joints around which the movement appeared to be made. In particular, some vertical movements appeared to depend on the action not only of neck muscles acting on the head, but also of shoulder muscles inserting onto cervical and thoracic vertebrae. These observations emphasize the complex nature of movements and muscle behavior in a multiarticular system like the neck. Supported by the MRC of Canada.

25.26 Gaze shift related neck muscle activity in trained cats. A. Roucoux, M. Crommelinck*, M.F. Decostre*, J. Crémieux* and A. Al-Ansari*. Lab. of Neurophysiology, Univ. of Louvain, Fac. of Med., B-1200 Brussels, Belgium.

We have shown (Roucoux et al, 1983, Abstracts, Society for Neuroscience, Vol. 9, Part. 2, p. 865) that, in the head fixed alert cat, various neck muscles discharge in relation with eye position. Horizontal as well as vertical eye position influence each neck muscle in variable proportion. These patterns of activity roughly fit the discharge seen when the head actually moves. In order to quantify the activity of neck muscles in a visual orientation behavior, we trained two cats to fixate small spots of light presented at various positions in the visual field. This training procedure yielded a strong and constant arousal level of the animal. The integrated EMG activity was measured in six pairs of muscles in two conditions : head fixed and head free. Eye and head movements were recorded by the coil technique.

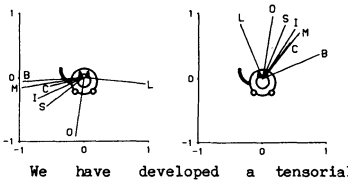
With head fixed, we found that the activity of each muscle is proportional to horizontal and vertical eye deviation. This confirms the previous findings. The discharge of the muscles shows no relation with eye velocity (no phasic bursts during the saccade), but only the eye position. In general, the discharge intensity is very often higher than with the head free to move). This high level of activity is attained after a rather long latency, variable from one muscle to the other. Biventer cervicis is mainly related to upward eye deviations and slightly to lateral deviations. Splenius is sensitive to downward eye movements, very slightly to upward extreme deviations but mostly to lateral eye movements. Longissimus is strongly related to downward and lateral eye deviations. Rectus capitis is related very strongly with upward eye positions and moderately with the horizontal component. Complexus is equally sensitive to upward and horizontal eye position. Finally the obliquus capitis cranialis is clearly related to downward deviations of the eye and very strongly to horizontal eye position. These relations have been obtained with a bipolar recording of a rather restricted volume of the muscle and do not represent the behavior of the whole muscle.

With head free, phasic discharges related to head displacements are predominant. These discharges reveal "pulling directions" that are in good accordance with patterns of activity observed with head fixed. In some muscles no tonic activity related to head position appears (for example the splenius of the longissimus). Other muscles, especially related to upward head movements like the biventer and rectus capitis show both phasic and tonic activity. This is probably related to their role in posture as well as in orientation of the head. "Phasic" muscles would be primarily involved in orientation and very little in posture.

25.27 RELATION BETWEEN PULLING DIRECTIONS OF NECK MUSCLES AND THEIR ACTIVATION BY THE VESTIBULOCOLLIC REFLEX: TESTS OF A TENSORIAL MODEL. B. Peterson, J. Baker, C. Wickland, A. Pellionisz, Northwestern Univ. Med. School, New York Univ. Med. School.

The vestibulocollic reflex (VCR) transforms a head rotation stimulus $\bar{S}$ into a pattern of neck muscle activity $\bar{N}$ that generates a head movement $\bar{H}$ directed opposite to $\bar{S}$. The sensory input responsible for generating $\bar{N}$ is activation of the 6 semicircular canals ($\bar{C}$). The sensorimotor transformation between $\bar{C}$ and $\bar{N}$ depends upon the geometry of the canals and muscles. To complement existing knowledge of the former, we measured stereotaxically the origins and insertions of 15 neck muscles in cats whose positions were adjusted so that a line from the center of the lambdoidal ridge to the T1 articular process was at a 73 deg angle with the stereotaxic horizontal plane. Pulling directions of 15 muscles/side were determined by calculating the cross products of vectors extending from the insertion towards the origin and towards the neck rotation point thus defining a 3D dimensional motor space.

When viewed as a 6-to-3D dimensional transformation, the VCR is overcomplete in that many $\bar{N}$ could generate a given $\bar{H}$. To determine the relation of $\bar{N}$ to $\bar{H}$, we measured electromyographic activity of 7 neck muscles during whole body rotations in many planes in 6 decerebrate cats. Muscle activation varied sinusoidally with the direction of rotation $\bar{S}$ and was maximal for rotation about a single best external space axis $\bar{V}xyz$. A muscle's $\bar{V}^i$ was consistent over time and across animals but typically did not align with the muscle's pulling direction (see figure). The question then is what criterion does the CNS use in selecting the $\bar{V}^i$s?



Frontal views of cat showing pulling vectors (left diagram) and activation vectors ($\bar{V}^i$s, right diagram) of 7 neck muscles. Note especially large misalignment of longus capitis (L) and rectus major (M) vectors.

We have developed a tensorial model of the VCR which incorporates 2 simple matrix transformations between $\bar{C}$ and $\bar{N}$ and minimizes the sum of squares of $\bar{N}$ for generating each $\bar{H}$. As described in an accompanying abstract, the model is based on the canal and neck geometry and generates predicted maximal activation axes $\bar{M}xyz$ for each muscle, which can be compared with the measured $\bar{V}^i$s. This comparison reveals an encouraging agreement at this stage of the project. Work is continuing to measure additional $\bar{V}^i$ for comparison with the 15 $\bar{M}^i$ and to refine the neck muscle geometry on which the model is based by recording muscle activity accompanying voluntary head movements in trained cats.
Supported by NIH grants EY04058, EY05289 and NS13742.

25.28 TENSOR MODELS OF PRIMARY SENSORIMOTOR SYSTEMS, SUCH AS THE VESTIBULO - COLLIC REFLEX (VCR), AND OF THE METAORGANIZATION OF HIERARCHICALLY CONNECTED NETWORKS. A. Pellionisz and B. Peterson, Dept. Physiol. & Biophys., NYU Med. Ctr., New York, NY 10016 and Dept. Physiol., Northwestern Univ. Med. School, Chicago IL 60611.

The accompanying abstract (Peterson et al. 1985) shows why and how a tensorial VCR model is built from experimental data and how it is experimentally tested. Here we open a broader perspective; ask how primary sensorimotor networks (eg. VCR) may emerge in the CNS and how their hierarchy may develop into connected networks.

The VCR is a closed-loop primary sensorimotor system since it both generates and measures the same physical invariant: Head movement is generated by a contravariant vector of neck muscle effectors and directly measured by a covariant vector of vestibular sensors. When both frames of reference are available in a quantitative manner (Blanks et al 1972, Baker et al 1985) general sensorimotor tensor models can interpret such transformations of one vectorial expression in one frame into a different version in another frame (Pellionisz 1984). The transfer, accomplished in the VCR by neuronal networks, can be expressed by a vestibular tensor and a neck motor tensor. First, the head-movement sensory reception vector is converted into a covariant motor intention vector by the contracted tensors of the contravariant vestibular metric and a sensorimotor operator that projects the sensory axes onto the motor axes. Second, motor intention is transformed into execution by a covariant-contravariant operation by the least squares formula of Moore-Penrose inverse of the motor metric.

Tensor theory of the Metaorganization of CNS networks, which implement functional geometrical counterparts of the physical geometry of a sensorimotor apparatus, offers an explanation of how such closed-loop networks as the VCR may emerge (Pellionisz & Llinas 1985). In particular, the Moore-Penrose generalized inverse may be constructed by the CNS from the eigenvectors and eigenvalues, found by reverberative oscillations of the motor action - sensory proprioception. In gaze control, however, there are other systems beyond the VCR; some are primary while others are not. Another primary system that may emerge by Metaorganization is the retino-ocular reflex (ROR), which generates an eye movement in the motor frame of extraocular muscles, and also measures it in coordinates, intrinsic to the retina. As a result of a separate Metaorganization of each primary system, both a fast but grossly over-complete and high-inertia VCR and a slow but pseudo-complete and low-inertia ROR may emerge. The VCR and ROR can then be linked by a further network to form the secondary (hierarchical) VOR, which is not a primary system (it is governed by the VCR and ROR), but it combines their advantages; it is a pseudo-complete and low-inertia, fast and precise gaze system.
Supported by NIH grants NS13742 and EY04058

26.1 TWO POPULATIONS OF ASTROCYTES IN THE GOLDFISH CENTRAL NERVOUS SYSTEM. R.L. Levine*, A. Tsang*, and C. Verdone-Smith* (SPON: E. Cooper). Dept. of Biology, McGill U., Québec, Canada H3A 1B1

We have been examining the glial cell population in the goldfish central nervous system (especially the visual system). Our evidence indicates that there are at least two forms of astrocytes in these animals, one in the optic nerve and the other in the rest of the brain.

In the optic nerve, EM reveals that typical astrocytes form sheaths around fascicles of optic axons. These cells are characterized by bundles of filaments in an electron-lucent cytoplasm which contains masses of glycogen. There is an astrocytic glia limitans at the periphery of the nerve and the astrocytes are held together everywhere by well developed desmosomes. In contrast, in the brain, all astrocytes appear to be radial glia, with cell bodies in the periventricular grey layers and expanded end feet, which form a glia limitans on the pial surface of the brain.

Immunocytochemical studies have further underscored the differences between optic nerve and brain astrocytes. We used commercially available anti-GFAP as primary antibody (Ab) and both fluorescent and HRP secondary markers to visualize its distribution. In our hands, anti-GFAP stains radial glia in the brain but stains optic nerve glia poorly, if at all. On the other hand, we have raised polyclonal Ab's to optic nerve antigens (48 Kd and 58 Kd) and find that while these stain a lattice-work in the optic nerve they do not stain cells in the brain. EM examination of immunochemically stained tissue confirms that anti-GFAP stains radial glia but we have been unable, at this point, to determine what cell types the anti-48 Kd and 58 Kd Ab's are staining. Finally, we have stained immunoblots of nerve and brain homogenates with anti-GFAP and we find that the two extracts show different staining patterns. In addition, anti-48 Kd and anti-58 Kd Ab's stain nerve blots very effectively but stain brain blots very weakly, if at all.

In summary, our data indicate that in the goldfish, optic nerve and brain astrocytes differ morphologically and biochemically.

26.2 DISTRIBUTION OF BIOGENIC AMINES IN THE FROG CNS: EFFECTS OF 5,6DHT. T. Ritchie, G. Karp*, and B. Haber. Mental Health-Mental Retardation Program, UCLA, Los Angeles, CA 90024 and Marine Biomedical Inst., Depts. of HBC&G and Neurology, University of Texas Medical Branch, Galveston, TX 77550.

The terminale portion of the frog spinal cord, the filum terminale, contains a large number of astrocyte like glia, and a neuropile with a dense network of 5HT immunoreactive fibers. The most caudal part of the FT contains significant quantities of GABA, acetylcholine and 5HT suggesting a possible glial storage mechanism for some neurotransmitters. We, therefore, have analyzed the entire frog central nervous system following pretreatment with the MAO inhibitor Pargylline and the intraventricular injection of the neurotoxin 5,6 dihydroxytryptamine (5,6DHT). Animals were sacrificed 5 or 14 days after the injection of 5,6DHT or saline in the controls. Tissues were frozen on dry ice, stored at -70° C and homogenized in 0.2M PCA for HPLC analysis with electrochemical (Coulochem, ESA) detection. The FT contains 84.8 ng/mg Prot. of 5HT, with somewhat higher levels seen in the conus medullaris and lumbar enlargement. The highest 5HT levels are seen in the brainstem (178 ng/mg Prot.) and lowest in the cerebellum and olfactory bulbs (17.7 ng/mg Prot.). Five days after the intraventricular injection of 5,6DHT, the 5HT levels in the caudal FT are reduced to 30% of control values, whereas depletions of 90% or greater are seen in the conus medullaris, the entire spinal cord and the Telencephalon. Parallel changes are seen in the distribution of 5HIAA after 5,6DHT. In contrast, only a 50% drop is seen in the diencephalon. Norepinephrine is present in the FT, though only at 10% of the 5HT values, and the greatest amount is seen in the brainstem (15 ng/mg Prot.). DA is present throughout the frog nervous system at concentrations similar to those of 5HT. It is interesting to note that the epinephrine (E) distribution does not parallel that of NE and DA, and E levels are double that of NE and DA (FT 19.2, CM 22.3). 5,6DHT results in small and inconsistent decrements in NE, DA and E levels, suggesting that 5,6DHT has neurotoxic effects on catecholamine terminals. We chose not to use 5,7DHT due to its greater toxicity in the frog. In all instances, analysis of the treated tissues reveals an HPLC peak that is identical to 5,6DHT. The observations suggest that 5,6DHT effectively destroys serotonergic terminals throughout the frog nervous system and that the large amounts of serotonin remaining in the caudal FT represents glial storage of that amine.

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26.3 DIFFERENT PROTEIN INCORPORATION PATTERNS FOR ^{3}H-LEUCINE AND ^{3}H-PROLINE IN ADULT CAT DORSAL COLUMN NUCLEI. N.Contos, J.Elam and K.J.Berkley. Dept. of Psychology (NC,KJB) and Dept. of Biology (JE), Florida St. Univ., Tallahassee, FL 32306.

Previous studies have shown that injection of ^{3}H-proline or ^{3}H-leucine into the cat dorsal column nuclei (DCN) results in a differential cellular incorporation pattern as assessed by EM autoradiography at 24h post injection. ^{3}H-proline produced heavy labeling of macroglia but negligible labeling of DCN neurons. ^{3}H-leucine, in contrast, was extensively incorporated into both neurons and glia (Molinari and Berkley, 1981).

We now report preliminary assessment of differences in the protein labeling patterns produced by the two amino acid precursors. Equal amounts of ^{3}H-proline or ^{3}H-leucine of the same specific radioactivity were injected into the DCN of different cats. Twenty four hours after injection, a 10-30 mg sample of the injected DCN tissue was excised and immediately frozen. Upon thawing, tissue samples were subjected to homogenization in distilled H_2O followed by separation into acid soluble and acid insoluble fractions by adjustment to 5% with respect to cold trichloroacetic acid (TCA). The acid insoluble fractions were further extracted with a mixture of ether and ethanol to remove lipids. A separate sample of homogenate was adjusted to 1% in SDS and subjected to PAGE on a 10 to 16% gradient gel.

Results indicated that proline, despite its lack of incorporation into neurons, labels total DCN tissue to a significantly higher extent than leucine (an average of 32.3dpm/μg protein/μCi for proline vs. 10.9dpm/μg protein/μCi for leucine). Of total radioactivity remaining at 24h past injection for either isotope, greater than 90% was recovered in protein. For proline, the average distribution of radioactivity was 96.5% in protein, 2% in TCA soluble and 1.5% in lipid soluble fraction, while for leucine, it was 92.5% in protein, 3.5% in TCA soluble and 4% in lipid soluble fraction.

Fluorographic analysis of acrylamide gels showed labeling of a wide variety of individual proteins with either amino acid. However, several differences were consistently seen in the specific radioactivity distribution patterns, presumably reflecting differences in the cellular origins of the radioactive proteins. Overall, the results indicate that the striking differences in cellular distribution of incorporated proline and leucine as observed by EM autoradiography are accompanied by differences in the extent of labeling and specific labeling pattern of proteins isolated from the tissue.

Supported by NSF grant BNS 83-10251.

26.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF GD3 GANGLIOSIDE TO ASTROCYTES IN MURINE CEREBELLAR MUTANTS. S.M.LeVine*, T.N.Seyfried, R.K.Yu and J.E.Goldman. Dept. Pathology (Neuropathology), Albert Einstein Coll. Med., Bronx, NY 10461; Dept. Neurology, Yale Univ. School of Med., New Haven, CT 06510

GD3 ganglioside, a major species of the immature CNS, is a minor species of the mature CNS. Substantially elevated levels of GD3 are found, however, in several murine mutants, staggerer (sg), lurcher (Lc), and Purkinje cell degeneration (pcd), characterized by neuronal degeneration in the cerebellar cortex (J. Neurochem. 38:551, 1982) and in gliotic human CNS. Astrocytes in sg and pcd cerebella elaborate sheet-like cytoplasmic extensions which wrap around neuronal processes (Neurology 18:1093, 1968; J. Neurosci. Res. 6:789, 1981). Seyfried et al. (J. Neurochem. 38:551, 1982) hypothesized that elevated GD3 in gliosis is due to its expression by astrocytes.

Using a monoclonal antibody to GD3 ganglioside (J. Exp. Med. 155:1133, 1982) and a polyclonal antibody to glial fibrillary acidic protein (GFAP) (J. Neurochem. 42:166, 1984) we explored GD3 localization in cerebella of mutants. Using 10 um-thick frozen sections of adult mutant and littermate control cerebella with an immunofluorescence method, positive GD3 staining was observed in sg, pcd, and Lc mice, but not controls or in weaver (wv) cerebella, a mutant in which GD3 levels are not elevated (J. Neurochem. 41:491, 1983). Sg, pcd, and Lc are also characterized by profound Purkinje cell loss, while wv is not. The localization of reaction in Lc and pcd was confined to the granule cell layer, usually the outer half, with no reaction in Bergmann glia of the molecular layer or astrocytes of white matter. In sg, in which cortical distortion was pronounced and no clear molecular layer was seen, GD3 reaction was present through the entire cortex. GD3 reaction appeared as many punctate or fine linear profiles, consistent with binding to thin cytoplasmic processes. Because of the staining pattern it was difficult to associate the profiles with specific cells, however. The large majority of GD3+ profiles were not also GFAP+. Therefore, ultrastructural localization was performed using pre-embedding immunocytochemistry in sg mice, with a PAP technique. The cytoplasmic processes of astrocytes were labeled, as well as astrocyte cell bodies. Thin processes did not contain intermediate filaments. Neuronal processes appeared unstained. Thus, GD3 is expressed by astrocytes in these pathological states. Expression of GD3 is localized to a specific astrocyte population(s) within the mutant cerebella, however. Our observations suggest that important changes in astrocyte membrane composition occur in pathological conditions. Neuronal degeneration, especially that of Purkinje cells, may induce changes in astrocyte membranes, changes that persist after neuronal loss has occurred. Supported by USPHS grants NS17125, NS00524, NS17704, and NS11853.

26.5 **LOCALIZATION OF PHOSPHORYLATION SITES WITHIN VIMENTIN AND GLIAL FIBRILLARY ACIDIC PROTEIN (GFA) OF CULTURED ASTROCYTES.** Edward T. Browning. Dept. of Pharmacology, Rutgers Medical School, Piscataway, New Jersey 08854.

Past studies identified two cytoskeletal intermediate filament proteins of astrocytes as phosphoproteins whose phosphorylation is stimulated by elevation of intracellular cyclic AMP (cAMP) (J. Cell Biol., 90, 803, 1981; J. Neurochem., 42, 718, 1984). The purpose of the present experiments was to localize the phosphorylation sites within the primary structure of the two polypeptides. First, the ^{32}P-labeled vimentin and GFA from intact, forskolin treated rat astrocytes were selectively cleaved with BNPS-skatole at the single tryptophan residue present in each of the molecules. For vimentin, 84% of the ^{32}P-labeling was associated with the 37K N-terminal fragment, the remainder was associated with the 20K C-terminal fragment. For GFA, all recovered ^{32}P-labeling was localized in the 30K N-terminal fragment. As the amino acid composition of the C-terminal peptides of the two proteins are highly conserved and that of the N-terminal peptides are much less conserved, most of the phosphorylation was associated with the less conserved portions of the polypeptides. Next, the two ^{32}P-labeled filament proteins were cleaved to limit peptides with trypsin and the peptides were resolved by HPLC. ^{32}P-labeled peptides were hydrolyzed with 6N HCl and the amino acid composition of each was determined by HPLC of orthophthaldehyde derivatives using a reverse phase system. From the amino acid composition of the peptides and a knowledge of the amino acid sequence of each polypeptide it was possible to localize the phosphorylation sites of each polypeptide. The procedure yielded a small labeled peptide for each filament protein. For vimentin the labeled peptide corresponded to a 16 or 18 amino acid peptide at or near the N-terminus of the molecule and localized the possible phosphorylation sites to serine residues at positions 9, 12 and/or 18. Independent experimentation had identified serine as the phosphorylated amino acid of vimentin. For GFA, the labeled peptide corresponded to a 16 amino acid peptide containing a single serine residue at position 52. The amino acid sequence in the region of both phosphorylation sites differs from that known to be recognized by the cAMP dependent protein kinase. These results suggest that one or more protein kinase other than cAMP dependent protein kinase is responsible for the direct phosphorylation of vimentin and GFA. The data further suggest the presence of a cascade mechanism in the cAMP stimulated phosphorylation of these sites in vimentin and GFA. (Supported by NSF Grant BNS84-06889)

26.6 Phospholipase C Stimulates Diacylglyercol Production In C6 Rat Glioma Cells. Joseph Bressler*(spon P. Kornblith Surgical Neurology Branch, NIH, Bethesda, MD. 20205

We have previously suggested an important role for diacylglycerols in glial cell differentiation (J. Neurochem. in press) by demonstrating that phorbol esters, which bind to the natural diacylglycerol receptor (protein kinase C), inhibits the glucocorticoid-mediated induction of glycerol phosphate dehydrogenase (GPDH) in C6 rat glioma cells. In order to further elucidate this mechanism, we have used a system whereby these cells can be artificially stimulated to produce diacylglycerols. A choline dependent phospholipase C (PLC, from C. perfringens), stimulates a 2.5 fold increase in diacylglycerols wtih an ED 50 of 0.01 unit. This enzyme was also able to induce the cells to release choline into the media. The number of phorbol ester binding sites on the glioma cells was reduced by approximately 50%, but the affinity of the ligand for the receptor was not significantly altered. GPDH induction was inhibited at an ED 50 of 0.03 units and the beta-adrenergic mediated increase in cAMP was also inhibited at an ED 50 of 0.02 units. The advantage of this enzyme over the use of synthetic diacylglycerols is its stability. For example, 1-oleoyl-2-acetyl-glycerol at 250 uM given to C6 cells every 12 hours gives a similar amount of inhibition that 0.1 unit gives after only one treatment. Therefore, this enzyme can help us learn more about the role of diacylglycerols in glial function.

26.7 CULTURED ASTROGLIAL CELLS RELEASE FOUR DISTINCT AGENTS ADDRESSING NEURONS. J.S. Rudge,* M. Manthorpe and S. Varon. Dept. of Biology, Sch. of Med., Univ. Calif. San Diego, La Jolla, CA 92093.

A protocol is described by which thousands of replicate microcultures of rat brain astroglial cells can be set up and maintained within a chemically defined medium and on a defined substratum. Neonatal cerebra are dissociated and the cells grown for 10 days on culture plastic in a fetal calf serum-supplemented Eagle's Basal Medium as described (J. Neurocytol. 8: 605-621, 1979). The cells in these primary cultures are harvested and reseeded on a poly-ornithine-fibronectin substratum in microtiter plate wells and cultured in Eagle's Basal Medium without serum. These secondary confluent microcultures contain nearly exclusively GFAP-positive cells which can be maintained at the initially attached cell number (75% of seed) for 7 days without refeeding or for weeks with medium changes every 3 days.

Conditioned media were collected for these secondary cultures at various times and tested for their content of neuronotrophic and neurite promoting activities. The test neurons used to quantitate activities were from (i) chick E8 (embryonic day 8) dorsal root ganglia for the measurement of Nerve Growth Factor; (ii) chick E8 ciliary ganglia for Ciliary Neuronotrophic Factor; (iii) chick E8 telencephalon for trophic activity addressing CNS neurons and (iv) E8 chick ciliary ganglia again for determination of Polyornithine-binding Neurite Promoting Factor. All four activities are present in the conditioned medium and can be physically separated from one another. The activities accumulate and/or decline in the astroglial cell conditioned medium with reproducible and distinct temporal patterns, suggesting the independent regulation of production, release and/or stability of the corresponding molecules. This astroglial microculture method will be used in future studies to identify and investigate specific substances which may regulate astroglial cell content/release of these neuroactive agents. Supported by NSF grant BNS 82-18366 and NINCDS grant NS 16349.

26.8 **SEROTONIN STIMULATES TAURINE RELEASE FROM GLIAL CELLS IN CULTURE.** D. L. Martin, J. E. Abunaw* V. Madelian and W. Shain. Wadsworth Center for Laboratories and Research, New York State Health. Dept., Albany, NY 12201.

Glial cells are known to have receptors for a variety of neurotransmitters, but their functional significance is unclear. Previous studies from this laboratory have established that activation of beta-adrenergic or kappa-opiate receptors stimulates release of the neuroactive amino acid taurine from glial cells in culture. We now report that taurine is also released upon stimulation by serotonin. To study release LRM55 glial cells were preloaded with ^{3}H-taurine and then superfused with Hepes-buffered Hank's medium without or with drugs. Radioactivity appearing in the superfusate was measured. Taurine release was strongly stimulated by low doses of serotonin (EC_{50} = 0.4 μM), and this effect was inhibited by the serotonin receptor blockers methysergide and mianserine (IC_{50} = 0.04 nM and 5 nM when tested against 1 μM serotonin). Tryptamine was a much less potent agonist (EC_{50} 300 μM). Release stimulated by serotonin and beta-adrenergic agonists were mediated by different receptors, as the beta-adrenergic antagonist propranolol did not inhibit stimulation of release by serotonin and methysergide did not inhibit stimulation of release by the beta-agonist isoproterenol (IPR). The temporal pattern of release in response to prolonged stimulation by serotonin was similar to that observed with IPR. There was a rapid rise in release that reached a maximum after 5 min. Release then declined to a new steady-state level that continued until drug application ceased. IPR and serotonin were synergistic when initially applied together. However this synergism was lost if either agent was applied for 15 min before the other. Unlike IPR, serotonin did not stimulate cyclic-AMP synthesis and there was no synergistic increase in cyclic-AMP if both were applied together, indicating that serotonin acts via a different second messenger than beta-adrenergic agonists.

26.9 DESENSITIZATION OF BETA-ADRENERGIC RECEPTOR STIMULATED TAURINE RELEASE FROM GLIAL CELLS. W. Shain, V. Madelian, D.L. Martin, and L. Memmo*. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201.

Stimulation of beta-adrenergic receptors on LRM55 glial cells and primary cultures of astrocytes results in cyclic AMP (cAMP) mediated release of the inhibitory amino acid taurine. When cells were continuously exposed to beta-agonists for 30 min, three phases of release were observed: activation, inactivation, and elevated steady state. We have previously demonstrated that activation of release is mediated by cAMP The experiments described here were designed to elucidate the regulatory processes mediating inactivation and elevated steady-state. Several observations indicated that inactivation was not dependent on the intracellular concentration or changes in adenylate cyclase activity. (i) Inactivation of release occurred more rapidly than attenuation of intracellular cAMP levels during continuous application of agonist. (ii) When activation was studied by pretreating cells with 10 nM isoproterenol (IPR) for various lengths of time and then challenging them with 100 nM IPR the magnitude of the challenge response declined rapidly with a $t_{1/2}$ = approx. 2.5 min. This was more rapid than the $t_{1/2}$ for the observed decrease in intracellular cAMP. (iii) Continuous treatment of cells with dibutyryl cAMP and forskolin results in release profiles similar to that observed with beta-agonists, even though high levels of intracellular cAMP are sustained during both treatments. These results suggested that regulation occurred at a site distal to adenylate cyclase along the intracellular cAMP-protein kinase pathway. Regulation of the phosphorylated substrate(s) by protein kinase and phosphoprotein phosphatase is one possible site of control. Protein phosphorylation was studied by incubating cells with ^{32}P for 5 hrs to permit intracellular ATP pools to come to steady-state with ^{32}P. Cells were then stimulated with IPR and prepared for polyacrylamide gel electrophoresis at 0, 5, and 30 min after agonist application. Analysis of the phosphorylated proteins showed that several labelled bands had little or no ^{32}P incorporation at 0 min, maximal incorporation a 5 min, and low levels of incorporation at 30 min. Since the elevated steady-state was maintained as long as agonists were applied, these last observations suggested that inactivation may occur via rapid dephosphorylation and that the elevated steady-state represents the steady-state condition between protein phosphorylation and dephosphorylation.

26.10 DESENSITIZATION OF cAMP PRODUCTION IN LRM55 GLIAL CELLS BY THE BETA-ADRENERGIC AGONIST ISOPROTERENOL. V. Madelian, D.L. Martin, and W. Shain. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201.

Beta-adrenergic stimulation of LRM55 glial cells produces cyclic AMP (cAMP) mediated release of taurine. Prolonged exposure to agonists resulted in attenuation, i.e. desensitization, of both intracellular cAMP accumulation and taurine release. The regulation of cAMP during desensitization was studied by describing the kinetics of attenuation, determining changes in response to receptor stimulation during attenuation, and pharmacologically manipulating cells to separate cAMP degradation from receptor-stimulated changes in cAMP synthesis. The kinetics of attenuation were described by exposing cells continuously to various concentrations of isoproterenol (IPR) and by pretreating cells for various periods of time and then challenging them for one min with IPR. During a 30 min continuous exposure intracellular cAMP levels increased rapidly to dose-dependent maxima then declined steadily to values about 80% lower than the peak values. Attenuation of the response, determined as a function of time of pretreatment, was observed by one min and had a $t_{1/2}$ of approx. 5 min. Receptor stimulation during attenuation was studied by pretreating cells with various concentrations of IPR and then generating dose-response curves for intracellular cAMP levels to determine if pretreatment resulted in changes of either the apparent affinity, i.e. ED_{50}, or the apparent V_{max}, i.e. maximal stimulation, of the response. There was no significant change in the EC_{50} values (60 nM) but there was a 70% decrease in maximal stimulation. Pharmacological manipulation of receptor-stimulated cAMP response was achieved by first producing a maximal response with IPR and then either inhibiting phosphodiesterase with RO20-1724 (RO20) in the continued presence of agonist or inhibiting receptor activation by removal of IPR or addition of propranolol, a beta-antagonist. When RO20 was added in the presence of agonist, cAMP continued to rise for another 15 min at which time the cAMP level in cells in the presence of agonist alone had dropped to 10% of peak values. When receptor activation was inhibited, there was a very rapid decrease in cAMP levels. However, when cells were changed to media containing RO20 alone this drop was prevented. These data demonstrate that attenuation of intracellular cAMP accumulation in LRM55 glial cells may be principally a result of cAMP hydrolysis and not to a reduced rate of synthesis.

26.11 (^{3}H)-NITRENDIPINE BINDING IN NON-NEURONAL CELLS FROM MOUSE SPINAL CORD CULTURES. M.J. Litzinger and D.E. Brenneman, Lab. of Dev. Neurobiology, NICHD, NIH, Bethesda, Maryland 20205

(^{3}H)-Nitrendipine (NTP) is believed to bind to the voltage-sensitive calcium channel (VSCC). Recently, electrophysiological evidence has indicated that VSCC is present in primary glial cultures grown in 10% fetal calf serum (MacVicar, Science 226: 1345, 1984). The purpose of the present study was to determine if non-neuronal cells exhibit specific NTP binding sites which would correlate with the physiological evidence of the VSCC.

Previous studies of (^{3}H)-NTP binding in dissociated spinal cord cultures from the fetal mouse demonstrated two NTP binding sites throughout development (Litzinger and Brenneman, BBRC 127: 112, 1985). The culture preparation utilized for these studies consisted of both neuronal and non-neuronal cells. These mixed cultures were maintained in Eagle's minimal essential medium (MEM) with 5% horse serum supplemented with defined medium components. In the present study, spinal cord cultures were grown in MEM with 10% fetal calf serum. With these conditions, spinal cord neuron growth was not supported, although a small number of dorsal root ganglion cells did survive this treatment. The plating density for the background cultures was 2.5 x 10^5 cells per 16 mm diameter well. After 3-4 weeks, the cultures were comprised of glial cells and fibroblasts.

(^{3}H)-NTP binding was conducted on living cells at 37° C. Unlabeled NTP (50 µM) was used to determine specific binding. NTP was dissolved in ethanol. Vehicle controls indicated no significant effect on (^{3}H)-NTP binding. Specific binding was found to range between 48% and 62% of the total binding. Curve fitting analysis (the Bright program) indicated that the best estimate of the Scatchard plot was linear. The apparent Kd was 25 nM and the B_{max} was 293 fmoles per well or 13.0 fmoles per µg protein. These values are similar to those reported for the low affinity component of NTP binding in cultures which contained both neurons and background cells. Our data suggest the existence of a single, low affinity class of binding sites in background cells. These studies are consistent with the conclusion that VSCC is present on non-neuronal cells derived from spinal cord cultures.

26.12 Pharmacology and Ionic Mechanism of the (-)NE Induced Depolarization of Astrocytes in Primary Culture. Charles L. Bowman* and Harold K. Kimelberg. Division of Neurosurgery, Albany Medical College, Albany, New York 12208.

Astrocytes, derived from an enzymatic dissociation of cerebral hemispheres of neonatal rats (Frangakis and Kimelberg Neurochem Res 9:1689 1984), were grown in primary cell culture. It is well established that the membrane potential of astrocytes is depolarized by (-)NE. The NE induced depolarization is mediated by an α- rather then a β-adrenergic receptor (Hosli et.al. Neurosci 7:2867 1982; Hirata et.al. Brain Res 270:358 1983). We are investigating the pharmacological characteristics and ionic mechanisms underlying the NE induced depolarization of astrocytes in primary cell culture.

It appears that an α_1-rather then an α_2-adrenergic receptor may be involved because prazosin (an α_1-adrenergic receptor antagonist) was a more effective inhibitor of the NE induced depolarization then either yohimbine or idazoxane (RX 781094) (both α_2-adrenergic receptor antagonists). These results are in general agreement with the hypothesis that α_1-adrenergic receptors are postsynatic.

It is well established that astrocytes in primary culture have a high affinity Na^+dependent uptake mechanism for catecholamines. This uptake mechanism is blocked by the tricyclic antidepressents (amitriptyline (AMT); desipramine (DMI)) and by dopamine (Kimelberg and Pelton J. Neurochem 40:1265 1983). Perfusion of DMI, AMT or dopamine (10^{-6}M) only partially (approx 30%) inhibited the NE (10^{-5}M) induced depolarization. We conclude from these results that the NE response is not due to the high affinity catecholamine uptake system alone.

Using a double impalement current clamp technique, we have measured the current-voltage characteristics of the astrocytes. In the absence of agonist, the IV curve was linear. Perfusion of NE (10^{-5}M) resulted in some degree of nonlinearity in the presence of agonist. Washout of NE caused an irreversible increase in the input resistence so that subsequent perfusion and washout of NE was without effect on the input resistence. These results suggest that NE either closed K^+ channels in the plasmalemma or closed junctional channels between cells, and suggest the presence of multiple ionic conductance changes or the presence of chemically gated - voltage dependent channels.

We have studied the effect of varying the chloride equilibrium potential on the NE response. In the presence of high $[K^+]_o$ (130 mM) and low $[Na^+]_o$(5 mM), varying $[Cl^-]_o$ results in either a depolarization (5 mM $[Cl^-]_o$) or hyperpolarization (122 mM $[Cl^-]_o$). These results suggest that NE opens a chloride channel in the plasmalemma. Supported by NINCDS grant NS19492, and by NSF grant BNS 8213873.

26.13 BARIUM INDUCED RHYTHMIC DEPOLARIZATION OF GLIAL CELLS IN BRAIN SLICES. W. Walz[1] and B.A. MacVicar[2], [1]Dept. of Physiology, University of Saskatchewan, Saskatoon, S7N OWO and [2]Dept. of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1, Canada.

Voltage-dependent calcium channels have been observed in glial cells in primary cell culture (MacVicar, 1984, Science 226:1345). However we wished to confirm that glial cells in in situ preparations such as brain slices also have the same properties. We used intracellular recordings from presumed glial cells in the CA1 layer of the rat hippocampal slice to examine this question. Glial cells were identified by the following criteria: 1. high resting potential (>-70 mV in 5 mMK^+); 2. low input resistance (<2 MΩ as compared to neuronal input resistance of >15 MΩ); 3. current injection did not evoke any spikes; 4. repetitive synaptic stimulation did not lead to post-synaptic potentials but to a slow depolarization (∿20 mV) which was followed by a slow hyperpolarization (2-4 mV) lasting for about 5 min. These properties have all been previously described as characteristics of glial cells. In these experiments low calcium (0.1-0.2 mM) solutions were perfused to block transmitter-mediated synaptic transmission and ensure that the glial response was endogenous. After synaptic responses were blocked barium (10-25 mM) solutions were perfused and in some experiments tetraethylammonium (10 mM) was included. We observed the following phenomena during stable long-term recordings from 12 cells: 1. all cells depolarized by 20-30 mV in barium; 2. rhythmical oscillations of the membrane potential were observed in 35% of the cells. The frequency was approximately 1Hz and the amplitude 10 to 20 mV. The depolarization phase of the oscillation was associated with a decreased input resistance; 3. an after-depolarization followed depolarizing current pulses (100 msec duration) in 25% of cells. The amplitude of the after-depolarization was increased when the cell was hyperpolarized and the after-depolarization was correlated with a decrease in input resistance.

Therefore the membrane properties of glial cells in brain slices are altered by barium. The depolarization observed in all cells may indicate that resting K^+ permeability of glial cells is decreased by barium. The rhythmic and evoked depolarizations may result from barium entry through voltage dependent calcium channels.

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26.14 A POTASSIUM DEPOLARIZATION OF THE OLIGODENDROCYTE PARANODAL REGION IN RAT OPTIC NERVE. V. Lev-Ram* (SPON: Z. Wollberg). Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

The present study describes the use of fluorescence voltage-sensitive probes and an optical recording technique for investigating the physiological relationship between axons and oligodendrocytes in myelinated nerves. This optical technique allows direct recording from structures that cannot be penetrated by electrodes. Rat optic nerves were stained in vitro and action potentials were stimulated and recorded with suction electrodes. Simultaneously, electrical activity in axonal membrane and potential changes in the glial cells were recorded optically. Two types of optical signals were detected: a fast signal (3-5 msec) that could account for the sum of intracellular action potentials of a population of axons, and a slow signal (latency to peak 40-90 msec, decay time 700-1300 msec) (Fig. A). We concluded that the slow signal originated from the oligodendrocyte myelin sheath, because it could be elicited neither in nonmyelinated optic nerves of newborn rats (Fig. B) nor when myelinated nerves were stained with axon-specific dyes that did not bind to glial membranes. Furthermore, the slow signal recorded from myelinated nerves disappeared after demyelination was produced by repetitive osmotic shocks. This glial signal showed the following characteristics: varied with extracellular K^+ in a glial depolarization manner (1.2-11.2 mM); increased by 4-AP (5 mM); was absent in Cs^+-loaded nerves; decreased upon temperature elevation; and was abolished by TTX (except for near the stimulating electrode where passive spread could evoke the glial signal). The glial signal was found to be Ca^{2+}-dependent, as it could not be detected in the absence of Ca^{2+} or when Ca^{2+} was replaced by its antagonists. It appears that the slow signal represents glial membrane depolarization caused by Ca^{2+}-dependent K^+ accumulation which results from axonal activity. The function of this glial response in the physiology of myelinated nerves remains to be ascertained.

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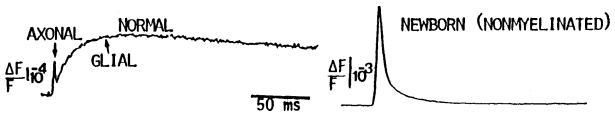

FIG. A. FIG. B.

COEXISTENCE OF TRANSMITTERS

27.1 DISSOCIATED RAT SYMPATHETIC NEURONS IN MICROCULTURE: NON-ADRENERGIC EXCITATION OF CARDIAC MYOCYTES AND THE VARIETY OF MULTIPLE-TRANSMITTER STATES. S.G. Matsumoto*, D.W.Y. Sah, S.C. Landis, D.D. Potter and E.J. Furshpan. Dept. of Neurobiol. Harvard Med. Sch., Boston, MA. 02115.

Neurons dissociated from the superior cervical ganglion (SCG) of both neonate and adult rats have been shown to release norepinephrine, acetylcholine and adenosine (or a related purine) in various combinations when co-cultured with cardiac myocytes (Furshpan et. al. J. Neurosci. in press). We have now observed the release of at least 2 additional substances that produce slow depolarizations in the cardiac myocytes. These responses are insensitive to adrenergic, cholinergic and purinergic antagonists.

The non-adrenergic excitatory (NAE) effects on the cardiac myocytes were small (1-5 mV) and usually detected only after any other transmitter effects were blocked. One of the agents responsible for NAE has been tentatively identified as serotonin (5-HT) (see also Sah and Matsumoto Neurosci. Abs., 1985). This type of effect is blocked reversibly by methysergide and/or gramine and irreversibly by reserpine. Ultrastructural experiments are in progress to determine the 5-HT storage compartment. A second NAE effect had a longer duration than the putative 5-HT responses and was not affected by 5-HT antagonists or reserpine. Long trains of neuronal impulses (20s) at high frequency (20 Hz) were required to evoke this response, which then persisted for several minutes. Long inter-stimulus intervals were usually required to evoke these responses reproducibly. The mediator(s) of this second NAE effect has not been identified. Attempts to mimic the effects of this agent(s) with peptides whose immunoreactivities have been reported in adult sympathetic neurons in vivo have thus far been unsuccessful.

The transmitter repertoire of microcultured SCG neurons is extremely diverse. Two types of diversity are observed; first in the combination of transmitters released and second, in the relative strengths of their effects on the cardiac myocytes. A summary of the different combinations of co-released transmitters reveals a broad spectrum of multi-functional states. A majority of the possible combinations of 5 distinct transmitter actions have been observed. The variability in the relative strengths of the transmitter effects appear to be due to their graded expression within the individual neurons.

(supported by NIH and NSF)

27.2 DISSOCIATED CELL CULTURES OF RAT SUPERIOR CERVICAL GANGLION NEURONS: SEROTONIN UPTAKE AND RELEASE. D. W. Y. Sah and S. G. Matsumoto*. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Cultures of dissociated sympathetic principal neurons from newborn and adult rat superior cervical ganglia have been shown to release norepinephrine, acetylcholine and a purine (probably adenosine). In some microcultures of single sympathetic neurons and cardiac myocytes, a non-adrenergic excitatory (NAE) interaction, sensitive to serotonin (5-HT) antagonists, has been observed (see Matsumoto et al, Neurosci. Abs. 1985). To examine further the possibility that 5-HT is a neurotransmitter in these neurons, we used HPLC and electrochemical detection to study its uptake, presence and release, and immunocytochemistry to study its distribution. Electrophysiological recording was used to further study uptake.

Mass cultures of approximately 1000 neurons were used for biochemical and immunocytochemical experiments. Culture homogenates contained a substance that co-migrated with authentic 5-HT. As a further test of the identity of the extract peak, a voltammogram was obtained and compared with that of authentic 5-HT. The profile for putative 5-HT from the culture homogenates superimposed upon that of authentic 5-HT in the presence or absence of an ion-pair. When the cultures were examined with immunocytochemical staining using the peroxidase-antiperoxidase technique, many neuronal processes, as well as 2-48% of the somata contained 5-HT-like immunoreactive material.

Serotonin was present in the rat serum added to the growth medium; the concentration of 5-HT in the medium was 0.25-0.50 μM. The serum was probably at least a major source of the 5-HT present in the neurons, since levels of 5-HT in the cultures were highest just after replacing the culture medium with fresh medium, and declined to very low levels over the next 4 days.

The 5-HT in the cultures was released in a Ca^{2+}-dependent manner by high K^+ or veratridine, consistent with a neurotransmitter role. In electrophysiological experiments on microcultures, a non-adrenergic excitatory interaction, sensitive to 5-HT antagonists, could sometimes be obtained after exposing the neurons to 5-hydroxytryptophan. In our observations so far, neurons with adrenergic function could take up 5-hydroxytryptophan and release 5-HT, but neurons with cholinergic function and no adrenergic function could not.

There are several reports that autonomic nerve terminals can take up exogenous 5-HT, e.g. in the vas deferens and blood vessels. In vivo, the terminals of SCG neurons are not normally exposed to serum, but are exposed to mast cells in the target tissues, a major source of 5-HT. Perhaps the 5-HT uptake that occurs in cultured sympathetic neurons also occurs in vivo under certain conditions.

(Supported by NIH and NSF).

27.3 COEXISTENCE OF ENKEPHALIN AND DYNORPHIN IMMUNOREACTIVITIES IN NEURONS IN THE DORSAL GRAY COMMISSURE OF THE LUMBOSACRAL SPINAL CORD IN RAT. Cathrine A. Sasek and Robert Elde. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

In a recent report (Neurosci. 12:855) we described the distribution of several peptides in the dorsal gray commissure (DGC) of the sixth lumbar (L) and first sacral (S1) spinal cord segments in the rat and hypothesized that two or more peptides might coexist in neurons in the DGC. This hypothesis was supported by the abundance and wide variety of peptides found in the DGC. Of the peptides present in the DGC the opioids enkephalin (ENK) and dynorphin (DYN) are especially interesting in that they appear to be present in a greater number of neuronal perikarya than most other peptides. Additionally, opiates have been shown to be important in the regulation micturition. Thus, the present study was undertaken to determine if DYN and ENK coexist in neurons in the DGC in L6 and S1 of rat spinal cord.

Frozen (5um) sections of L6 and S1 from colchicine treated rats were processed for immunofluorescence using anti-DYN as the 1 antiserum. After photographing the sections the DYN antiserum was eluted with acid potassium permanganate. The sections were then rinsed, incubated in fluorescein labeled secondary antiserum as a control, and if no staining was seen, incubated with anti-ENK and FITC labeled 2 antibody. Sections were rephotographed and the negatives were used to compare the number and location of DYN immunoreactive cells to that of ENK immunoreactive cells in each section. A chi square test for independence was used to determine if there was a significant difference between L6 and S1 in the proportion of cells with only ENK to those with ENK and DYN coexistence. Both ENK and DYN antisera were tested extensively for cross reactivity and were found to be specific.

It was found that of 67 ENK containing cells counted in 20 sections through L6, 54 also contained DYN immunoreactivity and that of 100 ENK containing cells in counted 47 sections through S1, 95 also contained DYN immunoreactivity. No cells containing only DYN immunoreactivity were seen. A statistically significant difference ($p<0.004$) was seen between L6 and S1 in the ratio of the number of cells containing both ENK and DYN immunoreactivity to those containing only ENK immunoreactivity.

The results of the present studies indicate that ENK and DYN immunoreactivities coexist in most, but not all neurons in the DGC of L6 and S1 and that a significant difference exists between L6 and S1 in the extent of coexistence of these peptides. The functional significance of the expression of two opioids by a single neuron is unknown, but it can be speculated that the role of opioids in neurons in the DGC must be important, as so much of the neurons biosynthetic machinery is dedicated to the synthesis of opioids. Supported by DA 02148.

27.4 SUBSTANCE P AND SEROTONIN COEXIST IN NERVE FIBERS APPOSED TO IDENTIFIED SYMPATHOADRENAL PREGANGLIONIC NEURONS IN RAT INTERMEDIOLATERAL CELL COLUMN. N.M. Appel, M.W. Wessendorf and R. Elde. Dept. of Anatomy, U. Minnesota, Minneapolis MN 55455.

Physiological evidence has accumulated implicating serotonin (5-HT) and substance P (SP) in the regulation of sympathetic nervous system activity. In addition, anatomical studies have demonstrated the existence of SP and 5-HT-immunostained neuronal processes in close proximity to identified sympathoadrenal preganglionic (SAP) neurons in the intermediolateral cell column (IML) of spinal cord. In the present study we examined the possibility that these putative neurotransmitters were localized within the same neuronal processes.

Adult male Sprague-Dawley rats were anesthesized and Fast Blue (5µl, 1% w/v in water) was pressure injected via a glass micropipette into the left adrenal medulla. Five days later they were deeply anesthesized and perfusion-fixed with buffered 4% paraformaldehyde. Spinal cords were collected, sectioned on a cryostat (10µ) and processed for simultaneous demonstration of 5-HT and SP immunoreactivities. This protocol resulted in 5-HT- and SP-immunostained material fluorescing red and green, respectively.

With appropriate ultraviolet illumination, Fast Blue-labeled SAP neurons appeared as "ice-blue" colored cells in IML. When illumination was adjusted to visualize rhodamine- (5-HT) or fluorescein-labeled (SP) structures, nerve terminals and fibers immunostained for 5-HT or SP were observed in the vicinity of Fast Blue-labeled cell bodies in IML. Furthermore, some, but not all of these structures fluoresced using both rhodamine and fluorescein excitation filters. This was not due to "bleed-through" of weak red fluorescence since a 560nm short-pass filter did not affect their appearance. In addition to being in proximity to SAP neurons, dual-labeled structures were also apparent in regions where SAP neurons were not observed.

We conclude from these studies that nerve terminals and fibers in which 5-HT and SP immunoreactivities coexist occur in close proximity to SAP neurons. While the presence of these immunostained fibers and terminals in the vicinity of labeled SAP neurons suggest their interaction with these cells, synaptic contact must be established with ultrastructural studies. In view of contradictory reports regarding the effects of 5-HT on IML, our data suggest that consideration of an interaction between 5-HT and SP in this setting may be necessary.

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27.5 CATECHOLAMINE, SEROTONIN, AND SUBSTANCE P-LIKE PEPTIDE CONTAINING INTERNEURONS IN A PARASYMPATHETIC GANGLION. D.S. Neel* and R.L. Parsons (SPON: G. Webb). Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

There are two neuron types in the mudpuppy cardiac ganglion; large parasympathetic ganglion cells and small catecholamine containing interneurons (SIF cells) (1). The function of these interneurons is not known and direct application of catecholamines to the parasympathetic ganglion cells produce no electrophysiological effect (2). It is now well known that many different classes of neurons can synthesize and use more than one transmitter type, therefore experiments were done to test whether putative transmitter substances other than catecholamines are also present within these interneurons.

Whole mount septal preparations were dissected from Necturus maculosus, pinned onto Sylgard coated petri dishes and processed for indirect immunocytochemistry. Two protocols were used: a) to examine for neuropeptides or other putative transmitters, the preparations were incubated in cold Zamboni's fixative for eighteen hours prior to application of primary antisera (3); b) to determine coexistance of catecholamine and other transmitter substances, the preparations were fixed for approximately 30 min. in buffered 4% paraformaldehyde and 0.25% glutaraldehyde solution (4). Primary antisera included: Substance P 1:200 (Sera Labs) and serotonin (5-HT) 1:200 (Immunonuclear Corp.). Absorption controls for the specificity of the immunoreactivity were produced by incubating purified peptide or 5HT-congugate complex with its respective antisera for 24 hours before incubating whole mounts in the antisera.

From these studies, it was concluded that several combinations of the substances examined can occur in the interneurons. Some interneurons and their processes contain a substance P-like peptide and 5-HT. Other interneurons were reactive for only one substance. Many interneurons exhibited immunoreactivity for either substance P or 5-HT and also were positive with aqueous-aldehyde-induced fluorescence which indicated the presence of a catecholamine. Finally, observations were made that showed certain interneurons contain all three, a catecholamine, 5-HT and substance P-like peptide.

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27.6 SEROTONIN AND SUBSTANCE P COEXIST IN AXON TERMINALS OF THE HYPERINNERVATED INFERIOR OLIVE AFTER INTRAVENTRICULAR 5,6-DIHYDROXYTRYPTAMINE. M. Paré* and L. Descarries (SPON: A. Ferron). Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Qué., Canada H3C 3J7.

In order to investigate the possible coexistence of serotonin (5-HT) and substance P (SP) in axon terminals of the inferior olive (IO) of adult rats (Hökfelt, T. et al., *Neuroscience, 3:* 517, 1978), paired histological sections taken at regular intervals across the lower half of medulla oblongata were processed for PAP-immunohistochemistry using anti-5-HT and -SP antibodies. Observations were made in rats subjected to cerebro-ventricular injection of 5,6-dihydroxytryptamine (5,6-DHT, 75 µg f.b. in 20 µl of saline) 1-2 weeks or 6-21 months earlier, as well as in saline-injected controls of the same age. In the short term experiments, the effects of the neurotoxin on 5-HT neurons were manifested by the presence of enlarged, tortuous and/or bulbous "dystrophic" immunostained axonal profiles in all regions of the IO and neighboring tissue normally innervated by 5-HT fibers, and a reduced number of axonal varicosities especially in the dorsal accessory olive (DAO). Some 5-HT-immunopositive nerve cell bodies were still visible in the adjacent nuclei raphe pallidus and obscurus. Immunoreactivity to SP appeared to be unchanged in certain portions of the IO (dorsal cap of Kooy, dorsal ramus of principal olive (PO) and patches in DAO) but, in other areas (area b of caudal MAO and lateral DAO), dystrophic immunostained fibers were visible. At the prolonged time intervals after 5,6-DHT, the expected 5-HT hyperinnervation in IO (Wiklund, L. and Björklund, A., *Brain Res. 191:* 129, 1980) was evidenced by marked increase in the number of immunostained axons and varicosities, not only in the DAO, where the 5-HT innervation normally predominates, but also in the MAO where it is weak in the controls. Concomitant changes in the distribution of SP immunoreactivity were seen in the very same regions, including the rostral MAO where SP terminals appear exceedingly scarce in the controls. These results strongly suggest the coexistence of 5-HT and SP within certain axon terminals of adult rat IO in addition to categories of IO fibers containing 5-HT and SP independently. The presence of SP-immunoreactivity in IO 5-HT axons regrown and hyperdeveloped after 5,6-DHT-lesioning is consistent with earlier hypotheses that the production of the peptide and that of the biogenic amine are interregulated at the level of the cell bodies and/or controlled by a signal(s) issued from the terminals. Such an experimental model might be of use in discriminating between these alternatives. (Supported by grant MT-3544 from the MRC of Canada).

27.7 CO-LOCALIZATION OF GABA- AND TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITIES IN NEURONS OF THE RAT OLFACTORY BULB. C.M. Gall, S.H.C. Hendry, K.B. Seroogy, and E.G. Jones. Department of Anatomy, University of California, Irvine, CA 92717.

Tyrosine hydroxylase (TH), the biosynthetic enzyme for dopamine, and glutamic acid decarboxylase (GAD), the biosynthetic enzyme for gamma aminobutyric acid (GABA), have both been localized within periglomerular neurons in the rat main olfactory bulb. The present study was conducted to determine whether the two "classical" transmitter substances, dopamine and GABA, are co-localized within individual neurons in this brain region.

Sprague Dawley rats were perfused with either 4% paraformaldehyde or 2% paraformaldehyde and 0.1% glutaraldehyde. Using indirect immunofluorescence, we processed sections through the olfactory bulb for the simultaneous detection of TH (sheep antiserum supplied by J. Haycock, Rockefeller Univ.) and GABA (rabbit antiserum, Immunonuclear Corp.). In agreement with previous reports we found TH immunoreactivity (TH-I) localized primarily within numerous small (5-7um) periglomerular cells and within somewhat larger neurons (major diam. 10-15um) in the glomerular and external plexiform layers. The larger neurons presumably include external and middle tufted cells as well as some short-axon cells. GABA immunoreactivity (GABA-I) was also localized within the small periglomerular cells and larger (major diam. 10-12.5um) neurons in the glomerular and external plexiform layers as well as within large numbers of internal granule cells. The vast majority of the TH-I neurons in both the glomerular and external plexiform layers also contained GABA-I. However, in each field some neurons were immunoreactive only for TH and a larger number were immunoreactive only for GABA. The larger, presumably middle tufted, TH-I neurons in the EPL generally did not contain GABA. These data demonstrate that the majority of the dopamine containing periglomerular cells, which others have suggested to comprise 20% of the total periglomerular cell population, contain GABA. The co-localization of TH and GABA in larger neurons within the periglomerular and external plexiform layers indicates that dopamine and GABA coexist within a number of external tufted and short-axon cells as well.

(Supported by NSF grant BNS8200319 and a Sloan Research Fellowship to C.M.G. and NIH grant NS21377 to E.G.J.)

27.8 **MOST GABA-ERGIC NEURONS IN THE RAT CEREBRAL CORTEX CONTAIN THE CALCIUM-BINDING PROTEIN PARVALBUMIN.** L. Schärer*, M.R. Celio (SPON: H.P. Lipp) Anatomisches Institut der Universität Zürich, CH-8057 Zürich (Switzerland).

Gamma-amino-butyric-acid (GABA) is recognized as one of the major inhibitory neurotransmitters in the central nervous system. In the cerebral cortex, a subpopulation of interneurons is GABA-ergic. Parvalbumin (PV) was also shown to be a marker for a subpopulation of cortical neurons (Celio and Heizmann, Nature 293,300-302,1981). In this study we sought to determine whether the two substances, GABA and PV, coexist in the same cortical neurons.

Rats were perfused transcardially with fixative and pieces of the somatosensory cortex were frozen in liquid nitrogen after imbibition with sucrose. Consecutive semi-thin cryo-sections (0.5-1 um) were cut with a Reichert Ultra-cryo-microtome and mounted on glass slides. The sections were alternatively incubated with the PV- or the GABA-antiserum, and further processed by the peroxydase-anti-peroxydase method. GABA-ergic and parvalbuminic neurons were scattered in the somatosensory cortex in a similar manner. A closer scrutiny revealed two different populations of interneurons: the major (approx. 70%) were both GABA- and PV-immunoreactive, whereas a minor (approx. 30%) had only GABA-immunoreactivity. PV distributed itself only in GABA-ergic neurons.

What could be the role of PV in GABA-ergic neurons of the neocortex? If it can be assumed that parvalbumin in the brain binds Ca^{++} in exchange of Mg^{++}, as in skeletal muscles, then PV could modulate the excitability and the activity of GABA-ergic neurons. In fact, by binding Ca^{++} after its entry in the cytoplasm, PV would prevent the activation of the Ca^{++} activated K^{+} current, thus reducing the after-hyperpolarization of the neuron. The shortening of the refractory period might enable a higher firing rate of the neuron. By discharging Mg^{++} in the cytoplasm, PV may trigger the multitude of Mg^{++} dependent enzymes, thus linking cell activity and cell metabolism. Abnormalities of parvalbumin in the GABA-ergic cells of the neocortex could lead to ineffective inhibition, and serve as a causative factor in the development of epileptiform discharges.

27.9 TYROSINE HYDROXYLASE AND NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN RABBIT MEDULLA OBLONGATA. W.W. BLESSING, T.H. JOH, J.R. OLIVER* AND J.O. WILLOUGHBY. Centre for Neuroscience and Department of Medicine, Flinders University of South Australia, Bedford Park, South Australia, 5042.

The distribution and co-localization of tyrosine hydroxylase (TH) and neuropeptide Y (NPY)-like immunoreactivity in the rabbit medulla were studied, with special reference to the possible presence of these substances in vagal preganglionic neurons.

Rabbits were perfused with a solution of formaldehyde and picric acid and the brain was post-fixed for 24 hours. Additional rabbits received unilateral intramedullary injections of colchicine (1-2 ug) 36 hours before perfusion. In some rabbits, vagal preganglionic neurons were identified by Fast Blue, retrogradely transported from the cervical vagus nerve. Vibratome sections (50 um) were washed in 50% ethanol, rinsed in TRIS buffer and blocked with 20% normal horse serum. Sections were processed either by the avidin-biotin-peroxidase method with rabbit anti-TH antiserum and sheep anti-NPY antiserum used separately, each diluted 1/5000, or by fluorescence procedures with combined antisera, each diluted 1/500. By using combined rhodamine labelled anti-rabbit IgG and FITC-labelled anti-sheep IgG and appropriate filters it was possible to study, simultaneously, co-localization of TH, NPY and Fast Blue.

TH-positive cells were distributed within the ventrolateral region (A1-C1) of the entire medulla and in the nucleus tractus solitarius - dorsal motor nucleus of the vagus (nTS-dmnX) (A2-C2). No cells were seen within the medial longitudinal fasciculus (C3). No TH-positive vagal preganglionic neurons were demonstrated, either in the dmnX or within and around the nucleus ambiguus. NPY-positive cells were observed in the A1 and C1 areas except at the most caudal and most rostral (A5) levels. TH and NPY were co-localized in the A1-C1 cells but some TH neurons were negative for NPY at every rostrocaudal level, especially in the more caudal regions. Rostral to the obex, many NPY cells were found within the dmnX itself. These neurons were TH-negative. A substantial proportion contained Fast Blue and and were therefore vagal preganglionic neurons. At this rostrocaudal level other NPY cells, scattered around the TS and medial to the dmnX, were TH-positive. Others, in the dorsal portion of the nTS, were TH-negative. Few NPY-positive neurons were observed in the nTS-dmnX caudal to the obex. Other NPY-positive, TH-negative cells were found in the caudal spinal trigeminal nucleus, in the ventral portion of the rostral medullary raphe nucleus and in the medial reticular formation, dorsal to the rostral portion of the inferior olive.

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27.10 DIFFERENTIAL PROJECTIONS OF NEUROPEPTIDE Y CONTAINING NEURONS IN THE LOCUS CERULEUS OF THE RAT. E.L. Gustafson and R.Y. Moore. Departments of Psychology, Neurology and Neurobiology & Behavior, SUNY @ Stony Brook, Stony Brook, New York, 11794.

The locus ceruleus (LC) is a dense concentration of noradrenergic neurons in the brainstem that has extensive projections to many areas of the central nervous system (CNS). Recent studies have demonstrated that a subpopulation of these neurons also contain neuropeptide Y (Everitt et al., '84). In addition, some areas of the CNS contain NPY-immunoreactive axonal plexuses which are similar to the noradrenergic innervation arising from the LC, while other areas which receive a noradrenergic projection from the LC are lacking in NPY-immunoreactive axons. The observations raise the possibility that the co-localization of NPY with noradrenaline in a subpopulation of LC neurons may provide a mechanism for functionally discrete projections from this nucleus. We have addressed this question by combining immunohistochemical analysis of NPY in the LC with retrograde transport of the dye fast blue from the cerebral cortex and cerebellum of the adult rat. Both of these areas receive a noradrenergic innervation from the LC and also contain NPY-immunoreactive axons which are similar to the noradrenergic innervation in both morphology and distribution.

Bilateral injections of fast blue into the cerebral cortex result in numerous retrogradely labeled perikarya in the LC. The majority of these cells are concentrated in the dorsal two-thirds of the LC, but scattered cells are also present in the ventral aspect of the nucleus. There is also a differential distribution of labeled cells through the rostrocaudal axis of the LC with the greatest concentration of neurons occurring in the posterior portion of the nucleus. After processing the same sections for immunohistochemical localization of NPY, many but not all, of the cells which were retrogradely labeled with fast blue also exhibit NPY-like immunoreactivity, particularly in the posterior and dorsal aspect of the LC. In contrast, following bilateral dye injections of the cerebellar cortex, retrogradely labeled neurons were present primarily in the ventral aspect of the LC with only scattered labeled cells in the dorsal LC. However, there does not appear to be any rostrocaudal gradient in their distribution. Processing of this tissue for immunohistochemistry demonstrated that only a small percentage of these neurons contain NPY-like immunoreactivity.

These data demonstrate that the extensive efferent projections of the LC are divided into discrete subfields through the co-localization of NPY with only a subpopulation of the noradrenergic neurons in this nucleus. This raises the possibility of differential functional output of the LC which is specified by the presence or absence of NPY in noradrenergic afferents. Supported by NIH grant N5-16304.

27.11 AMPHETAMINE-INDUCED CHANGES IN IMMUNOREACTIVE NEUROPEPTIDE Y IN BRAIN, PINEAL GLAND AND PLASMA. R.E. Tessel*, D.A. DiMaggio* and T.L. O'Donohue (SPON: C. Rutledge). Experimental Therapeutics Branch, Neuroendocrinology Unit, NINCDS, NIH, Bethesda, MD 20205.

Acute injection of d-amphetamine sulfate to rats (10 mg/kg) 60 min prior to sacrifice doubled neuropeptide Y-like immunoreactivity (NPY-LI) in the pineal gland but had no effect on gross or micro-dissected brain-regional or plasma NPY-LI concentrations. However, when amphetamine was injected twice daily for six days and once more 60 min prior to sacrifice, several additional changes were found. NPY-LI was decreased in micro-dissected regions of the caudate-putamen, and the paraventricular and dorsomedial nuclei of the hypothalamus, and increased in medial preoptic hypothalamic nucleus and in plasma; NPY-LI in other hypothalamic nuclei was unchanged. Pineal NPY-LI was also elevated. The data indicate that NPY-LI concentrations are susceptible to manipulation by amphetamine but that the extent and direction of change (increase or decrease) depends on the frequency of drug administration and the tissue being sampled. Based on the effects of amphetamine on central and peripheral norepinephrine and epinephrine disposition observed in other studies, the data also suggest that NPY-LI and catecholamine dispositions are not directly correlated and may be inversely related in some tissues.

27.12 COEXISTENCE OF HIGH-AFFINITY UPTAKE MECHANISMS FOR PUTATIVE NEUROTRANSMITTER MOLECULES IN CHICK EMBRYO RETINAL NEURONS IN PURIFIED CULTURE. M. Pessin* and R. Adler, (SPON: P. Hoffman). Wynn Center, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Autoradiographic detection of neuronal labelling with radioactive neurotransmitter molecules is frequently used as a criterion for the identification of different neuronal subpopulations in vitro. The usefulness of this parameter would be somewhat limited, however, if a same neuron could take up more than one putative neurotransmitter via high-affinity mechanisms. To investigate this question, purified neuronal cultures from chick embryo retina were incubated with radioactive GABA, aspartate (ASP) or taurine (TAU) either individually or in binary or tertiary combinations. Measurements of the cells radioactive content showed lack of inhibition when the cultures were exposed to combinations of these radioactive amino acids. The percentage of labelled cells in autoradiograms of cultures exposed simultaneously to two or more radioactive amino acids was substantially lower than the number that would be expected from the values observed in cultures labelled with each amino acid individually. The only possible interpretation for these observations is that there is coexistence of high-affinity uptake mechanisms for GABA, TAU and ASP in cultured retinal neurons. Numerical analysis of the autoradiographic data showed that approximately 20% of the neurons have only the high-affinity uptake mechanism for TAU whereas very few if any neurons have high-affinity uptake mechanisms for GABA alone or ASP alone. Our experiments have also identified populations of cells which take up GABA and TAU (but not ASP) or ASP and TAU (but not GABA). Interestingly, we have not seen any neurons which can take up ASP and GABA and can not take up TAU, whereas as many as 50% of the neurons can take up ASP, GABA and TAU simultaneously.

By showing concomitant uptake of different putative neurotransmitters within individual neurons, then, our results emphasize the limitations of transmitter uptake autoradiography as a criterion for neuronal identification in vitro.

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27.13 CEREBRAL CORTICAL NEURONS OF DEVELOPING AND ADULT RATS CONTAIN THE TACHYKININS, SUBSTANCE P AND SUBSTANCE K. J.R. Loesche, J.F. McGinty and J.-S. Hong (SPON: M.E. McNeill). Dept. of Anatomy, East Carolina Univ. Sch. Med., Greenville, NC 27834 and Lab Behav. Neurol. Toxicol. NIEHS/NIH Research Triangle Park, NC 27709.

Tachykinin peptides contain a common C-terminal amino acid sequence PHE^{8}-X-GLY-LEU-MET^{11}-NH_2. Recently, Nawa, et al., (Nature 306:32, 1983) have reported that alternative mRNA splicing generates two different preprotachykinin mRNAs in bovine striatum. Alpha preprotachykinin (PPT) contains a single Substance P (SP) sequence whereas beta-PPT contains one SP and one Substance K (SK) sequence. In the course of comparing regional CNS differences in SP and SK distributions, we discovered a wide-spread population of cortical neurons which contained SP and SK immunoreactivity (IR). SP and SK were coupled to thyroglobulin and injected into rabbits. The antigenic determinants of the resulting SP antiserum (SP1) appeared by radioimmunoassay to be between LYS^{3}-PHE^{7} with minimal cross-reactivity with other tachykinins. The SK antiserum (SK3) has less than 1% cross-reactivity with SP. Rats ranging from 2 days postnatal to young adults (150-200 g) were perfused with a 1% saturated picric acid/4% paraformaldehyde solution and brain tissue was prepared for immunocytochemistry as described (McGinty, et al., J. Neuroscience 4:1117, 1984). Adult rats were pretreated with 100-150 µg colchicine in 10-15 µl of saline intraventricularly. The distribution of both peptides was similar if not identical in subcortical and cortical neurons. In the cerebral cortex, SP-IR and SK-IR were barely detectable in neuronal cell bodies by postnatal day 2. The intensity of SP/SK-IR and number of cortical neurons stained increased until the fourth postnatal week when it became necessary to administer colchicine in order to visualize cortical neurons. In an attempt to enhance the intensity of SP immunostaining in cortical neurons of adult rats, a number of free-floating sections were incubated with affinity purified electric eel acetylcholinesterase (80 U/ml phosphate buffer) for 4 hrs prior to incubation with the primary antiserum (Millar and Chubb, Neuroscience 12:441, 1984). In medial frontal and perirhinal cortex, small polymorphic neuronal cell bodies containing SP/SK-IR were surrounded by a dense band of fibers in layers V-VI. A less densely packed band of fibers and fewer immunoreactive cell bodies were present in layers II-III of medial frontal cortex. In the remainder of the neocortex, cell bodies containing SP/SK-IR were present in layers II-III and V-VI. Within the hippocampal formation, SP/SK-IR cell bodies were present in the subiculum and throughout the CA fields and dentate gyrus, scattered in all layers with particular prominence in stratum oriens. AchE incubations markedly enhanced the SP-IR in cortical and hippocampal neurons allowing extensive visualization of neuronal processes and morphology. Radioimmunoassays of SP-IR and SK-IR are underway to determine the SP:SK ratios in discrete cortical regions.

Supported by NS 20451.

27.14 ACETYLCHOLINE AND PEPTIDE SCP(b) WHICH CO-OCCUR IN AN IDENTIFIED BUCCAL NEURON B11 OF TRITONIA, HAVE OPPOSITE EFFECTS UPON THE FEEDING MOTOR PROGRAM. A.O.Dennis Willows. Friday Harbor Laboratories, Univ. of Washington, Friday Harbor, WA 98250.

In prior experiments, we showed that a large re-identifiable pair of buccal ganglion neurons (B11) in the marine nudibranch mollusc Tritonia diomedia contain the neuroactive peptide SCP(b) and also the classical neurotransmitter acetylcholine (ACh) (P.E. Lloyd, B. Masinovsky, R.E. McCaman, and A.O.D. Willows. Soc. Neurosci. Abstract 209.6, 1981). Additionally, we have shown that intracellular electrical stimulation of B11 modulates the output of the motor program generator (MPG) for swallowing (Willows, A.O.D. and P.E. Lloyd. Soc. Neurosci. Abst. 115.11, 1983). In this work I report that the complex modulation of the MPG elicited by stimulation of B11 has two components. One (initiation of MPG bursting in previously inactive preparations and increased spiking rate during bursts) is mimiced by superfusion of the ganglia with SCP(b) and the other (increased interburst intervals), is mimiced by superfusion with acetylcholine.

In these experiments, B11 was stimulated and recorded in isolated buccal ganglia preparations continuously superfused with sea water to which test serially diluted solutions of SCP(b), and ACh could be added. Additionally, motor neurons B5 were recorded to monitor the output of the MPG. The characteristic background activity of the MPG in the unstimulated preparation was cyclic volleys of subthreshold psp's or bursts. Intracellular stimulation of B11's (5-7 Hz for 30 s) elicited three prominent changes in the output of the MPG all of which outlasted the period of stimulation by as much a 3 min.: (i) enhanced likelyhood of B5 firing in bursts, in preparations that were inactive, (ii) increased spike rate in the bursts, and (iii) decreased burst rate. In summary, B11 stimulation turns on the MPG, and both intensifies the bursts and reduces their frequency.

As reported earlier, exposure to SCP(b) alone in the perfusion solution enhances the bursting likelyhood, increases the spike rate within bursts, and increases the bursting rate.

In the present experiments I report that exposure to ACh causes the rate of bursting to be reduced in a dose dependent manner, and blocks it completely at about 5X10-3M. However, ACh does not markedly alter the firing rate during each burst. Furthermore, during ACh exposure the membrane of B5 is prominently depolarized, and the input resistance of the cell is reduced, indicating a membrane conductance increase.

These results are consistent with the suggestions that SCP(b) and ACh are released from B11 and that they may produce the combined enhancement and inhibition of the MPG respectively, as seen when B11 is electrically stimulated.

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27.15 **EFFECTS OF THE PENTAPEPTIDE, PROCTOLIN, ON RAT SPINAL MOTONEURON EXCITABILITY.** S.R. White (SPON: F.White). Dept. of VCAPP, Col. of Vet. Med., Washington State Univ., Pullman, WA 99164.

Proctolin, originally identified in proctodeal nerves of the cockroach, enhances contractions of a variety of arthropod neuromuscular preparations and modulates excitability of certain insect central nervous system neurons. Recently, proctolin-like immunoreactivity has been identified in cell bodies in the caudal medulla oblongata of rats and in terminal plexuses near cell bodies and dendrites of large motoneurons in rat spinal cord ventral horn. Much of the proctolin-like immunoreactivity appeared to be co-localized in somata and terminals with serotonin-like immunoreactivity. Serotonin is known to enhance spinal motor neuron excitabilitiy in rats, but nothing is known about the effects of proctolin on mammalian neuronal activity. The present study compared the effects of iontophoretically applied serotonin and proctolin on lumbar motoneuron excitability in urethane-anesthetized rats.

Seven barrel microdot glass micropipettes contained NaCl, 4.0 M, pH 7, for recording extracellular action potentials, for automatic current balance and for control current ejection; glutamate, 0.2 M, pH 7, for activating the silent motoneurons; 5-hydroxytryptamine bimaleate (5HT, serotonin), 0.16 M, pH 4; and proctolin, 0.002 M, pH 6, in 0.9% NaCl. Motoneurons were identified electrophysiologically and were driven at low stable firing rats by automatically cycled ejections of glutamate. 5HT, applied with low ejection currents (15-20 nA, 60 sec) consistently facilitated glutamate evoked motoneuron excitability without directly firing the motoneurons in the absence of glutamate. Proctolin (10-40 nA, 30-120 sec) had less consistent effects on motoneuron excitability than did 5HT. Although proctolin facilitated glutamate evoked excitation of 14/20 motoneurons tested, the facilitation was delayed as much as 90 sec after current offset in some cells and was not consistently repeatable within the same cell. Electrode barrels containing proctolin tended to block after several current applications which may account for the lack of repeatability of proctolin effects in some cells. Application of proctolin by micropressure may produce more consistent facilitation of motoneuron excitability than was found in the present study using microintophoretic application. However, the majority of cells in the present study responded to proctolin and serotonin application in a qualitatively similar manner. Both substances produced a delayed, long lasting facilitation of motoneuron excitability which was sometimes preceded by a brief period of inhibition during current application.

27.16 INTERACTION OF CHOLECYSTOKININ AND DOPAMINE IN THE NUCLEUS ACCUMBENS AND IN THE DORSAL HIPPOCAMPUS: MICROIONTOPHORETIC STUDIES IN THE RAT. G. de Bonnel* and C. de Montigny. Centre de recherche en sciences neurologiques and Institut Philippe Pinel, Université de Montréal, Montréal, Canada.

Cholecystokinin (CCK) and dopamine (DA) coexist in a subpopulation of neurons of the ventral tegmental areas (VTA) projecting to the nucleus accumbens and other limbic areas. DA neurons of the VTA projecting to the dorsal hippocampus do not contain CCK. The present experiments were carried out to study the interaction of CCK and DA in these two regions in naive rats and in rats in which DA neurons of the VTA had been destroyed.

Male Sprague-Dawley rats (200-400 g) were injected unilaterally in the VTA with 12 µg of 6-hydroxydopamine (6-OHDA) under chloral hydrate anesthesia (400 mg/kg, i.p.), one hour after a pretreatment with desipramine (25 mg/kg, i.p.) to protect the noradrenergic system. One to three weeks later, five-barrelled micropipettes were used for extracellular recordings of dorsal hippocampus pyramidal neurons and of neurons in the dorsomedial part of the nucleus accumbens, under urethane anesthesia (1.25 g/kg, i.p.). The following solutions were used for microiontophoresis: sulphated octopeptide CCK (1 µM or 10 µM in 200 mM NaCl; pH: 5), DA (100 mM; pH: 4), kainate (KA) 1 mM in 400 mM NaCl; pH: 8), glutamate.HCl (GLU) (20 mM in 20 mM NaCl; pH: 8). DA was assayed with HPLC from punches of the dorsomedial region of the nucleus accumbens.

The 6-OHDA injection produced an 80% decrease of DA content in the ipsilateral nucleus accumbens. In the latter nucleus, the excitatory effect of CCK was markedly increased. The inhibitory effect of DA on KA- and GLU-induced activations was also increased. Strikingly, however, when the neurons were activated with CCK, DA applications resulted in the same degree of inhibition in naive and 6-OHDA rats.

In the dorsal hippocampus ipsilateral to the 6-OHDA injection, the effect of CCK was unchanged in both the CA_1 and CA_3 regions. The effect of DA was markedly enhanced in the CA_1 region, whether the neurons were activated with CCK or KA. However, the effect of DA was not modified in the CA_3 region by the 6-OHDA injection.

The present finding of a supersensitivity to CCK in the nucleus accumbens but not in the hippocampus following a VTA lesion is consistent with the coexistence of CCK and DA in the projection to former but not to the latter. The fact that, in the accumbens, a supersensitivity to DA was present when the neurons were activated with GLU or KA but could not be detected when they were activated with CCK might be an indication of a postsynaptic interaction between the cotransmitters CCK and DA. The absence of supersensitivity to DA in CA_3 suggests that this hippocampal region does not receive a DA input from the VTA.

CHARACTERIZATION OF CHOLINERGIC RECEPTORS I

28.1 METHYLLYCACONITINE, A NATURALLY OCCURRING INSECTICIDE WITH A HIGH AFFINITY FOR THE INSECT CHOLINERGIC RECEPTOR. K. R. Jennings, D. G. Brown* and D. P. Wright, Jr.*, Agricultural Center, American Cyanamid Co., P. O. Box 400, Princeton, NJ 08540.

The seeds of Delphinium (Ranunculaceae) have long been know to possess insecticide properties. The earliest recorded reference to the use of the plant as an insecticide is given in the Naturalis Historia by Pliny, where it is described as a treatment for headlice control.

An experiment was conducted to evaluate the insecticidal properties of chloroform extracts of seeds from the Delphinium hybrid cv. "Pacific Giant", King Arthur. An oil prepared from these seeds was demonstrated to have insecticidal and/or antifeeding properties against a series of insects and mites including: Spodoptera eridania (Cramer), Empoasca abrupta Delong and Tetranychus urticae Koch.

Preparative TLC of the alkaloidal component gave six fractions. Fraction 3 was observed to be a very potent inhibitor of ^{3}H-propionyl-α-bungarotoxin binding to the nicotinic cholinergic receptor in a Musca domestica head homogenate preparation. This alkaloid was shown by mass spectrometry, ^{13}C and proton NMR spectroscopy to be methyllycaconitine (MLA). A pure sample of MLA.citrate was obtained from Dr. Benn of the University of Calgary. This material displayed insecticidal activity similar to that observed for the original seed extract.

In addition, the Kinh values obtained for the pure MLA.citrate were identical to those observed for the Delphinium oil TLC fraction 3: Kinh=0.25 ± 0.05 nM. This very potent inhibitor of the insect receptor may prove to be a useful neurobiological tool for studies of the comparative pharmacology of cholinergic receptors.

28.2 STRUCTURAL AND PHARMACOLOGICAL STUDIES ON THE CHOLINERGIC RECEPTOR FROM MANDUCA SEXTA. D.J. Prescott* and M.L. Perez* (SPON. E. Yadin). Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The brain (protocerebrum, subaesophageal ganglion, optic and antennal lobes) of the hawk moth, Manduca sexta is a rich source of acetylcholine receptor. The AchR is an integral membrane protein, since disruption of the membrane is required for maximal extraction. High concentrations of salt are also necessary for maximal extraction. Even in the presence of 1% Triton X 100 large aggregates of the receptor are detected in sucrose density gradients. The approximate sedimentation value of 9S for the toxin-receptor complex may represent the monomeric structure with larger aggregates forming during storage or conditions of low ionic strength and dilute detergent. Pharmacological studies have revealed that AchR exhibits largely nicotinic character. The following drugs were shown to inhibit formation of the radioiodinated alpha-bungarotoxin-receptor complex starting with the most effective: nicotine, gallamine, curare, atropine, carbachol, and decamethonium, while hexamethonium and muscarine had little effect at 10^{-3}M. The I_{50} values for these drugs fall in the range of 10^{-5}M to 10^{-3}M which indicates that this receptor has similar sensitivity to these agents compared to that of the Drosophila receptor except for nicotine. The Manduca receptor appears to be less sensitive to this agent by about one order of magnitude than that of Drosophila. This may represent a functional adaptation of the receptor since high concentrations of nicotine are present in hemolymph of the larval stages when the organism is consuming a diet of tobacco leaves. Since the antennal lobes of Manduca exhibit sexual dimorphism [Matsumoto, S.G. and J.G. Hildebrand Proc. R. Soc. Lond., B213, 249 (1981)], with the male lobe exhibiting an additional large macroglomerular area, we are quantitating the amount of receptor present in male vs. female lobes.

28.3 KAPPA-BUNGAROTOXIN: BINDING OF A NEURONAL NICOTINIC RECEPTOR PROBE TO CHICK OPTIC LOBE AND SKELETAL MUSCLE. V.A. Chiappinelli, K. Wolf* and A. Ciarleglio*. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Kappa-Bungarotoxin (KBgT) is a snake venom neurotoxin isolated from the venom of Bungarus multicinctus (Chiappinelli, Brain Res. 277:9, 1983) that blocks nicotinic transmission in a variety of autonomic ganglia (Chiappinelli and Dryer, Neurosci. Letts. 50: 239, 1984). This property distinguishes the toxin from alpha-bungarotoxin (ABgT), which is ineffective in blocking transmission in avian and murine autonomic ganglia.

The complete amino acid sequence of KBgT has recently been obtained (Grant and Chiappinelli, Biochemistry 24:1532, 1985). While the toxin demonstrates considerable homology (47%) with the long postsynaptic neurotoxins (a family of polypeptides to which ABgT also belongs), several structural features of the toxin are unique. Of particular interest is the shortened COOH-terminal tail, which KBgT shares with only one other postsynaptic toxin, namely L.s. III. In addition, KBgT is the only postsynaptic neurotoxin which exists entirely in dimeric form in physiological buffers (Chiappinelli and Lee, J. Biol. Chem. 1985, in press).

To examine which of the structural features of KBgT most likely account for its unusual physiological effects, a series of binding experiments was carried out in skeletal muscle and optic lobe homogenates from chick embryos.

Skeletal muscle nicotinic sites. A single nicotinic site was detected in skeletal muscle by all three neurotoxins tested. The IC_{50} values for inhibition of ^{125}I-ABgT binding were as follows: ABgT=0.56 nM; L.s. III=20.3 nM; KBgT=127 nM. Hill plots of the competition data yielded slopes approximating unity for all three toxins.

Optic lobe nicotinic sites. Both ABgT and L.s. III bound with similar affinity (Kd=1.8 nM) to a high concentration (14.4 fm/mg tissue) of nicotinic sites in optic lobe. In contrast, KBgT bound to only a portion of these sites with high affinity (3.36 fm/mg tissue; Kd=1.3 nM). At higher doses, KBgT bound to the remaining sites detected by the other two neurotoxins. The Hill plot of the KBgT data revealed a slope of 0.51, indicating the presence of either negative cooperativity or multiple sites with differing affinities. Evidence obtained thus far favors the hypothesis that KBgT distinguishes two different classes of neuronal nicotinic sites. It remains to be shown which of these neuronal nicotinic sites is involved in nicotinic transmission in the optic lobe.

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28.4 LOCATION OF A POLYPEPTIDE SEQUENCE WITHIN THE α-SUBUNIT OF THE ACETYLCHOLINE RECEPTOR CONTAINING THE CHOLINERGIC BINDING SITE. N.D. Boyd,* B. Oblas,* and R.H. Singer* (SPON: J. Walsh) Dept. of Physiol., Univ. Mass. Med. Sch., Worcester, MA 01605

The identification of the location of the acetycholine (AcCh) binding site on the primary sequence of the α-subunit of the nicotinic AcCh receptor is important information for understanding the molecular basis of AcCh receptor activation. We have previously addressed this question by identifying through the use of a nitrocellulose protein transfer binding assay, various proteolytic fragments of the α-subunit of the AcChR from Torpedo electric organ that contain the α-bungarotoxin (α-BgTx) binding site. Here we report further studies in which a toxin binding fragment of the α-subunit (M_r=17K), was obtained by V_8 protease digestion, isolated by preparative SDS-PAGE and chemically characterized. The amino acid sequence at the amino-terminus of this 17K polypeptide fragment is Val-Asn-Gln-Ile-Val-Glu which is identical to a unique sequence on the α-subunit beginning at Val_{46}. The 17K polypeptide fragment was shown to contain carbohydrate and thus Asn_{141}, the only site of N-glycosylation on the α-subunit. The 17K polypeptide fragment was not labelled by 4-(N-maleimido) benzyl-tri[^{3}H]methylammonium iodide and thus does not contain $Cys_{191}$the site of attachment of this cholinergic affinity ligand. Based on considerations of the apparent molecular weight of the fragment, the enzyme specificity of V_8 protease, computer analysis of the partial amino acid composition of the 17K polypeptide fragment, together with the above identifying landmarks, the most likely locations of the carboxy-terminus are Glu_{161}, Glu_{172} or Glu_{175}. Although the carboxy-terminus is thus 15-30 amino acids residues distant on the primary sequence of the α-subunit from Cys_{191} which has been proposed to be part of a reducible disulphide bond that is within 1nm of the AcCh binding site, both non-radiolabelled αBgTx and d-tubocurarine were able to displace ^{125}I-αBgTx binding to the 17K polypeptide fragment with potencies of IC_{50}=100nM and 600μM respectively. These values are not significantly different from those obtained in similar experiments using the intact α-subunit indicating that the 17K polypeptide sequence contains all the essential structural elements present in the undigested α-subunit that are necessary for the binding of both cholinergic ligands.

The 17K polypeptide sequence is located in the middle of a large extracellular hydrophilic domain of the α-subunit and is predicted to have a highly ordered structure which may account in part for the existence within the cholinergic binding region of sufficient secondary and tertiary structure to bind α-BgTx and d-tubocurarine despite prior exposure to high concentrations of the denaturing detergent SDS.

28.5 AMINO ACID SEQUENCE OF A NEUROTOXIN THAT BLOCKS NEURONAL NICOTINIC RECEPTORS AND THE LOCALIZATION OF ITS BINDING SITES IN CHICK CILIARY GANGLION. R.H. Loring and R.E. Zigmond. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

The amino acid sequence has been determined for toxin F, a postsynaptically acting neurotoxin purified from the venom of Bungarus multicinctus (Loring et al., Neuroscience 11:989, 1984). Toxin F blocks nicotinic transmission in the chick ciliary ganglion and in the rat superior cervical ganglion (Loring et al., Neurosci. Abst. 9:1143, 1983). α-Bungarotoxin, a toxin derived from the same snake venom and well known for its ability to block nicotinic transmission at the neuromuscular junction, has no effect on transmission in these ganglia. When compared to previously sequenced snake toxins, toxin F has greatest sequence homology with the "long" postsynaptic neurotoxins although it has a number of unusual features. For example, it contains the following unusual sequence starting at position 22:

F L K A Q C D K F C S I R G P V I E Q G.

In other postsynaptic neurotoxins, positions 22-41 are considered to contain most of the functional residues involved in binding to the receptor. The six underlined residues are unusual substitutions including a Phe/Tyr substitution at position 22 and a Gln/Trp substitution at position 26. Tyr-22 and Trp-26 are usually invariant in postsynaptic neurotoxins.

To determine the localization of toxin F binding in the chick ciliary ganglion, ganglia were incubated at 37°C in 10 nM ^{125}I-toxin F for 45 min. Previous work in our laboratory has demonstrated that toxin F binds to two sites in this ganglion, one to which α-bungarotoxin also binds and an additional site unique to toxin F. Therefore, 1 μM α-bungarotoxin was present to prevent ^{125}I-toxin F binding to the α-bungarotoxin site. Control ganglia were incubated in ^{125}I-toxin F in the presence of 1 μM unlabeled toxin F or 100 μM dihydro-β-erythroidine (DHBE), a nicotinic antagonist. The ganglia were fixed in aldehydes, counted in a gamma counter, and then sectioned for electron microscopic autoradiography. Of 307 autoradiographic grains observed within 0.25 μm of neuronal plasma membranes, 117 (38%) were within 0.25 μm of synaptic profiles. In the presence of unlabeled toxin F or DHBE, few grains were observed near plasma membranes of ciliary neurons. A previous study in our laboratory demonstrated that less than 3% of the autoradiographic grains observed from the localization of ^{125}I-α-bungarotoxin binding to ciliary neurons were found within 0.25 μm of synaptic profiles (Neuroscience 14:645, 1985). Together, these results demonstrate that the α-bungarotoxin binding site and the unique toxin F binding site are distributed differently over the surface of ciliary neurons and suggest that toxin F is a useful probe for localizing neuronal nicotinic receptors. Supported by USPHS grants NS12651 and MH00162.

28.6 MONOCLONAL ANTIBODIES THAT DISTINGUISH BETWEEN THE TWO α-BUNGAROTOXIN-BINDING SITES OF TORPEDO-ACETYLCHOLINE RECEPTOR. A.J.Dowding* and Z.W.Hall. Dept. Physiol., Univ. Calif., San Francisco, CA 94143.

Antigenic differences between the two cholinergic ligand-binding sites of acetylcholine receptor (AChR) from Torpedo marmorata have been demonstrated using Mabs (e.g. Watters,D.and Maelicke,A.,Biochem.22:1811-1819, 1983). However, the molecular causes for these antigenic differences remain to be elucidated. The most likely reasons for these differences are either that the posttranslational modifications are not identical for the two α-subunits or that their antigenicities may be modulated by their neighboring δ- and β- or β- and γ-subunits. The positions of the ligand-binding sites within AChR dimers may also influence their antigenicities.

In order to obtain probes with which to investigate these possibilities we have generated a library of hybridomas that secrete Mabs against detergent solubilized Torpedo-AChR, which compete with α-bungarotoxin (α-BTX). Female C57 Bl mice were immunized with α-BTX-AChR complex and then treated with cycloposphamide with the intention of suppressing the immune response against various regions of AChR other than the masked toxin-binding site. Three weeks later, the mice were immunized with AChR alone and finally they were boosted with AChR in saline on three consecutive days prior to fusion with SP2/0 myeloma cells.This method was based on that of W.D. Matthews and P.H. Patterson (Cold Spring Harb. Symp. Quant. Biol. 48, 625-631). In the best two fusions using this protocol, about 20-25% of the hybridomas against AChR secreted Mabs which were found to be blocked by α-BTX and which also inhibited α-BTX-binding to AChR.

When tested against detergent solubilized AChR, two of the Mabs blocked 100% of toxin-binding, five caused 50% inhibition and four caused about 20% inhibition. The Mabs which cause partial inhibition of α-BTX-binding fall into two mutually exclusive classes as shown both by competition between Mabs in direct binding experiments and by assays to detect additive or nonadditive inhibition of α-BTX-binding by various pairs of Mabs. Because both classes of Mab bind to the same AChR molecules, our results suggest that each class of Mab is specific for one of the two toxin-binding sites in the AChR pentamer. With the exception of one Mab (which blocks 50% of α-BTX-binding to solubilized AChR) all of these Mabs bind to Torpedo-AChR in membranes. All of the Mabs which react with membrane-bound AChR, are partially or fully inhibited from binding to detergent solubilized AChR by various cholinergic agonists or antagonists. Further characterization of these and other toxin-blocking Mabs is in progress.

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28.7 IDENTIFICATION OF THE Mr-43,000 N_3ATP-BINDING PROTEIN IN TORPEDO POST-SYNAPTIC MEMBRANES. K. Miles, S. Froehner, and R. Huganir. Lab. Mol. & Cell. Neurosci., The Rockefeller Univ., N.Y., N.Y. 10021. Dept. Biochem., Dartmouth Med. School, Hanover, N.H. 03755

We recently demonstrated the existence of an endogenous tyrosine-specific protein kinase in postsynaptic membranes enriched in the nicotinic acetylcholine receptor (AcChR) isolated from the electric organ of Torpedo californica. The tyrosine-specific protein kinase phosphorylated the β, Y, and δ subunits of the Torpedo AcChR. In an effort to identify this protein kinase, postsynaptic membrane fractions enriched in AcChR were incubated with [Y-^{32}P] 8-azido ATP (N_3ATP) under optimal conditions for tyrosine protein kinase activity. N_3ATP, when photo-activated by UV light, binds covalently to ATP-binding sites. In agreement with previous reports by Gordon et.al. (PNAS 79:3666,1982), a Mr-43,000 protein was specifically labeled in UV irradiated samples. However, it is well established that there are at least three proteins migrating in Mr-43,000 in Torpedo membrane fractions. These are ν_1 (the 43K protein associated with the AcChR and located on the cytoplasmic surface of the postsynaptic membrane), ν_2 (creatine kinase) and ν_3 (actin). We have determined that N_3ATP does not covalently label the 43K (ν_1) protein but appears to react with a protein with the characteristics of creatine kinase (ν_2). By using monoclonal antibodies against the 43K protein and rabbit antisera against creatine kinase to detect these two proteins on immunoblots, an inverse distribution of 43K and creatine kinase could be seen between cytoplasmic and AcChR-enriched membrane fractions. While creatine kinase was enriched in the cytoplasmic fraction, the 43K protein was found in highest concentrations in the membrane fractions. The distribution of the N_3ATP binding protein corresponded with that of creatine kinase and not the 43K protein. In addition, the Mr-43,000 proteins of Torpedo postsynaptic membranes may be resolved by 2-D electrophoresis. Following N_3ATP labeling and 2-D electrophoresis, only one protein in the Mr-43,000 range was found to be labeled with N_3ATP. Immunoblot experiments using anti 43K monoclonal antibodies or antisera against creatine kinase established that the N_3ATP binding protein migrated with an isoelectric point similar to that reported for the BB isozyme of creatine kinase. Finally, purified commercially available rabbit BB and MM forms of creatine kinase were labeled with N_3ATP and electrophoresed in parallel with N_3ATP labeled proteins in Torpedo AcChR-enriched membranes. The N_3ATP labeled protein in AcChR-enriched membranes migrated with isoelectric point and Mr similar to that of the N_3ATP labelled BB form of rabbit creatine kinase. Taken together, these findings suggest that N_3ATP-binding activity in the Mr-43,000 range of Torpedo membrane fractions may be attributed to creatine kinase and not to the 43K protein.

28.8 CHARACTERIZATION OF THE TYROSINE-SPECIFIC PROTEIN KINASE WHICH PHOSPHORYLATES THE NICOTINIC ACETYLCHOLINE RECEPTOR R. L. Huganir, A. Hirano and K. Miles. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

We have recently reported the presence of a tyrosine-specific protein kinase activity in postsynaptic membranes enriched in the nicotinic acetylcholine receptor isolated from the electric organs of Torpedo californica (Huganir et al., PNAS 81:6968-6972, 1984). This tyrosine kinase(s) phosphorylated the nicotinic acetylcholine receptor on tyrosine on the β, Y and δ subunits. The tyrosine kinase(s) also phosphorylates tyrosine on exogenous substrates such as the tyrosine containing synthetic peptides [Val^5]-angiotensin II and poly (Glu^{80}, Tyr^{20}). Using these synthetic peptides as substrates we have started to characterize the acetylcholine receptor tyrosine-specific protein kinase(s). The tyrosine kinase(s) is a membrane associated enzyme which has the characteristics of an integral membrane protein. Alkaline treatment of the postsynaptic membrane which removes peripheral membrane proteins such as the 43K protein does not extract the tyrosine-specific protein kinase activity. The tyrosine kinase activity however is easily extracted from the membrane by nonionic detergents such as NP-40 and Triton X-100. The nicotinic acetylcholine receptor is not itself the tyrosine kinase since the tyrosine-specific protein kinase activity is separated from the receptor after affinity chromatography of detergent extracts of the postsynaptic membrane on an acetylcholine affinity column. The tyrosine-specific protein kinase(s) has a relatively high Km for ATP of ~ 50 μm and has a Km of ~1.0 mM for [Val^5]-angiotensin and a Km of ~ 150 μgm/ml for poly (Glu^{80}, Tyr^{20}). The V_{max} of the tyrosine-specific protein kinase is approximately 50-100 pmoles/min/mg using [Val^5] angiotensin II or poly (Glu^{80}, Tyr^{20}) as substrates. We are currently attempting to purify the tyrosine-specific protein kinase(s) to determine if the kinase is a unique type of tyrosine kinase which is specific for the nicotinic acetylcholine receptor. The possible role of tyrosine phosphorylation in the regulation of the function of the nicotinic acetylcholine receptor will be discussed.

28.9 SPECIFIC ANTIBODIES TO THE cAMP-DEPENDENT PHOSPHORYLATION SITES IN THE ACETYLCHOLINE RECEPTOR. S. Fuchs, M.C. Souroujon*, D. Neumann* and S. Pizzighella*. Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Phosphorylation of the acetylcholine receptor (AChR) was suggested to be involved in the stabilization of the receptor in the neuromuscular junction. Based on substrate requirements, the putative phosphorylation sites for a cAMP-dependent protein kinase, a tyrosine specific kinase and a calcium phospholipid dependent kinase have been proposed (Huganir et al., PNAS, 81:6968, 1984). We have synthesized a tridecapeptide corresponding to residues 354-366 in the δ subunit of Torpedo AChR and containing the sequence Arg-Arg-Ser-Ser. This sequence has been proposed as the substrate for phosphorylation by an endogenous cAMP-dependent kinase. We have shown that the synthetic peptide is phosphorylated by the catalytic subunit of bovine heart cAMP-dependent kinase, whereas other serine or threonine-containing peptides are not phosphorylated under the same conditions.

Antibodies elicited against peptide 354-366 were shown to cross react with native AChR and to bind specifically to the γ and δ subunits in immunoblotting experiments. This indicates that the phosphorylation sites in the γ and δ subunits are highly cross reactive, and is in agreement with the demonstration that an endogenous cAMP-dependent kinase phosphorylates these two subunits, probably on homologous sequences. Tryptic digestion of the δ subunit isolated from phosphorylated AChR yields a single 25 Kd phosphorylated fragment. Immunoblotting experiments demonstrate that the antibodies against peptide 354-366 bind to this same phosphorylated fragment. Preliminary experiments suggest that the anti-peptide antibodies interfere specifically with the cAMP-dependent phosphorylation of the γ and δ subunits of AChR. Such antibodies are being employed in order to assess the role of AChR phosphorylation in synaptogenesis.

Supported by grants from the Muscular Dystrophy Association of America, The Los Angeles Chapter of the Myasthenia Gravis Foundation and the United States-Israel Binational Science Foundation (BSF).

28.10 [^{3}H]ACETYLCHOLINE AND [^{3}H](-)NICOTINE LABEL THE SAME NICOTINIC CHOLINERGIC BINDING SITE IN BRAIN. A.M. Martino*, V. Hamui* and K.J. Kellar (SPON: F.G. Standaert) Department of Pharmacology, Georgetown University School of Medicine, Washington, D.C. 20007.

[^{3}H]Acetylcholine ([^{3}H]ACh) and [^{3}H]nicotine ([^{3}H]N) bind to sites in brain that have characteristics of nicotinic cholinergic receptors. These sites appear to be different from the sites in brain to which alpha-bungarotoxin binds. Autoradiographic analyses indicate that the distributions of [^{3}H]ACh and [^{3}H]N binding sites in brain are virtually identical (Clarke et al., J. Neurosci, 1985). Nevertheless, certain differences between the binding of [^{3}H]ACh and [^{3}H]N to homogenate preparations have raised questions of whether these two ligands bind to the same site. For example, the K_d of [^{3}H]ACh is approximately 10 nM, but the IC_{50} value of acetylcholine in competing for [^{3}H]N binding sites was found to be greater than 200 nM in some studies. Similarly, the K_i of carbachol for [^{3}H]ACh binding sites is approximately 13 nM, but its apparent affinity for [^{3}H]N binding sites was found to be 500-2000 nM. In addition, the reported distribution of the [^{3}H]N binding sites in homogenates appears to be different from that of [^{3}H]ACh binding sites.

These apparent differences between [^{3}H]ACh and [^{3}H]N binding characteristics could be due to differences in incubation conditions, such as temperature and duration of incubation, as well as to the use of racemic [^{3}H]N, which binds to two sites in brain. We have compared the binding of [^{3}H]ACh and [^{3}H](-)nicotine ([^{3}H](-)N) in rat brain homogenates incubated in parallel under the same conditions: 2°C, 45 min, presence of 100 uM DFP to inhibit cholinesterases and 1.5 uM atropine to occupy muscarinic receptors. Nonspecific binding was defined in the presence of 100 uM carbachol. In cerebral cortex under these conditions, both [^{3}H](-)N and [^{3}H]ACh bind to a single class of sites (n_H = 1) with a density (B_{max}) of 3-4 fmol/mg tissue. The K_d of [^{3}H](-)N is 4 nM and that of [^{3}H]ACh is 10 nM. In competition experiments, the K_i of acetylcholine for the [^{3}H](-)N binding site is 12 nM, and the n_H = 1, while the K_i of (-)nicotine for the [^{3}H]ACh binding site is 6 nM and the n_H = 1. The K_i of cytisin, a nicotinic agonist, is approximately 1 nM in competition against either [^{3}H](-)N or [^{3}H]ACh. Regional distribution studies of [^{3}H](-)N and [^{3}H]ACh binding sites indicate that the density of sites for both ligands is high in the thalamus, cerebral cortex and striatum and low in the hippocampus. Taken together, these data strongly suggest that [^{3}H](-)N and [^{3}H]ACh label the same nicotinic cholinergic site under these conditions. Furthermore, in rats treated with nicotine (1 mg/kg twice daily for 10 days), both [^{3}H](-)N binding sites and [^{3}H]ACh binding sites are increased by approximately 30%.

28.11 PYRIDOSTIGMINE AFFECTS THE RECEPTORS ON GUINEA PIG ILEUM MUSCLES IN A COMPLEX MANNER. P.L. Donaldson, B. O'Neill*, M.E. Wettig*, and K.J. Gall*. Neurotoxicology Branch, US Army Med Rsch Inst of Cml Def, Aberdeen Proving Ground, MD 21010-5425.

Pyridostigmine (PYR), a reversible inhibitor of acetylcholinesterase (AChE), has been shown to be an agonist to nicotinic cholinergic receptors in skeletal muscle, but PYR's effects on muscarinic acetylcholine receptors (mAChR) are not well known. To examine these effects, PYR was assayed on the isolated guinea pig ileum. One inch pieces of ileum were placed in tissue baths containing warm (34-37°C) Tyrodes solution with Hexamethonium Bromide 0.1 mM and Diphenhydramine 1 uM (pH=7.6). Dose-response curves were obtained for acetylcholine (ACh) and carbachol (CARB). ACh (1-5 nM) initiated a small contraction and a peak response at 0.5-1 uM. Concentrations of ACh greater than 1 uM caused a gradual decline in the contractile amplitude. In contrast, CARB initiated contractions at 5-10 nM, a peak contraction at 1-10 uM, and a decline in contractile amplitude at concentrations above that. Muscles exposed to pyridostigmine without any previous exposure to an agonist or antagonist did not respond until the concentration was >= 1 mM. At 1-5 mM, pyridostigmine elicited a very dramatic relaxation in the muscle tone that persisted for the duration of the exposure. At these doses, the carbamate also abolished all spontaneous contractions in the muscle. When the muscle was washed with normal Tyrodes, the muscle tone recovered rapidly, while the spontaneous contractions returned at a much slower rate. When the muscle was first exposed to an agonist, used as a "primer," the responses were much more variable. After CARB, the muscle usually reacted much as it did to pyridostigmine without a primer. Its tone relaxed and the spontaneous contractions were always abolished at high concentrations of pyridostigmine. Occasionally, there was a slight increase in tone exhibited at concentrations of 1-2 uM. In contrast, after ACh, pyridostigmine exhibited a biphasic response; at concentrations of 1-100 uM the muscle contracted, while at the usual high concentrations (1-5 mM) the muscle relaxed. Pyridostigmine after DFP, an essentially irreversible inhibitor of AChE, did not have a much greater response, suggesting that the effects it had on the muscarinic receptor were independent of its affinity for AChE. These data suggest that pyridostigmine may have some direct effects on the muscarinic ACh receptor of smooth muscle, but just how it is affecting the muscle to cause both contractions and a decrease in muscle tone at different concentrations is unresolved.

28.12 CHARACTERISTICS OF ACETYLYCHOLINE RECEPTOR-IONIC CHANNELS (ACHR) ACTIVATED BY THE SECONDARY AMINE (+) ANATOXIN. K.L. Swanson[1], C.N. Allen[1], R.S. Aronstam[2], and E.X. Albuquerque[1]. [1]Dept. Pharmacol. & Exper. Therap., Univ. Maryland Sch. Med., Baltimore, MD 21201, [2]Dept. Pharmacol & Toxicol. Med Col. Georgia, Augusta GA 30912.

(±)-Anatoxin-a (AnTX) is a semi-rigid nicotinic agonist which is more potent than acetylcholine (ACh) in inducing contractures of frog rectus abdominus muscle. Further, (+)-AnTX is a more potent agonist than ACh or its enantiomer (-)-AnTX. However, the decay of end-plate currents were the same for carbamylcholine and (±)-AnTX and the ionic channel conductance measured by fluctuation analysis was less for (±)-AnTX than carbamylcholine (Spivak et al., Mol Pharmacol 18: 384, 1980). We therefore used receptor binding and patch clamp techniques to investigate the mechanisms mediating the potency of (+)- and (-)-AnTX.

The AChR of frog interosseal muscles was studied using cell-attached patch clamp techniques. The ionic channels were activated at 20 and 200 nM (+)-AnTX and occurred in bursts of openings. Very high concentrations of (-)-AnTX were required to activated channels. The conductances of channels activated by ACh (27 pS) and (+)-AnTX (28 pS) were similar. The mean duration of individual openings was shorter with (+)-AnTX than with ACh. Short closures, which interrupted the bursts, failed to demonstrate the kinetics common to most ionic channel blockers: 1) the duration of short closures (τ = 0.3-0.5 msec) and the number of openings per burst were voltage independent and 2) the frequency of closures was concentration independent (20-200 nM). The toxin-bound receptor entered a closed channel conformation from which reactivation was voltage independent. Hypotheses describing the possible significance of the short closures will be discussed. Because the unique kinetics may be related to the binding of a secondary amine (pK_a = 9.36) to the receptor, the characteristics of channels activated by other secondary and tertiary amine agonists including arecoline, arecolone and nicotine have also been explored.

The AChR from Torpedo californica had higher affinity for (+)-AnTX (K_d = 80 nM) than (-)-AnTX (K_d = 4000 nM) and (+)-AnTX also had higher affinity than ACh (K_d = 300 nM). The rate of desensitization was slower with AnTX (50% maximal effect at 3 min) than with ACh (50% maximal effect at 1.2 min). These binding data suggest that (+)-AnTX is a more potent agonist than ACh due to its greater affinity for the receptor and its lower rate of desensitization. (Supported by U.S. Army Med. Res. & Devel. Command Contract DAMD 17-84-C-4219.)

28.13 EFFECTS OF SUBSTANCE P ON CARBAMYLCHOLINE-INDUCED DESENSITIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE NEURONAL CELL LINE PC12. G. A. Weiland and S. M. Simasko. Department of Pharmacology, NYSCVM, Cornell University, Ithaca, NY 14853.

Substance P (SP) has been shown in a number of systems to modulate nicotinic acetylcholine receptor function by noncompetitive inhibition of receptor activation. In the neuronal cell line PC12 this appears to reflect an enhancement of desensitization. To further investigate this mechanism we examined the effect of SP on desensitization in PC12 cells. Exposure of the cells to carbamylcholine (CARB) caused a decrease in the subsequent CARB-induced rate of $^{22}Na^+$ flux. The rate of onset of CARB-induced desensitization appeared to be increased by SP. However, SP by itself also appeared to induce desensitization. The steady state level of desensitization induced by CARB was concentration dependent (EC50 = 32 μM) and at maximum steady state desensitization 15% of the control flux rate remained. SP increased the apparent potency of CARB; 1 μM SP decreased the steady state EC50 16 μM. However the peptide had no effect on the maximum level of desensitization. Examination of the time course of recovery following 15 min exposure to CARB revealed two components of desensitization. The rate of the first component of recovery (k = 0.5/min) was independent of the inducing concentration of CARB and was not affected by the presence of SP during desensitization. This recovery from CARB-induced desensitization was slower than recovery from desensitization induced by SP alone (k = 0.8/min) or by the less potent analog 7-11SP (k = 1.2/min). For concentrations of CARB greater than 0.1 mM, recovery was not complete and flux rates plateaued below control values. We have termed this nonrecoverable component inactivation. The extent of inactivation was dependent on the concentration of CARB (20% at 0.1 mM, 40% at 1 mM). This inactivation appeared to be mediated specifically by the nicotinic receptor since it was blocked by d-tubocurarine, could not be produced by depolarization of the cells with high K^+, and was not accompanied by increased LDH in the medium. SP alone did not appear to induce an inactivation as seen with CARB, but the peptide did increase the amount of inactivation produced by CARB (from 20% with 0.1 mM CARB alone to 40% with 0.1 mM CARB plus 1 μM SP). It thus appears that there are two components to desensitization in PC12 cells. SP can modulate nicotinic receptor function by affecting both processes, adjusting receptor responsiveness in the minute time range by increasing the rate of desensitization and adjusting receptor responsiveness over longer periods by increasing the extent of inactivation. (Supported by NSF BNS 82-15572)

28.14 ACETYLCHOLINE RECEPTOR SYNTHESIS IN RETINA & TRANSPORT TO OPTIC TECTUM IN GOLDFISH. J. Henley*, J. Lindstrom and R.E. Oswald. Department of Pharmacology, N.Y.S. College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA, and The Salk Institute for Biological Studies, San Diego, CA 92138 USA.

Previous studies have suggested that the retinotectal system of the goldfish contains a nicotinic acetylcholine receptor which is sensitive to α-bungarotoxin (Oswald & Freeman, Neurosci., 6 (1981) 1-14). Extracellularly recorded field potentials elicited in response to visual stimulation can be blocked by α-bungarotoxin, and α-bungarotoxin can interfere with the maintenance of retinotectal synaptic connections. The question remains as to whether the transmission between the retinal ganglion cells and the tectal cells is mediated by acetylcholine and whether acetylcholine receptors exist on the dendrites of tectal cells. These experiments were designed to determine the site of synthesis of the acetylcholine receptors associated with the goldfish retinotectal projection. We have found that a subset of monoclonal antibodies raised against Electrophorus electricus and Torpedo california electroplaque acetylcholine receptor are capable of interacting with α-bungarotoxin binding protein in goldfish retina and tectum. Immunohistochemistry with a fluorescent second antibody demonstrated that the antibody binding colocalizes with α-bungarotoxin binding in the goldfish optic tectum. To determine the site of synthesis of these receptors, [^{35}S]methionine was injected into one eye, and the incorporation of radioactivity into acetylcholine receptors was measured by immunoprecipitation using a monoclonal antibody. Immunoprecipitation could be inhibited by preincubation of detergent solubilized retinal or tectal membranes with Sepharose 4B coupled to α-bungarotoxin or by inclusion of affinity chromatography-purified acetylcholine receptor from Torpedo nobiliana electroplaque in the immunoprecipitation cocktail. Radioactivity was found to be incorporated into the acetylcholine receptor in the contralateral optic tectum 6 hours after injection into the eye, and the radioactivity incorporated peaked at 12 hours after injection. Furthermore, the incorporation of radioactivity into the acetylcholine receptor in the contralateral tectum could be totally blocked by crushing the optic nerve immediately before the injection. These results suggest that the nicotinic acetylcholine receptor associated with the goldfish retinotectal projection is synthesized in the retina and transported to the optic tectum.

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29.1 FUNCTIONAL AND PHYSICAL STUDIES OF GUANINE NUCLEOTIDE REGULATORY PROTEINS IN MUSCARINIC RECEPTOR MEDIATED PHYSIOLOGICAL RESPONSES IN THE EMBRYONIC CHICK HEART. J.M. Martin*, E.M. Subers*, S.W. Halvorsen*, and N.M. Nathanson. Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Activation of muscarinic receptors (mAChRs) by agonists in chick atria but not ventricles causes an increase in K^+ permeability. Because of suggestions that this may be due to differences in the guanine nucleotide regulatory protein associated with the receptor, we compared the properties of these proteins in atria and ventricles from 8-day chick embryos. The affinity of agonists for mAChRs in either the absence or presence of guanine nucleotides was the same in membranes from atria and ventricles; similar concentrations of Gpp(NH)p were required to regulate agonist binding in both tissues. Forskolin-stimulated adenylate cyclase activity in atria and ventricles was equally sensitive to inhibition by Gpp(NH)p. Inhibition of basal adenylate cyclase activity by the muscarinic agonist carbachol was similar in atria and ventricles. After covalent modification by Islet Activating Protein (IAP), two labelled polypeptides were detected with molecular weights of 39 KD and 42 KD, corresponding to N_o and N_i, respectively. Identical amounts of both proteins were found in atrial and ventricular membranes. Isoelectric points of atrial and ventricular forms of the IAP labelled substrates were identical. Peptide mapping demonstrated that while the 39 KD and 42 KD proteins had non-identical peptide maps, atrial and ventricular peptide maps were identical. Thus, the regulation of K^+ permeability by mAChRs in atria but not ventricles does not appear to be due to a qualitative or quantitative alteration in N_i or N_o, since these two proteins appear to be functionally and physically identical in atria and ventricles.

To investigate if N_o/N_i are involved in mAChR-mediated increases in K^+ permeability, the effect of IAP treatment on mAChR stimulation of $^{86}Rb^+$ efflux from cultured cardiac cells was determined. Treatment of cultures overnight with 5 ng/ml IAP caused complete covalent modification of N_o and N_i and blocked the stimulation of $^{86}Rb^+$ efflux evoked by mAChR agonists. Because previous work has demonstrated that changes in cAMP levels do not mediate mAChR regulation of K^+ permeability, these results suggest that N_o and/or N_i may directly couple the mAChR to the K^+ channel.

29.2 *GUANINE NUCLEOTIDE MODULATION OF MUSCARINIC RECEPTOR BINDING IN PC12 CELLS: EFFECTS OF NERVE GROWTH FACTOR AND ORGANOPHOSPHATES.* *L.H. Davis* and F.C. Kauffman. Dept. Pharmacology & Exp. Therap., University of Maryland School of Medicine, Baltimore, MD 21201.*

Nerve growth factor (NGF)-enhancement of muscarinic receptor binding by rat pheochromocytoma cells (PC12) is inhibited if the cells are exposed to an organophosphate within 30 min of adding NGF. Soman, pinacolyl methylphosphonofluoridate, was approximately 10-fold more potent than DFP in inhibiting this response. Binding of [^{3}H]N-methylscopolamine ([^{3}H]NMS) decreased with passage of cells and varied between different subcultures of PC12 cells. Thus, experiments were performed with early passages of a subculture which contained about 12,000 [^{3}H]NMS binding sites/cell in the absence of NGF and approximately 20,000 binding sites/cell when maintained 2 days in the presence of 100 μg 7S NGF. Half-inhibition of NGF-stimulated binding to whole cell preparations occurred with 70 μM soman. Isolated crude membrane fractions from PC12 cells demonstrated multiple binding sites for [^{3}H]NMS as revealed by Scatchard analyses and competitive binding of the agonist, carbamylcholine. The response to the guanine nucleotide analog, guanylimidodiphosphate (Gpp(NH)p) was also stimulated by NGF as shown below. Pretreatment of PC12 cells with the organophosphate, soman, enhanced the sensitivity to guanine nucleotide regulation in native but not in NGF-treated cells.

Carbamylcholine Inhibition of [^{3}H]-NMS Binding by Membrane Fractions Isolated from Native and NGF-Treated Cells

	Native Cells	*NGF-treated Cells*
	IC_{50}, μM	
Control		
-Gpp(NH)p	*31.6*	*47.3*
+Gpp(NH)p 100 μM	*39.8*	*177.2*
Soman, 50 μM		
-Gpp(NH)p	*70.8*	*30.9*
+Gpp(NH)p 100 μM	*281.8*	*141.2*

The data suggest that NGF treatment increased the sensitivity of muscarinic receptor binding sites in PC12 cells to regulation by guanine nucleotides. Although soman inhibited the elevation of [^{3}H]NMS binding sites produced by NGF-treatment, the organophosphate did not inhibit the reduction in muscarinic receptor affinity for carbamylcholine produced by Gpp(NH)p. (Supported, in part, by US Army Contract DAMD-17-85-C-5091 and NIH grant HD1-6596.)

29.3 INFLUENCE OF HALOTHANE ON MUSCARINIC ACETYLCHOLINE RECEPTOR BINDING AND INTERACTIONS WITH GTP-DEPENDENT REGULATORY PROTEINS. R.L. Dennison, Jr.*[1], B.L. Anthony*[2], G.O. Carrier[2] and R.S. Aronstam[2]. (SPON: B.B. Gallagher). Depts. of [1]Anesthesiol. and [2]Pharmacol. & Toxicol., Med. Coll. of Georgia, Augusta, GA 30912 and [1]Anesthesia Service, Vet. Adm. Med. Ctr., Augusta, GA 30910.

The influence of halothane on muscarinic receptor binding was examined in membranes isolated from rat cerebral cortex and brainstem using [^{3}H]methylscopolamine ([^{3}H]MS) as a probe. Halothane (0.5-10%) enhanced [^{3}H]MS binding in both cortex and brainstem membranes. Halothane (2%) did not affect the number of muscarinic binding sites. [^{3}H]MS binding affinity, however, was increased in the presence of halothane (K_D, air = 0.41 nM; K_D, 2% halothane = 0.26 nM). This increase reflected a 50% decrease in the the dissociation rate constant rather than a change in the bimolecular rate constant of association (Table 1).

Table 1.

	Binding Constants		
Halothane	K_D, nM	k_1, $x10^{-7}M^{-1}min^{-1}$	k_{-1}, $x10^{-3}min^{-1}$
0	0.41±0.03	1.8±0.2	13.0±1.1
2%	0.26±0.04	1.9±0.2	6.5±0.3

Carbamylcholine affinity for brainstem and cortex muscarinic receptors was not affected by halothane. The ability of 5'-guanylylimidodiphosphate (Gpp(NH)p; a stable GTP analogue) to lower carbamylcholine affinity for brainstem receptors, however, was eliminated after equilibration with 2% halothane (Table 2).

Table 2.

		Binding Constants		
Halothane	Gpp(NH)p	B_H	K_H	K_L
0	0	.57±.05	.05±.02	12±4
	10 uM	.40±.03	.13±.04	40±6
2%	0	.54±.04	.06±.03	15±3
	10 uM	.51±.03	.05±.02	18±5

(B_H = fraction of receptors displaying high affinity binding.)

The selective enhancement of muscarinic antagonist binding may reflect the relatively greater importance of hydrophobic interactions in the binding of cholinergic antagonists compared to agonists. Halothane may interact with lipophilic binding domains, thereby stabilizing hydrophobic interactions. The elimination of the guanine nucleotide effect by halothane may reflect a stabilization of the receptor-G protein complex due to either a direct action on the G protein or to an alteration of the physical state of the membrane. Alternately, the ability of G protein to bind guanine nucleotides may have been eliminated by halothane.

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29.4 **ONTOGENY OF MUSCARINIC RECEPTOR BINDING SITES AND MUSCARINIC RECEPTOR-MEDIATED STIMULATION OF PHOSPHOINOSITIDE BREAKDOWN AND INHIBITION OF CYCLIC AMP ACCUMULATION IN RAT FOREBRAIN.** W. Lee*, K.J. Nicklaus*, D.R. Manning* and B.B. Wolfe. Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104

The existence of subtypes of muscarinic cholinergic receptors has been proposed based on evidence from studies of function and binding of radioligands. Pirenzepine (PZ) is a muscarinic antagonist that appears to distinguish the subtypes of muscarinic receptor assessed by radioligand binding and some functional assays. The receptor with high affinity for pirenzepine has been termed M_1 and the receptor with low affinity has been termed M_2. Thus, the quantity of binding sites labelled by (^{3}H)-PZ is used as an estimate of the number of M_1 sites, and the binding sites labelled by (^{3}H)-quinuclidinylbenzilate (^{3}H-QNB, a nonselective muscarinic antagonist) represent the sum of M_1 and M_2 sites. Additionally, it has been suggested that M_1 receptors mediate the breakdown of phosphatidylinositol (PI), while M_2 receptors mediate the inhibition of adenylate cyclase activity.

We have examined the developmental time courses of M_1 and M_2 binding sites as well as muscarinic receptor-mediated stimulation of PI breakdown and inhibition of cyclic AMP accumulation, in order to determine if these parameters develop at different times. Both types of muscarinic receptor binding sites develop at the same time: binding site densities were 30% of adult levels at 1 week, 70% at 2 weeks, 90% at 3 weeks and equal to adult levels at 4 weeks postpartum. Acetylcholine (ACh)-stimulated PI breakdown was detected at all ages tested (1,2,3,4 and 6 weeks); the greatest amounts of (^{3}H)-inositol 1-P (^{3}H-Ins 1-P) accumulation were found at the earliest ages (1 and 2 weeks). There were, however, much higher levels of (^{3}H)-PI (i.e., precursor) in tissues from younger animals, and when the data were expressed as a percent of the (^{3}H)-PI converted to (^{3}H)-Ins 1-P, tissues from young (1 and 2 weeks) rats were less responsive than tissues from more mature (4 and 6 weeks) rats, with tissues from 3-week-old rats being intermediate. ACh inhibition of (^{3}H)-cyclic AMP accumulation, on the other hand, was undetectable in tissues from 1- and 2-week-old rats, while in tissues from 3-, 4- and 6-week-old rats, the responses were at adult levels. (^{3}H)-ATP levels and forskolin-stimulated cyclic AMP accumulation were the same at all ages. Thus, especially for ACh inhibition of cyclic AMP accumulation, a time in development existed (2 weeks) at which receptors were present but response to receptor stimulation was absent. The potential involvement of guanine nucleotide-binding regulatory proteins is currently under investigation. (Supported by GM 31155 and the AHA.)

29.5 REGULATION OF MUSCARINIC AND ADRENERGIC RECEPTORS COUPLED TO PHOSPHOINOSITOL METABOLISM IN THE RAT BRAIN. R.H. Lenox, D.D. Hendley and J. Ellis. Neurosci. Res. Unit, Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405.

Among the various neurotransmitters thought to mediate the hydrolysis of phosphoinositides upon activation of their respective receptors, acetylcholine and norepinephrine have both been shown to elicit such a response in hippocampal slices from rat brain. Ongoing studies in our laboratory have focussed upon subtypes of muscarinic receptors in the central nervous system (Ellis and Lenox, BBRC, 126 (3), 1985) and the coupling of monoamine receptors to second messenger systems in intact cell preparations (Lenox et al., Mol. Pharm. 27(1), 1985). In a series of recent studies we have been examining the characteristics of cholinergic receptor vs adrenergic receptor mediation of phosphoinositol turnover in brain slices from the rat.

Hippocampal slices (350 x 350 um) were washed in Krebs-Ringer bicarbonate buffer at 37°C for 30 minutes under O_2/CO_2 (95:5). The slices were then preincubated for 1 hr under O_2/CO_2 with 0.3uM [^{3}H]-myo-inositol. The slices were then washed at 20°C and incubated with various concentrations of receptor agonists. The hydrolysis of the phosphoinositides was measured by the accumulation of [^{3}H]-myo-inositol-1-phosphate (M-1-P) in the presence of LiCl (10mM) (Berridge et al., Biochem. J. 206, 1982). The reaction was stopped by the addition of chloroform:methanol (1:2 v/v). The aqueous phase was loaded onto a Dowex-1 column and the accumulated [^{3}H]-M-1-P was determined as the radioactivity retained by the resin and eluted by 0.1M formic acid in 0.4M ammonium formate. Values were expressed as the ratio of the amount of label in the [^{3}H]-M-1-P to the total label incorporated into M-1-P and lipid fractions.

A concentration dependent increase in the M-1-P fraction was demonstrated for both carbachol and epinephrine with EC_{50} values of approximately 10uM and 1uM respectively. Competition by antagonists was consistent with the activity of muscarinic and alpha$_1$ adrenergic receptor populations. The maximal increase of the percent of the label in the M-1-P fraction in the presence of either carbachol (1mM) or epinephrine (30uM) was approximately 3-4 fold over a 40-60 minute period. Concurrent addition of both carbachol and epinephrine resulted in an increase of 5-6 fold. In the absence of agonist little change was observed over time. Exposure of hippocampal slices to carbachol during the preincubation period significantly reduced the [^{3}H]-M-1-P response to the subsequent addition of the agonist and LiCl during the incubation period. Similar exposure to epinephrine did not result in any reduction of [^{3}H]-M-1-P response to epinephrine during the incubation period. These data suggest that cholinergic and adrenergic receptors in the hippocampus regulate separate pools of phosphoinositides, possibly located on separate cells and that the two classes of receptors may be regulated differently. (Supported by grant RO1-05214 NIA.)

29.6 NEUROTRANSMITTER RECEPTOR-STIMULATED Ca^{2+} MOBILIZATION AND INOSITOL PHOSPHOLIPID METABOLISM IN NEUROBLASTOMA CELLS. R. M. Snider and B. W. Agranoff. Neuroscience Lab, University of Michigan, Ann Arbor, MI 48109

Murine neuroblastoma (clone N1E-115) cells, known to have muscarinic, histamine H_1, bradykinin and neurotensin receptors which are coupled to a Ca^{2+}-dependent cyclic GMP response, are shown to also participate in stimulated inositol phospholipid turnover in the presence of these agonists. Inositol phospholipid metabolism was studied in this preparation by measuring agonist-induced stimulation of $^{32}P_i$ incorporation into PI and PA over a 30 min period. Carbachol stimulates a 2 to 4-fold increase in PI and a 2-fold increase in PA, which is blocked by atropine. Similar results on phospholipid labeling were produced by histamine, bradykinin and neurotensin. There is little agonist-induced effect on PIP_2 or PIP labeling under any of these conditions. With carbachol, the EC_{50} was 40-100 μM while K_i values for atropine and pirenzepine were 0.2 and 3 nM, respectively. Carbachol, acetylcholine, muscarine and methacholine were found to be full agonists; arecholine, bethanechol, pilocarpine and oxotremorine, partial agonists; and McN-A-343 was without effect. These results are in close agreement with those found for muscarinic receptor-stimulated phospholipid labeling in nerve endings (Fisher et al., J. Biol. Chem. 258:7358, 1983) and for cyclic GMP formation in neuroblastoma cells (McKinney et al., Mol. Pharmacol. 27:233, 1985). In additional studies, cells were loaded with the Ca^{2+}-binding probe quin2, the fluorescence of which reflects $[Ca^{2+}]_i$ (intracellular calcium concentration) within physiological ranges. Resting $[Ca^{2+}]_i$ of neuroblastoma cells was determined to be 80-100 nM. Receptor stimulation by maximally effective concentrations of agonists increased the $[Ca^{2+}]_i$ by 20-150 nM within 5-10 sec of addition in the following rank order of efficacy: neurotensin > bradykinin > histamine > carbachol. There was a return to baseline within 1-2 min except for carbachol, in which instance $[Ca^{2+}]_i$ remained elevated, but decreased rapidly upon addition of atropine. The present studies thus confirm in neuroblastoma cells a number of findings seen in nerve endings derived from brain, and extends these observations to the level of Ca^{2+} mobilization. (Supported by NIH Grants NS 20920 and NS 15413.)

29.7 RECEPTOR OCCUPANCY AND INHIBITION OF STIMULATED PI TURNOVER BY SELECTIVE MUSCARINIC ANTAGONISTS. F.J. Monsma, Jr.* and W. Hoss. Ctr. for Brain Res., Univ. of Roch. Sch. of Med. & Dent., Rochester, NY 14642

The non-classical muscarinic antagonists, pirenzepine (PZ) and gallamine (GAL), have been reported to distinguish subtypes of the muscarinic acetylcholine receptor in brain and peripheral tissues. This selectivity has been assessed for the most part through radioligand binding studies and, in the case of PZ, by its ability to block the turnover of inositol phospholipids stimulated by muscarinic cholinergic agonists. Recently, another muscarinic antagonist, trihexyphenidyl (THP), has been proposed as a selective antagonist, distinguishing between peripheral and CNS muscarinic receptors with a selectivity similar to PZ (Tien & Wallace, Biochem. Pharmacol. 34:588, 1985). We report here on the abilities of these three compounds to inhibit specifically the carbachol-stimulated accumulation of labeled inositol phosphates in slices of rat corpus striatum, as well as the affinities of these compounds for the muscarinic receptor as assessed by competition with ^{3}H-QNB in the striatal slice preparation.

Radioligand binding studies with ^{3}H-QNB in striatal slices reveal a single class of saturable, high affinity QNB binding sites exhibiting a K_d for QNB of 0.79 nM, and a B_{max} of 0.22 pmole/mg tissue (wet wt.). The antagonists examined were able to inhibit the binding of ^{3}H-QNB with the following rank order of potency: THP (K_i=47.1 nM)>PZ (K_i=1900 nM)>>GAL (K_i=109,000 nM).

The ability of these antagonists to inhibit the carbachol-stimulated turnover of inositol phospholipids in the corpus striatum slice preparation was assessed by analyzing the accumulation of ^{3}H-inositol phosphates in the presence of LiCl (Berridge et al., Biochem. J. 206:487, 1982). In the presence of 0.1 mM carbachol (CCh), the accumulation of labled inositol phosphates was increased 2-3 fold above control (basal) levels (ED_{50}=2.17 μM). Inclusion of antagonists in the assay resulted in a dose-dependent inhibition of the CCh-stimulated inositol phosphate accumulation. Pirenzepine and THP were equally potent, exhibiting an IC_{50} value of 0.2 M. Gallamine was a poor inhibitor of the CCh-stimulated PI turnover, having an IC_{50} of 1.6 mM.

Based on the comparison between receptor occupancy and inhibition of PI turnover, these results support the hypothesis that PZ is a selective antagonist for the muscarinic receptor subtype coupled to PI turnover (M1), whereas GAL shows a selectivity towards a different subtype, probably the M2. However, the antagonist THP, which apparently distinguishes peripheral and central muscarinic receptors, may not discriminate subtypes in the CNS.

29.8 SOLUBILISED CARDIAC MUSCARINIC RECEPTORS. P. Horodeckyj* and J.W. Wells. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

(-)-N-[^{3}H]Methylscopolamine (NMS) has been used to study muscarinic receptors in a solubilised preparation from the left ventricle and interventricular septum of Syrian golden hamsters. Binding was measured at equilibrium and 30° in 0.8% digitonin, 50 mM HEPES and 1 mM Mg^{2+} at pH 7.45. Bound radioligand was separated by chromatography on Sephadex G-50. Non-specific binding of [^{3}H]NMS, taken as total binding measured in the presence of 1 mM unlabelled NMS, increases linearly with the concentration of free radioligand. Binding inhibitable by cold NMS, or specific binding, is well described in terms of a single and uniform population of sites. The apparent dissociation constant of [^{3}H]NMS (K_D = 534 ± 47 pM) compares favourably with that found in a particulate preparation from the same tissue (K_D = 385 ± 2 pM). Four muscarinic agonists (carbachol, arecoline, bethanechol, methacholine) inhibit the specific binding of 3 nM [^{3}H]NMS with Hill coefficients markedly less than one; in each case, maximal inhibition is the same as that achieved by unlabelled NMS. In all experiments, total binding in the absence of agonist represented less than 5% of the total radioligand added. The inhibitory behaviour is well described by the expression $Y = F_1K_1/(K_1 + [A]) + F_2K_2/(K_2 + [A])$, in which $K_1 = K_H(1 + [[^3H]NMS]/K_D)$, $K_2 = K_L(1 + [[^3H]NMS]/K_D)$, and F_1 and F_2 sum to one (Eq. 1). Receptors in solution thus appear heterogeneous with respect to the binding of agonists, in common with those in suspension. Among the four agonists studied, the higher affinity (K_H) is the same in both preparations; in contrast, the lower affinity found in solution (K_L) is 32- to 60-fold weaker than the lower (K_M) of the two affinities (K_H, K_M) found in suspension. GMP-PNP at 0.1 mM is without effect on the specific binding of [^{3}H]NMS to the solubilised preparation: the data are well described assuming a single class of sites, and the affinity is unchanged from that measured in the absence of the nucleotide. With carbachol, however, GMP-PNP increases the Hill coefficient for inhibition of [^{3}H]NMS from 0.35 to 0.48. Analysis in terms of Eq. 1 reveals that the nucleotide is without effect upon the apparent dissociation constant at the sites of lower affinity (K_L), but causes a 25-fold increase in that at the sites of higher affinity; there is essentially no change in the value of F_2. This contrasts with the effect in suspension, where the nucleotide is observed to increase F_2 with little or no change in K_H or K_M. Interestingly, the higher affinity found with GMP-PNP in the solubilised preparation agrees well with the lower affinity (K_M) found with or without GMP-PNP in suspension. The data suggest that K_H and K_M reflect receptors in two different states of association with the G/F-protein, with the nucleotide favouring the appearance of K_M. The sites of lowest affinity (K_L) observed only in solution may reflect receptors no longer able to interact with a G/F-protein, a situation that may not exist to any appreciable extent in the membrane. (Supported by the Medical Research Council of Canada and by the Heart and Stroke Foundation of Ontario.)

29.9 GALLAMINE'S INTERACTIONS WITH CNS MUSCARINIC RECEPTORS: ALLOSTERISM AND SUBPOPULATION-SPECIFICITY ARE SEPARATE PHENOMENA. J. Ellis and R.H. Lenox. Neurosci. Res. Unit, Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405.

There is agreement among several laboratories that gallamine inhibits the binding of tritiated ligands to muscarinic receptors from both heart and brain. However, the mechanism by which gallamine achieves the inhibition of binding remains somewhat controversial. On the basis of studies of the binding of [^{3}H]-quinuclidinyl-benzilate (QNB) to brain membranes, Ellis and Hoss (Biochem. Pharmacol. 31, 1982) concluded that gallamine interacts competitively with distinct subpopulations of muscarinic receptors. Dunlop and Brown (Mol. Pharmacol. 24, 1983) have reported that gallamine inhibits the binding of [^{3}H]QNB to heart membranes in a competitive manner, but that it induces, rather than detects receptor heterogeneity. On the other hand, Stockton et al. (Mol. Pharmacol. 23, 1983) concluded that gallamine inhibits the binding of [^{3}H]N-methylscopolamine (NMS) in brain and heart by an allosteric mechanism. We have recently reported that in brain membranes gallamine appears to allosterically regulate the binding of [^{3}H]NMS by slowing both on- and off-rates, but that the on- and off-rates of [^{3}H]QNB are not affected by gallamine (Ellis and Lenox, Biochem. Pharmacol., in press, 1985). In the same system, quaternary antagonists other than gallamine (including NMS and N-methylatropine) have been shown to distinguish between the subpopulations of [^{3}H]QNB binding sites that are defined according to affinity for gallamine (Ellis and Lenox, BBRC, 126, 1985). These findings led us to suggest that the subpopulation-specificity of gallamine and the effect of gallamine on the kinetics of the binding of [^{3}H]NMS may be mediated by separate mechanisms (ibid).

Recently, Waelbroeck et al. (BBRC, 121, 1984) found that verapamil affects the kinetics of binding of [^{3}H]NMS to heart membranes in the same way that gallamine does. Since verapamil is not a quaternary ligand, we expected that it would not possess the subpopulation specificity of gallamine. Therefore, to test our suggestion of separate mechanisms, we have assessed the abilities of verapamil and N-methylatropine to (1) recognize the subpopulations defined by gallamine and (2) regulate the off-rate of [^{3}H]NMS in rat brain membranes. We have found that verapamil is as potent as gallamine in slowing the off-rate of [^{3}H]NMS but that it does not discriminate between the subpopulations. Conversely, N-methylatropine is not effective at slowing the off-rate of [^{3}H]NMS but exhibits approximately 1000-fold difference in affinity between the subpopulations defined by gallamine. Thus, it appears that the two effects exhibited by gallamine are mediated by separate mechanisms, with different pharmacological characteristics.

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29.10 THIORIDAZINE DOES NOT ACT AS AN ANTICHOLINERGIC AGENT AT ACH RELEASE MODULATING RECEPTORS IN THE RABBIT STRIATUM. D.M. Niedzwiecki, R.B. Mailman and L.X. Cubeddu. Depts. of Pharmacology and Psychiatry and Biological Sciences Research Center, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

In order to determine their relative potency at muscarinic receptors within the CNS, we measured the effects of THD and two of its pharmacologically active metabolites, mesoridazine and sulforidazine, on the electrically evoked release of (3H)ACh from rabbit striatal slices. Unlike anticholinergic agents such as quinuclidinyl benzilate (QNB), neither THD nor its two metabolites significantly antagonized the inhibition of evoked ACh overflow from the striatal slices produced by either of the muscarinic cholinergic agonists, carbachol or oxotremorine. Carbachol (10μM) inhibited the release of ACh evoked by stimulation at 0.3 Hz., 39 pulses by 55.6+/-3%. QNB, at a concentration of 30nM antagonized this inhibition by 37.2% (20.6% inhibition compared to controls), and completely antagonized the carbachol effect at 300nM. In contrast, neither THD nor its metabolites, even at concentrations as high as 30μM, had any effect on the inhibition of evoked ACh overflow produced by 10μM carbachol. In a similar manner, perfusion of striatal slices with oxotremorine (1000nM) resulted in 37.1+/-5% inhibition of electrically stimulated ACh release compared to controls. Again, this inhibition was significantly antagonized by QNB (1μM) but not by THD (1μM).

Concentrations of THD and its metabolites above 1μM significantly elevated the spontaneous or basal overflow of radiolabelled DA from these same slices. This granular effect has been demonstrated for most DA receptor antagonists. Neither QNB, THD, nor the two metabolites tested, had any effect on the spontaneous release of ACh. Additionally, none of the drugs when perfused through the slices in the absence of a cholinergic agonist, significantly enhanced ACh release evoked under these stimulation conditions.

In summary, THD did not appear to antagonize the inhibitory effect of two different muscarinic agonists at the muscarinic receptors which modulate ACh release from striatal slices. This result was unexpected because THD had some potency in competing for (3H)QNB binding sites in homogenates of rabbit striata. In preliminary experiments, the K_I for THD in competing for (3H)QNB binding sites in rabbit striatal membranes was 37.23 nM, compared to 1.21 nM for the classic anticholinergic agent, atropine. These data suggest either that the muscarinic receptors which modulate ACh release and those that bind QNB are different or that the population of striatal muscarinic receptors which modulate ACh release make up too small a proportion to be seen with the conventional (3H)QNB binding techniques.

29.11 FEATURES OF LIGAND BINDING IN HOMOGENATE AND SECTION PREPARATIONS. G. Dohanich, S. Halpain, L. Lambdin* and B.S. McEwen. Rockefeller University, New York, New York, 10021.

With in vitro autoradiographic techniques used to study neurotransmitter binding, radiolabeled ligands are incubated with tissue sections mounted on microscope slides. Following incubation, the unbound ligand is washed from the sections and the slides are placed in contact with tritium-sensitive film in order to generate an autoradiographic image. A typical procedure used to validate the binding of radiolabeled ligands to sections consists of incubting the sections with the ligand and, subsequently, wiping or scraping the washed sections from the slide into scintillation vials to determine the amount of radioactivity bound by standard scintillation counting. In a series of experiments, we compared the binding of [^{3}H]N-methylscopolamine (NMS) to muscarinic receptors in homogenates and slide-mounted sections from rat brain.

In homogenate assays, rat forebrain tissue was incubated for 60 min. at 23°C in 1 ml volumes of phosphate buffer containing [^{3}H]NMS. Free ligand was separated from bound ligand by rapid filtration through glass fiber filters. For binding to sections, frozen mash from rat forebrain was cut on a cryostat and thaw-mounted on microscope slides. Sections of varying thickness were incubated for 60 min. at 23° in 8-12 ml volumes of phosphate buffer containing [^{3}H]NMS. Free ligand was separated from bound ligand by a 10 min. wash in cold buffer. Sections were then wiped from the slide with glass fiber filters and counted with standard liquid scintillation procedures. For both homogenates and sections, nonspecific binding was determined in the presence of 5 uM atropine sulfate.

A comparison of binding parameters indicated that the apparent K_d for [^{3}H]NMS binding was at least one order of magnitude lower for homogenates (K_d=0.15 nM) than for 30u-thick sections (K_d = 1.25 nM). B_{max} values were equivalent for the two preparations. Furthermore, inhibition constants were also lower for carbachol inhibition of [^{3}H]NMS binding in homogenates (K_i=5 uM) than sections (K_i=100 uM). The larger K_d observed in sections was dependent upon section thickness since a reduction in K_d was evident as section thickness decreased (30u, K_d=1.20 nM; 20u, K_d= 0.86 nM; 10u, K_d=0.55 nM). A slower observed equilibration rate (K_{obs}) appears to account, at least in part, for the higher apparent K_d seen in thicker sections (30u, K_{obs}=0.02 min^{-1}; 10u, K_{obs}= 0.04 min^{-1} at 0.2 nM [^{3}H]NMS).

The results support previous evidence (Gilbert et al, Br. J. Pharm. 65:451; Nonaka and Moroji, Brain Res. 296:295) that certain ligands exhibit different binding profiles in homogenate and section preparations. These differences are probably related to the ability of the ligand to access available binding sites in the preparation.

29.12 DEMONSTRATION AND CHARACTERIZATION OF [^{3}H](-)QUINUCLIDINYL BENZILATE ([^{3}H](-)QNB) BINDING TO HUMAN NEUROBLASTOMA CELL LINE (SH-SY5Y). M. Serra*, K. Gulya* and H.I.Yamamura., Department of Pharmacology, University of Arizona, Tucson, AZ. 85724.

To demonstrate for the presence of muscarinic cholinergic receptors in clonal cell lines, we have begun to characterize for the presence of muscarinic receptors using the classical antagonist, [^{3}H](-)QNB. Our initial studies indicate that muscarinic receptors are present in the human neuroblastoma cell line, SH-SY5Y. Specific QNB binding showed tissue linearity up to 4 x 10^6 cells/assay. Kinetic experiments were performed to determine the association and dissociation rate constants. Using 200pM of [^{3}H](-)QNB, steady state levels of binding were reached after 60 minutes of incubation at 37°C. Dissociation of specifically bound [^{3}H](-)QNB from the receptor yielded a t 1/2 value of 110 minutes. Saturation studies were performed and gave a dissociation constant of 20pM and a maximal density of receptors of 230 fmoles/mg protein. The Kd value obtained from the saturation studies were in good agreement with the kinetic value. Pharmacological specificity was determined using atropine and pirenzepine. The classical antagonist atropine inhibited [^{3}H](-)QNB binding with a steep Hill slope and a Ki value of 0.4nM while the nonclassical antagonist pirenzepine inhibited the binding with a shallow Hill slope and a IC50 value of 400nM. The latter results with pirenzepine were analyzed using a 2-site computer model. This model gave a best fit of the data of K_H=5nM and K_L=180nM with the high affinity site being about 48%. To determine whether pirenzepine was binding to the QNB binding site, saturation studies using [^{3}H](-)QNB were performed in the absence and presence of varying concentrations of pirenzepine. A Schild plot showed that pirenzepine appeared to be a competitive antagonist of [^{3}H](-)QNB binding in these membranes.

Because pirenzepine showed muscarinic receptor heterogeneity on [^{3}H](-)QNB binding, direct studies with [^{3}H]pirenzepine were carried out to demonstrate for the presence of M_1 receptors. In membrane preparations, saturation studies resulted in a Kd value of 5nM and a Bmax value of 130 fmoles/mg protein thus indicating that [^{3}H]pirenzepine labels about 50% of the QNB binding sites.

In summary, the human neuroblastoma cell line, SH-SY5Y appears to have muscarinic cholinergic receptors and a portion of these muscarinic receptors show high affinity [^{3}H]pirenzepine binding. Further studies are being done in intact cells to correlate the radioligand binding data with biochemical responses.

29.13 IN VIVO TURNOVER OF MUSCARINIC RECEPTORS AS ASSESSED BY RECOVERY FROM RECEPTOR ALKYLATION. J. Waite and D.J. Jenden. Pharmacology Dept. and Brain Research Inst., UCLA School of Med., Los Angeles, CA 90024.

A clear understanding of homeostatic receptor regulation in vivo may provide insights into mechanisms of drug tolerance or supersensitivity and perhaps suggest ways of manipulating receptor numbers for therapeutic benefit. The time course of receptor recovery from irreversible blockade must ultimately depend on these basic parameters of receptor regulation.

N-[4-(2-chloroethylmethylamino)-2-butynyl]-2-pyrrolidone (BM123), a potent and specific alkylator of muscarinic receptors (Ehlert et al., Life Sciences 34:985, 1984), was injected into rat tail veins in a dose schedule which blocked more than 90% of cortical and striatal muscarinic receptors (8, 20, and 50 μmoles/kg at 1-hr intervals). Animals were decapitated at later time points and densities of unalkylated receptors present were determined by specific (10^{-6} M atropine displaceable) binding of 0.4 nM ^{3}H-l-quinuclidinyl benzilate (QNB) to well-washed membrane fragments from homogenates of cerebral cortex, striatum, and longitudinal muscle of the ileum. The binding incubation was conducted in 50 mM Na/K PO_4, pH 7.4, for 1 hr at 37°C. Rapid filtration through GF/B filters and 3 washes with cold saline terminated the assay. Receptor densities were quantified in terms of fmoles/mg protein.

The recovery time course in each tissue was found to approximate a single exponential curve described by this model:

$$R_t = R_{ss} - R_a e^{-kt}$$

R_t is free receptor density at time, t(hrs), as measured by ^{3}H-QNB binding. R_{ss} is steady state receptor density which was found to be the same before (control tissue) and after alkylation. R_a is the alkylated receptor density, extrapolated to t=0, and k is the degradation rate constant (hr^{-1}). This model assumes 1) a constant rate of receptor synthesis; 2) first order rate of receptor degradation; 3) the R_{ss} after recovery is the same as R_{ss} before alkylation.

The model was fitted to recovery curves by least squares non-linear regression yielding a best fit rate constant, k, of 0.021 ± .001 hr^{-1} (half-time 33 hrs) in all 3 tissues. Since the degradation rate constant was found by ANOVA to be identical in all 3 tissues, the different steady state densities in these tissues are likely due to different, tissue-dependent, rates of receptor synthesis.

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29.14 CHOLINERGIC SENSITIVITY OF CA1 PYRAMIDAL CELLS IN MOUSE HIPPOCAMPAL SLICES. R. K. Freund, S. L. Herzberger*, and J. M. Wehner*. Institute for Behavioral Genetics and School of Pharmacy, Univ. of Colorado, Boulder, CO 80309.

We examined the cholinergic pharmacology of the CA1 region in transverse hippocampal slices from 55-65 day old DBA/2Ibg female mice. Cholinergic agents were bath-applied to submerged hippocampal slices, and the effects of these drugs on the stimulus-evoked synaptic field potentials (recorded in Schaffer collateral-CA1 synaptic fields) and on the population spike amplitudes (PSAs; recorded in CA1 pyramidal cell layer) were monitored with an extracellular recording electrode. The effect of nicotine (Nic), oxotremorine (Oxo), mecamylamine (Mec), and atropine (Atr) on PSAs were compared by examining the drug-induced shift of the input-output curve (stimulus intensity vs. PSAs) at test concentrations of 80 and 800 uM. The sensitivity of synaptic field potentials toward acetylcholine (ACh), Nic, Oxo, Mec, Atr, and pirenzepine (Pir) was examined by comparing concentrations required for inhibition of the response amplitude. At both 80 and 800 uM Atr enhanced the PSA, shifting the input-output curve to the left, whereas the muscarinic agonist Oxo inhibited the initial population spike. Both Atr (800 uM) and Oxo (80 and 800 uM) induced the development of multiple spikes. Results for the nicotinic agents were less pronounced; Nic slightly enhanced the population spikes and induced multiple spikes at 800 uM, while the nicotinic antagonist Mec had a small depressant effect on the PSA. For inhibition of synaptic field potentials, Oxo was the most potent drug tested, but the inhibition pattern was complex. The data are consistent with a model describing an Oxo-sensitive component to the Schaffer collateral synaptic response, comprising 50% of the response and with an IC_{50} = 2-6 uM, and a less sensitive component comprising the remainder of the response. The other cholinergic drugs were much less potent; ACh, Mec, Pir, and Atr monotonically inhibited synaptic field potentials with IC_{50} values in the 1-5 mM range (potency rank order: Atr > Mec > Pir > ACh), while Nic had no inhibitory effect at concentrations up to 6.4 mM. Synaptic population spikes were observed as responses to Oxo (> 40 uM), Nic (> 1 mM), ACh (6.4 mM), and Atr (> 400 uM), but not to Pir or Mec. These results indicate that while CA1 pyramidal cells are sensitive to both muscarinic and nicotinic agents, the muscarinic sensitivity predominates. The data also suggest that activation of nicotinic cholinergic receptors, as well as blockade of muscarinic receptors, results in an increase in the number of discharging CA1 pyramidal cells. The classical muscarinic agonist Oxo may interact with both classes of cholinergic receptors. (Supported by NIDA grants DA 07043 and DA 03194.)

SYNAPTOGENESIS AND SYNAPSE ELIMINATION I

30.1 INTERACTIONS BETWEEN SYMPATHETIC PREGANGLIONIC NEURONS AND SYMPATHETIC GANGLION NEURONS IN VITRO. M. G. Honig and R. I. Hume. Div. of Biol. Sci., Univ. of Michigan, Ann Arbor, MI 48109.

The complex dendritic geometries of neurons raise important questions regarding how presynaptic terminals are apportioned over the surfaces of target neurons. We have chosen to approach this issue using a cell culture system, because it allows us to have access to both pre- and postsynaptic neurons. We are studying the interaction between sympathetic preganglionic neurons and lumbosacral sympathetic ganglion neurons dissected from chick embryos and placed in dissociated cell cultures.

These experiments require that we be able to identify each class of neuron. We have done this by using fluorescent carbocyanine dyes, which other researchers have shown are incorporated into the lipid portion of the plasma membrane. We label preganglionic neurons by retrograde transport following injection into the sympathetic chain of diI-C_{18}-(3), a dye which fluoresces orange-red. Sympathetic neurons are labeled by dissociation in the presence of a second dye, diO-C_{18}-(3), which fluoresces green. These dyes have several advantages over other markers that have been used previously to identify cells in culture. For the first day or two in culture, cells are labelled in their entirety, which allows us to follow process outgrowth. In older cultures, label is retained in small granules, and does not appear to spread to other neurons. Under our current culture conditions, many viable preganglionic neurons and ganglion neurons can be observed after two weeks in culture. The dyes have no discernable effect on the physiological properties of neurons. Ganglion cells are depolarized by acetylcholine, preganglionic neurons are capable of evoked transmitter release and both types of cells fire action potentials.

To study synaptogenesis in this system, we have begun by examining the morphology of preganglionic neurons using intracellular injections of Lucifer yellow. At 4-7 days in culture, preganglionic neurons are multipolar. They have 3-8 primary processes which are similar to dendrites in vivo in that they have many branches and sometimes short spines, but also have occasional varicosities. In addition, these neurons generally have one process which appears to be axonal in that it is much longer than the other processes and usually extends for several hundred micrometers before branching. At least some "axons" then break up into multiple collaterals which can branch extensively and show varicosities. These arborizations are sometimes, but not always, found in the vicinity of sympathetic neurons. This morphology is strikingly similar to the morphology of some presynaptic axons in autonomic ganglia in vivo. Our next step will be to test whether dissociated preganglionic neurons make functional synapses onto sympathetic neurons in vitro.

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30.2 CHANGES IN SYNAPTIC STRUCTURE AND DENSITY; A DEVELOPMENTAL LIFE-SPAN APPROACH. E.J. Markus, T.L. Petit and J.C. LeBoutillier*. Department of Life Sciences, University of Toronto, Scarborough, Ontario M1C 1A4.

Studies showing changes in synaptic structure and/or density in relation to age, have usually focused on a narrow age span and few synaptic properties.

In this study, the molecular layer of the sensori-motor cortex of Wistar rats aged 1 to 90 days and aged rats (28 months) were used.

Staining half the tissue with osmium and the remainder with EPTA, the following structural characteristics were studied:

Presynaptic element length, area, maximum height and number of vesicles; and the postsynaptic length, area and average height. In addition synaptic cleft width and overall synaptic density were analyzed.

Results to date indicate:

- A rise in synaptic density with development particularly between P10 and P15, with no apparent synaptic loss with aging.
- The presynaptic membrane appears to lengthen with age, while both pre and post synaptic membrane areas increase with age.
- Presynaptic maximal height also increases with age.

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30.3 PATTERN OF DENDRITIC DEVELOPMENT IN THE RAT NEOCORTEX. J.C. LeBoutillier*, T.L. Petit, H. Libstug* and A. Gregario*. (SPON: J. Gurd). Dept. of Life Sciences, University of Toronto, Scarborough, Ontario M1C 1A4.

The dendritic development of layer V pyramidal cells in the sensorimotor neocortex of the rat was studied in Golgi preparations. Animals were sacrificed on postnatal days (P) 1, 3, 5, 7, 10, 20, 25, 30, and 60, their brains processed, and cells from the sensorimotor cortex examined for maximal apical and basilar dendritic field, number of dendritic branches at 20 µm intervals from the cell body, number of apical and basilar branches (branching order), the length of each dendritic branch, and dendritic spine density on apical, basilar, and terminal dendrites. On P3, cells have an apical and a few oblique and basilar dendrites, most of which are restricted to primary and secondary branches ending within 60 µm of the cell body. The number of primary dendrites leaving the cell body increases until reaching a plateau around P7-10. However, the maximal dendritic field and measures of dendritic branching indicate increased differentiation until approximately P20. After this time, a plateau is observed in measures of branching order and the number of dendritic branches within 140 µm of the cell; however, there is a continued increase in the maximal dendritic field, the number of branches at the most distal portions of the dendritic field, average branch length and total dendritic length into adulthood. Protospines were evident at P3, but decreased in frequency over time, while mature spines appeared between P7 and P10 and increased dramatically until approximately P20-25. While a plateau or decline in spine density was seen on proximal dendrites, spine density did not decline on terminal branches into adulthood.

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30.4 **PLASTICITY OF HIPPOCAMPAL CIRCUITRY IN ALZHEIMER'S DISEASE.** J.W. Geddes, D.T. Monaghan, I.T. Lott*, H.C. Chui*, R.C. Kim* and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

The severe cell loss in the entorhinal cortex of Alzheimer's patients has recently been suggested to destroy the perforant path input to the hippocampus and may underly the memory deficit in this disease. Extensive studies in rodents have shown that entorhinal lesions result in the compensatory growth of adjacent afferents and the replacement of lost synapses. At present, it is uncertain if the human CNS is capable of a plastic response to neuronal loss induced by aging, injury, or disease. In this study we investigated the plasticity of kainic acid receptors in the rodent brain after entorhinal lesions and compared this response to that in postmortem tissues obtained from Alzheimer's patients. Our results suggest that in both rodents and man, kainic acid receptor distribution parallels afferent fiber expansion in the dentate gyrus molecular layer following entorhinal cell loss.

Glutamate, or a related excitatory amino acid, is thought to be the transmitter of the perforant path and the commissural/associational (C/A) system, which terminate on granule cell dendrites in the dentate gyrus molecular layer. Glutamate receptor subtypes are laminated in this region, with kainic acid (KA) receptors having a high density in the C/A system terminal zone (the inner 1/3 of the dentate molecular layer) and a much lower density in the perforant path terminal zone (the outer 2/3 of the dentate molecular layer). We used ^{3}H-KA autoradiography to determine if the KA binding site distribution parallels the expansion of C/A system afferents following an entorhinal cortex lesion and to see if such an expansion occurs in Alzheimer's disease. In male Sprague-Dawley rats which had received entorhinal cortex lesions, the region of high density KA binding sites in the dentate molecular layer expanded 50% into the deafferented hippocampus. This parallels the sprouting response of the C/A afferent fibers. The KA binding pattern observed in hippocampal sections of human brain samples obtained postmortem from non-demented patients was very similar to that identified in normal rodent brain, with a high density of KA binding sites occupying the inner 1/3 of the molecular layer. In brains obtained from Alzheimer's patients, the KA receptor field had expanded 60% so that it occupied over 1/2 of the molecular layer. The additional KA receptors may compensate for input loss and may increase vulnerability to excitoxic activity.

These results illustrate a striking similarity of the CNS response to entorhinal cell loss in Alzheimer's disease with lesion-induced denervation in the rodent. The parallel expansion of C/A afferents and KA binding sites in rodents and the similar increase in KA receptors in Alzheimer's disease suggests that new functional connections are formed. This is the first evidence that the human CNS is capable of plasticity in response to a pathologically-induced denervation. The Alzheimer's brain is therefore capable of at least limited compensatory growth in the wake of a severe degenerative disorder. (Supported by NIA grant AG00538).

30.5 NUMERICAL DENSITY OF BOUTONS AND ITS RELATIONSHIP TO NEURONAL, AXONAL AND DENDRITIC DENSITY IN THE OCCIPITAL CORTEX OF RATS REARED IN COMPLEX, SOCIAL AND ISOLATED ENVIRONMENTS. Anita M. Sirevaag and William T. Greenough. Depts. Psychol. & Anat. Sci. and Neur. & Behav. Biol. Prog., Univ. Illinos, Urbana-Champaign, IL 61820.

Electron microscopic studies of the occipital cortex have shown that rats reared in complex environments (EC) have a greater number of synaptic contacts per neuron than socially (SC) or individually caged animals (IC) (Brain Res. 1985, 329, 1985). The present study confirms these estimates, using numerical density of boutons (vesicle-containing processes), a parameter with different stereological shape assumptions, to estimate synapses.

Members of 11 littermate triplet sets of male Long Evans Hooded rats were reared in EC, a group of rats in a large toy filled cage with daily exposure to a playbox; SC, pairs in a cage; or IC, individual cages, for 30 days postweaning. Micrographs representing 236 µm^2 at a final magnification of 41,786X were randomly taken from cortical layers I, II-III, and IV of area 17 excluding somata and blood vessels. Neuronal densities were estimated from 0.5 µm toluidine blue light microscopic sections. Bouton measurements were calculated from digitized tracings of the minimum area occupied by vesicles. Numerical density of boutons was almost twice that of synapses. Paralleling the synapse per neuron estimates, EC rats had 13% more boutons per neuron in the upper cortical layers than IC's with SC values intermediate. In layer IV EC's had vesicle aggregates that were 25% larger than IC's. Aggregate size did not differ in layers I and II-III; this is also in agreement with differences in synaptic size. The number of boutons per cubic µm of myelinated axonal or dendritic material did not differ among the groups but the volume of myelinated axon and dendrite per neuron was significantly greater in EC's than IC's. This suggests that the addition of more myelinated axons per neuron is associated with an increase in synaptic or bouton number per neuron. The stability of dendritic material per bouton similarly suggests a minimum required intersynaptic distance.

	EC	SC	IC	EC vs. IC
Nv boutons/µm^3	1.25±.10	1.23±.13	1.27±.14	NS
boutons/neuron	17030±1329	15100±1549	14783±1725	p<.05
µm of axon/neuron	4.5±0.2	4.6±0.3	3.6±0.2	p<.03
boutons/ µm of axon	4248±222	3943±225	3932±222	NS
µm of dend./neuron	45.9±2.1	42.2±2.4	40.0±2.3	p<.03
boutons/µm of dend.	388±9	387±9	378±10	NS

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30.6 BLOOD VESSEL VOLUME FRACTION IN OCCIPITAL CORTEX OF RATS REARED IN COMPLEX OR ISOLATED ENVIRONMENTS. J. E. Black, A. M. Sirevaag, C. Gorman* and W. T. Greenough. College of Medicine and Neural & Behavioral Biology Program and Departments of Psychology and Anatomical Sciences, Univ. Illinois, Urbana-Champaign, IL 61820.

Rats reared in complex environments (EC) have more synaptic contacts per neuron in occipital cortex than individually caged animals (IC), and their neurons are farther apart (Brain Research, 1985, 329, 195). The additional synapses may impose a heavier metabolic load, as might any greater synaptic activity. The added volume in cortical layers may further alter the capability of local vasculature to support neural function. This study examines vasculature in occipital cortex of rats reared in EC and IC environments.

Eleven littermate pairs of male Long Evans hooded rats were randomly assigned to EC or IC rearing conditions. EC rats were raised for 30 days postweaning in a large cage toy-filled cage with daily opportunities to explore a playpen with additional toys. IC rats were raised individually in standard laboratory cages for 30 days postweaning. Micrographs from 0.5 µm toluidine blue stained sections of cortical area 17 were assembled into montages which included Layers I, II-III and IV. The volume fraction of vasculature, excluding pial vessels, was calculated from point counts of a double latticed grid placed over the montage. Neuronal densities across all upper cortical layers were similarly estimated for the same tissue, and synapse densities were estimated from EM micrographs of adjacent sections at 41,786X. The volume fraction of vasculature was 35% greater in EC's than in IC's across all upper cortical layers. There was a corresponding reduction in the number of neurons served per unit vascular volume. The synapse per unit vascular volume comparison failed to reach statistical significance. The difference in vascular volume fraction was greatest in layer I. Intersubject variability was high, indicating that a larger tissue area must be sampled to provide accurate percentage estimates. The large difference in the vascular volume fraction suggests greatly increased metabolic demand in the occipital cortex of rats reared in more stimulating environments.

	EC	IC	
vasc. vol. frac.	2.2±.4	1.5±.2*	
no. synapses/mm^3 vasc. vol.	50±14	78±42	*p<.02
no. neurons x 10^7/mm^3 vasc. vol.	5.0±1.0	10.2±4.3*	

Supported by MH 35321, PHS-5-32-GM07143 and the Retirement Research Foundation.

30.7 CHANGES IN POLYRIBOSOMES AND CISTERNAL STRUCTURES DURING SPINE FORMATION AND SYNAPTOGENESIS IN RAT VISUAL CORTEX. H.-M. Hwang and W. T. Greenough Neural & Behavioral Biology Program, and Departments of Psychology & Anatomical Sci., University of Illinois, Champaign, IL 61820

Polyribosomal aggregates (PRAs) are associated with synaptic formation in developing rat visual cortex (Hwang & Greenough, Neurosci. Abst. 10, 1984) and hippocampal dentate gyrus (Steward & Falk, J. Neurosci. Res. 13, 1985). In developing dendrites, they were found to be intimately associated with cisternal structures and usually located near the membrane, especially near or inside spines. PRAs are found in the spine proper during development, and cisternal structures, including tubules and sacs, appear to accumulate in and near spines. To elucidate possible contributions of PRAs and cisternae to synaptogenesis and spine formation, dendritic spines, including PRAs and cisternae, on apical dendrites in layer IV of developing rat visual cortex were reconstructed through serial thin sections. Preliminary data showed that PRAs appear to become located near cisternal structures in the spine base at about the time that identifiable synapses have formed on the spine. Synapses were located in various positions on spines: They appear to migrate toward the tip of spines with maturation, although there is a large age difference in innervation on spines of apical dendrites, such that new synapses could confound this measure. Whether cisternae process locally synthesized protein is not certain. No rough endoplasmic reticulum is apparent. However, the appearance of coated vesicles pinching off cisternae suggests that cisternal structures may play a role in membrane expansion and recycling. PRAs in the spine proper are sometimes associated with cisternal structures in innervated spines. The PRA in the spine become less frequent as the synapse matures, while cisternally-associated PRAs in the spine base remain frequent. There is a shift in the distribution of PRA size (number of ribosomes) toward smaller numbers from day 20 to 25. Non-cisternally-associated PRAs are often seen in the spine proper prior to the appearance of an evident synaptic specialization. Non-innervated spines (no evident membrane specialization) were often surrounded by several boutons, one of them typically contacting the membrane at the junction of the spine and dendrite. Some non-innervated spines, filled with abundant cisternae, had no PRAs in the spine and no boutons touching any part of the spine. The association of PRA with cisternae may be induced by the presence of presynaptic boutons which appear to first contact the spine at its junction with the dendrite in most cases. Supported by NIMH 35321.

30.8 PROTEIN PRODUCTION IN THE NEUROPIL OF THE DENTATE GYRUS DURING DEVELOPMENTAL SYNAPTOGENESIS. L. Phillips, A. Pollack*, and O. Steward. Dept. of Neurosurgery, Univ. of Va. Sch. of Med., Charlottesville, VA 22908.

The neuropil of the post-natal dentate gyrus shows a dramatic accumulation of polyribosomes under dendritic spines which spans the period of developmental synaptogenesis (Steward, O. & Falk, P., J. Neurosc. Res. 13:75-88, 1985). The density of polyribosome-containing spines rises to a peak at 7 days of age, and then declines between 8 and 20 days, while synapse density continues to increase at a relatively constant rate. Because protein production might mark the different phases of developmental synaptogenesis, we were interested in determining protein synthetic activity during the periods of polyribosome accumulation under dendritic spines.

Fifteen to 20 living hippocampal slices were prepared from albino rat pups sacrificed at 4, 7, 10, 12, 14 and 21 days after birth. Slices were equilibrated for 1-2 hours in a modified Eagle's medium, and then incubated in medium supplemented with 3H-leucine (54 Ci/mmol;0.3 mCi/ml) for 30 mins. After a brief rinse with cold medium, the dentate molecular layer, enriched in developing dendrites, was dissected free from the remainder of the hippocampus. Protein within each region was acid precipitated, radioactivity determined through liquid scintillation spectroscopy and protein content assayed by the Bradford method. Incorporation of 3H-leucine into acid precipitable protein was expressed as counts/min/ug protein (cpms/ug) for each post-natal time period. These data were compared to previously obtained values for adult tissue.

The amount of 3H-amino acid incorporated into protein was higher than that of the adult (583 cpms/ug) for all post-natal time periods. This was true for both dissected regions, with the largest range of increase (8-33 fold) in the dentate molecular layer: from 4,416 cpms/ug at 7 days to 19,385 cpms/ug at 12 days and 4,315 cpms/ug at 21 days post-natal. Maximal protein synthetic activity during developmental synaptogensis did not directly coincide with the peak in polyribosome accumulation under dendritic spines. Sites of synapse formation might be marked by aggregations of polyribosomes prior to the protein synthetic burst associated with the elaboration of the mature contact. The specific polypeptide products of these post-synaptic polyribosomes and their regulation must still be determined. Supported by NIH grant NS12333 to O.S.

SYNAPSE ELIMINATION AND COMPETITION

31.1 SYNAPSE ELIMINATION IS DELAYED IN THE ANDROGEN-SENSITIVE LEVATOR ANI MUSCLE OF THE RAT. C.L. Jordan, M.S. Letinsky, and A.P. Arnold. Laboratory of Neuroendocrinology, Ahmanson Laboratory, Jerry Lewis Neuromuscular Research Center, and Department of Psychology, University of California, Los Angeles, CA 90024.

Although mammalian muscle fibers are multiply innervated at birth, adult muscle fibers are typically innervated by only one axon. The loss of polyneuronal innveration generally is complete by the end of the second week after birth. In the present study, we compared this process of synapse elimination in two fast-twitch, glycolytic muscles, the levator ani (LA) and the extensor digitorum longus (EDL) in the rat. These two muscles differ in the androgen sensitivity of their development, since survival of the LA requires androgens, whereas this is not true for the EDL. We find that in the adult, 10-15% of LA muscle fibers are innervated by more than one axon, but that all EDL muscle fibers are singly innervated. Although both muscles experience a developmental elimination of multiple inputs, the process of synapse elimination is substantially delayed in the LA compared with the EDL, and is not complete in the LA by the fourth postnatal week.

Male rats ranging in age from 14-28 days postnatal and also 90 days of age were overdosed with sodium pentobarbital and the LA and EDL muscles were removed, prepared as whole mounts, and stained with a modified tetranitroblue tetrazolium stain. The number of preterminal axons/junctional site was counted for surface muscle fibers, and was restricted to those sites which were fully visible. No fewer than 35 junctional sites/muscle/animal contributed to the estimates with 4-6 animals/time point. At postnatal day 14, when two thirds of EDL muscle fibers are singly innervated, LA muscle fibers are virtually all polyneuronally innervated, with more than 50% having three or more inputs. The adult pattern of single innervation is established in the EDL muscle by postnatal day 21. At this time, however, about half of LA muscle fibers are multiply innervated, although three or more inputs are relatively rare. By postnatal day 28, the innervation pattern for the LA is not fully adult, since a third of LA muscle fibers are still multiply innervated.

The time course of synapse elimination for the LA and EDL differs by almost two weeks. We do not currently understand the basis of this difference, but work is underway to determine whether the LA muscle is like the lumbrical muscle in that it is increasing in muscle fiber number while undergoing a protracted time course of elimination. On the other hand, the androgen sensitivity of LA development suggests the intriguing possibility that androgens might regulate synapse elimination in this muscle.

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31.2 SYNAPSE ELIMINATION BY FIBER TYPE IN NEO-NATAL RABBIT SOLEUS. J. M. Soha*, C. Yo* and D. C. Van Essen. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have compared the time course of the elimination of polyneuronal innervation from histochemically identified fast and slow fibers in a mixed muscle, the neo-natal rabbit soleus. Individual muscle fibers whose state of innervation was determined electrophysiologically were labeled intracellularly in a partially curarized in vitro preparation by pressure injection of the fluorescent dye Lucifer Yellow. In one series of muscles, only singly innervated fibers were labeled; multiply innervated fibers were labeled exclusively in a second set of muscles. Sections were stained for alkali stable ATPase activity, and the histochemical type of each labeled fiber determined as Type I (slow) or Type II (fast).

Two age groups were studied, at early and late stages of the appearance of singly innervated fibers. In the first group (7-8 days postnatal), singly innervated fibers constituted about 25% of the total. At this earlier age, we saw no significant difference by fiber type in the level of polyneuronal innervation. Of 22 singly innervated fibers, 13 were of the slow type, while 14 of 26 multiply innervated fibers were slow. In the second group (10-11 days), less than 20% of the fibers examined remained multiply innervated. Interestingly, at this later age, the clear preponderance (16 of 17) of multiply innervated fibers is of the slow histochemical type. Of 18 singly innervated fibers, 12 were of the slow type, indicating a moderate sampling bias for this type, probably owing to their larger size. These results contrast with the suggestion of Riley (Exp. Neurol. 56: 400, 1977), based on indirect evidence from neo-natal rat soleus, that synapse elimination proceeds somewhat more rapidly for Type I (histochemically slow) fibers.

We have also examined the innervation state at 18-22 days of age using the cut muscle technique to increase epp size. About 8% of the fibers examined were multiply innervated. As seen by Taxt et al. (Acta Physiol. Scand. 117: 557, 1983), these fibers consistently exhibit one very small epp component along with one much larger. We are now using the Lucifer Yellow labeling technique to determine if this residual polyinnervation reflects a hypothesized secondary synaptic reorganization in which slow motor units expand in size at the expense of fast motor units. Supported by NSF grant BNS 8408213.

31.3 FIBER TYPE COMPOSITION OF SINGLE MOTOR UNITS IN NEONATALLY REINNERVATED RAT SOLEUS MUSCLE. Carmen Soileau and Wesley J. Thompson, Dept. of Zoology, University of Texas, Austin, TX 78712.

Single motor units in the neonatal rat soleus muscle are composed of muscle fibers which are predominantly of one or the other of the two ATPase fiber types present in the muscle, despite the fact that all of the muscle fibers are polyneuronally innervated and the motor units are several times their adult size (Nature 309: 709; Gordon, thesis, Cal. Tech.). In hope of gaining some indication of whether this innervation pattern might represent a selective innervation of the different types of muscle fibers by the motor neurons, we have examined the selectivity with which single motor neurons reinnervate the different fiber types early in postnatal life. Rat soleus muscles were denervated by crushing the soleus nerve at its entry into the muscle at postnatal day 2. At day 14-18, the fibers contained in a single motor unit were marked in each of several muscles using the technique of glycogen depletion. The fiber types of the marked fibers were then determined by staining an adjacent muscle section for myofibrillar ATPase following alkaline preincubation at pH 10.4.

As reported previously (Neurosci. Abs. 10: 641), these neonatal muscles contained a reduced number of muscle fibers following reinnervation (about 55% of the number present in normal muscles). Nonetheless, the proportion of the fiber types was roughly normal: on average 52% of the fibers were type I and 48% were type II. The total number of motor units following reinnervation ranged between 19 and 25 (as compared with the normal 20-26), indicating that the majority of the motor axons had regenerated but that they now innervated a smaller number of muscle fibers.

Following glycogen depletion, units were found to contain 49-76 depleted fibers (avg. = 67). These fibers were distributed differently from those in normal muscles: the fibers were often confined to one area of the muscle (rather than being dispersed throughout the muscle as in normal animals) and in several cases the reinnervated fibers occurred in discrete clusters or groups.

While all depleted units were found to contain some mixture of the two fiber types, the mixtures have been, for 4 of the 6 units examined to date, far from random. For example, the most impressive deviations from randomness were in one unit which contained 99% type II fibers and in a second unit which contained 83% type I fibers. These results indicate an order to the innervation of the fiber types in the muscle at ca. 2 weeks following denervation, suggesting that there may be some selectivity to the reinnervation of the fiber types. At the moment however, we can not exclude the possibility that the reinnervation is initially random and that, within these 2 weeks, the fibers are converted to match their innervating motor neuron.

31.4 THE COMPARTMENTAL ORGANIZATION OF THE RAT EXTENSOR DIGITORUM LONGUS DURING POSTNATAL DEVELOPMENT. Rita J. Balice-Gordon and Wesley J. Thompson, Dept. of Zoology, University of Texas, Austin, TX 78712.

The nerve to the EDL divides into two branches prior to entering the muscle. We have investigated the distribution of the fibers innervated by each branch in young adult and newborn animals. In the adult muscle, the twitch and tetanic tensions elicited from each branch summate, indicating that each innervates a separate, non-overlapping population of muscle fibers. Using glycogen depletion to mark fibers innervated by each branch, we find that each innervates a contiguous subvolume or "compartment" containing roughly half of the muscle fibers. A sharp boundary exists between the two compartments which is rarely violated.

To investigate whether synapse elimination plays any role in setting up this compartmentalized innervation, we have determined the innervation pattern of each branch in newborn muscles. Even though each muscle fiber is polyneuronally innervated, and motor unit size measurements show that each motor unit is 2-3 times its adult size, each branch appears to polyinnervate fibers only within its own compartment. Tension measurements indicate that the innervation territory of each branch is discrete; moreover, glycogen depletion shows that the compartment boundary is as sharp at 1 day as in the adult. Therefore, it appears that the compartments are set up prenatally: alpha motor neurons are segregated into at least two nerve branches which subsequently confine their innervation to distinct muscle territories. One possible cause for this segregation would be some kind of physical barrier to axons crossing between the muscle regions; in the light microscope we see no obvious barrier. At this time, we have no clear explanation for how the innervation becomes segregated in this manner.

Several levels of compartmentalization exist in young adult as well as newborn EDL muscles. While EDL has one tendon of origin, it has four tendons which insert on the four digits of the foot. The proximal nerve branch innervates fibers which insert on digits 3, 4, or 5 whereas the distal branch innervates fibers which insert on digits 2 or 3. Silver stains reveal several intramuscular branches which may further compartmentalize the muscle into more than two innervation territories. Furthermore, the distribution of fiber types is non-homogeneous: a region of the muscle, containing the fibers that insert on the largest digit, 2, is devoid of type I or slow fibers. This distribution may be indicative of an additional level of compartmentalization: segregation of motor unit types. Although the functional significance of this regionalization is still unclear, the compartmental organization of EDL might allow the different regions of the muscle, each inserting onto separate digits, to function independently during locomotion.

31.5 NEONATAL SYNAPSE ELIMINATION IN THE RAT SUBMANDIBULAR GANGLION: EFFECTS OF TARGET ATROPHY. M.D. Womble*, K. Vanderslice*, and S. Roper. (SPON: M. McPheeters) Dept. of Anatomy, Univ. Colo. Med. Sch. Denver, CO 80262.

The elimination of excess synapses during development is a general phenomenon seen in many regions of the vertebrate nervous system, such as at neuromuscular junctions, several areas of the central nervous system, and peripheral autonomic ganglia. We have studied synapse elimination in the submandibular ganglion of neonatal rats, to determine the effects of target tissue atrophy on synaptic development. This ganglion provides parasympathetic innervation to the submandibular and sublingual salivary glands. Atrophy of the developing salivary glands was produced by ligating the main salivary ducts 2-4 days after birth at a point where postganglionic nerve fibers were not damaged. Ductal ligations produced marked salivary gland atrophy, reducing gland weight to approximately 40% of contralateral control wet weight at 9-12 days after birth. Intracellular recordings from postganglionic neurons which innervated these atrophied glands were conducted to determine the number of presynaptic inputs to these ganglion cells. In control unoperated animals, the average number of preganglionic inputs per cell normally declined from 2.13 0.77 (S.D.) (n=47) during the 3rd week after birth to 1.73 0.81 (n=45) during the 5th week after birth. This latter value was not different from that in ganglia from control adult rat (1.59 0.67; n=68). Ganglionic neurons projecting to atrophied salivary glands in newborn rats showed an acceleration in the process of input elimination. The average input number during the 3rd week was reduced to 1.65 0.70; (n=40), significantly lower than the above value for the 3rd week after birth, and not different from data taken from control ganglia either during the 5th week after birth or in adult animals. These changes are confined solely to the ganglion which innervates the atrophied gland, and thus are not due to bilateral or systemic changes governing synaptic development. The loss of preganglionic inputs was not further enhanced by prolonged target atrophy at later stages of development: the number of inputs/neuron was 1.64 0.75 (n=61) by the 5th week and 1.42 0.61 (n=19) in adult animals, neither of which were different from age-matched, unoperated controls. No differences were seen in average resting potentials or input resistances between neurons from experimental or control animals. Thus, target atrophy in the salivary glands leads to an acceleration in the process of normal synaptic elimination. This effect may be mediated by changes in the level of activity or alterations in trophic factors emanating from the target.

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31.6 ACTIVITY SHARPENS THE REGENERATED RETINOTECTAL PROJECTION: SENSITIVE PERIOD FOR STROBE AND TTX. L.E. Eisele* and J.T. Schmidt. Dept. Biol. Sci., SUNY Albany, Albany, NY 12222.

The regenerating optic nerve of goldfish initially forms a crude retinotopic map on the tectum, then sharpens it over several weeks, as determined with HRP staining of optic arbors (Schmidt et al., NS Abstr., 1984). Before 35 days postcrush, electrophysiological recordings from the tectum fatigue rapidly and are difficult to map. After 35 days normally organized maps with multiunit receptive fields (MURF's) of normal size, (approximately 11°) are obtained. Manipulations of visual activity (e.g. intraocular tetrodotoxin (TTX) to block spike activity or strobe illumination to synchronize activity) allow a correctly oriented map to form but the MURF's average 25° to 40° (Schmidt and Edwards, Brain Res., 1983; Schmidt and Eisele, Neurosci., 1985). Single unit receptive fields remain small (11°) in both experiments. Since these recordings are from presynaptic arbors of the ganglion cells, the enlarged MURF's are thought to reflect errors in targeting of the regenerating arbors. Our hypothesis is that appropriate synapses are stabilized based on correlated activity of neighboring ganglion cells that could lead to summation of their postsynaptic potentials and strengthen synapses in appropriate areas. The studies described here extend these findings by defining more precisely the period of sensitivity to TTX and strobe in two ways: 1) using a constant two week period begun at different times postcrush, and 2) looking for maximal effects with long strobe exposures.

In parallel TTX and strobe experiments, fish were recorded after their respective two week intervals. The least sharpened maps (MURF's averaged 29°) came from fish treated from 20 to 35 days. At 35 days the map is already sharp, but two weeks of strobe or TTX unsharpen it. After 50 days the regenerating projection becomes progressively less sensitive to both TTX and strobe. After 130 days, the projections are virtually unaffected, as are mature projections. The periods of sensitivity to TTX block and to strobe are very similar and correspond roughly to the time course of synaptogenesis (Murray and Edwards, JCN, 1982).

Fish reared under strobe conditions from 20 to 80 days have MURF's no larger than 20-35 day fish. Therefore there is no accumulative effect. Fish strobed from 14 days postcrush onwards had larger MURF's (36°) than those strobed from 20 days onward (24°). This indicates the importance of activity cues between 14 and 21 days, when the fibers first invade the tectum. (Supported by NIH grant EY 03736).

31.7 IMBALANCE IN SPONTANEOUS ELECTRICAL ACTIVITY CAUSES OCULAR DOMINANCE SHIFT IN KITTEN VISUAL CORTEX. B. Chapman*, M.D. Jacobson, H.O. Reiter and M.P. Stryker. Dept. of Physiology, School of Medicine, UCSF, San Francisco, CA 94143.

Classical studies in which monocular deprivation produced ocular dominance shifts in the visual cortex have involved rearing conditions in which at least one eye experienced patterned vision. More recently a similar shift in ocular dominance toward the less impaired eye has been produced in the absence of patterned vision (Jacobson, Reiter, Chapman and Stryker, this meeting). We hoped to determine whether a change in ocular dominance could be produced by an imbalance in spontaneous activity alone, or whether some vision, even if only the diffuse temporally modulated light seen through a fused eyelid, is necessary.

Five kittens, 28-33 days old, received intravitreal injections of tetrodotoxin (TTX) in one eye to block ganglion cell activity in that eye. All visual input from the other eye was eliminated by suturing the eyelid and keeping the kittens in continuous darkness. Action potential blockade was maintained for one week. The kittens then remained in the dark to prevent vision during recovery of retinal activity.

Single units were recorded in the lateral geniculate nucleus (LGN) and in area 17. In the LGN no difference in responsiveness was detected between cells driven by the two eyes, indicating that the retinal blockade was no longer in effect. In area 17, however, there was a significant ocular dominance shift favoring the eye that had been spontaneously active throughout the experiment.

Our results indicate that vision is not necessary to produce an ocular dominance shift in the kitten visual cortex. An imbalance in spontaneous activity alone can shift ocular dominance toward the more active eye. There was no statistical difference between the shift produced by our condition of spontaneous activity in one eye versus no impulse activity in the other eye, and the shift produced by vision through a sutured eyelid versus no activity (Jacobson, et al.). This supports the hypothesis that changes in the amount of retinal illumination alone have no effect on ocular dominance in the cortex.

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31.8 OCULAR DOMINANCE SHIFT IN KITTEN AREA 17 IN THE ABSENCE OF PATTERNED VISUAL EXPERIENCE. M.D. Jacobson, H.O. Reiter, B. Chapman* and M.P. Stryker. Dept. of Physiology, School of Medicine, UCSF, San Francisco, CA 94143.

Earlier work on various forms of monocular deprivation has revealed ocular dominance shifts in the visual cortex only when at least one eye had received patterned visual experience. The present results demonstrate that patterned vision is not necessary; rather an imbalance of electrical activities from the two eyes is sufficient to cause changes in the ocular dominance of neurons in area 17.

We sutured one eyelid of six 28-34 day old kittens and blocked all impulse activity from the other eye by intravitreal injection of tetrodotoxin (TTX). We maintained this imbalance--both eyes deprived of patterned vision but one of spontaneous activity as well--for one week, and then sutured the blocked eye's lid just before the TTX blockade wore off. We finally recorded the activity of single units in the lateral geniculate nucleus (LGN) and visual cortex area 17.

LGN cells responded as vigorously to the formerly TTX-blocked eye as they did to the other eye, demonstrating that the TTX blockade had completely subsided. In area 17, however, neurons were driven less strongly by the formerly blocked eye, resulting in an ocular dominance shift towards the previously lid-sutured eye. This shift was significant in all six experimental animals compared with untreated normal kittens recorded at the same age. In addition, neuronal cell bodies in geniculate laminae innervated by the blocked eye were smaller than those in laminae innervated by the sutured eye. Similar changes in geniculate cell sizes have been observed in older kittens treated in the same way as in our experiment (Kuppermann & Kasamatsu, Nature. 306: 465-468. 1983).

Our results demonstrate that vision is not an absolute requirement for causing ocular dominance shifts in the cat's visual cortex. Imbalances in the activities of the two eyes can shift eye preference towards the less-impaired eye even without patterned visual experience.

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31.9 TRANSPLANTATION OF GRANULOPRIVAL CEREBELLAR CULTURES WITH OPTIC NERVE CAUSES REDUCTION IN THE NUMBER OF SYNAPSES. C.K. Meshul and F.J. Seil, Neurology Research, VA Med. Ctr., and Dept. of Neurology, Oregon Health Sciences University, Portland, OR 97201.

Exposure of neonatal mouse cerebellar explants to cytosine arabinoside (Ara C) (5 ug/ml) for the first 5 days in vitro (DIV) destroyed granule cells and arrested those surviving glia in an early stage of maturation. Purkinje cells lacked an astroglial ensheathment and underwent marked sprouting of recurrent axon collaterals. Recurrent axon collateral terminals synapsed not only with somata of other Purkinje cells, but also with dendritic spines, sites normally occupied by terminals from granule cell axons (parallel fibers)(Blank et al., Neurosci., 7:1509, 1982). Transplantation of such granuloprival cultures with granule cells and mature glia resulted in Purkinje cell ensheathment by astroglia, and a marked reduction in the number of recurrent axon collaterals and of recurrent collateral terminals synapsing on Purkinje cell somata and dendritic spines. Synapses were formed between parallel fibers and Purkinje cell dendritic spines (Blank and Seil, J. Comp. Neurol., 214:267, 1983).

It was of interest to determine the role of glial cells alone in inducing changes toward normal organization in the transplanted granuloprival cultures. Neonatal mouse cerebellar explants, exposed to Ara C for 5 days, were transplanted with 7 day old mouse optic nerve at 13 DIV and maintained in normal nutrient medium until 22 DIV. They were then fixed and processed for light and electron microscopy. At the light microscopic level, it was observed that transplantation caused no significant reduction in the number of Purkinje cell axon collaterals. It was seen ultrastructurally that not only were Purkinje cells almost completely ensheathed by astroglia, but there was a significant reduction in the number of Purkinje cell somatic and dendritic synapses compared to the granuloprival cultures. The transplanted cultures also contained many more dendritic spines that were not making synaptic contact with presynaptic terminals. At the time period studied, there was no indication of glial fingers separating the pre- and postsynaptic elements. There was also no widespread degeneration of presynaptic terminals. The ensheathment of Purkinje cells by astroglia could be the physical element provoking the reduction in the number of Purkinje cell somatic synapses. Since a significant reduction in the number of recurrent axon collaterals was not observed, a detailed time course study of events leading to the reduction in the number of dendritic synapses will be needed.

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31.10 WHY ARE TRANSIENT CEREBROCEREBELLAR PROJECTIONS TRANSIENT? D.L. Tolbert* (SPON: P.A. Young), Francis and Doris Murphy Neuroanatomy Res. Lab., Depts. of Anatomy and Surgery (Neurosurgery), St. Louis Univ. Sch. of Med., St. Louis, MO 63104

Two non-mutually exclusive mechanisms may direct the elimination of transient projections during normal development of brain pathways. One mechanism involving competition between overlapping afferents in the target for a limited amount of trophic substance or synaptic space may result in unsuccessful axons being eliminated. Alternatively, the elimination of some collateral pathways may be intrinsically directed; reflecting a mechanism whereby collaterals, in excess of the number that the neuron can support through maturation, are selectively pruned. If pruning occurs, one might expect that eliminating the collaterals that normally persist will result in the persistence of the transient collaterals. This theory was tested on transient cerebrocerebellar projections in neonatal cats. In the second postnatal week, pyramidal tract axons branch and project to both the cerebellum and to the brainstem or spinal cord. Later all cerebrocerebellar collaterals are selectively eliminated, while the collateral projections in the pyramidal tract persist. In this study, the collaterals in the pyramidal tract were cut prior to the elimination of the cerebellar collaterals. Later orthograde labeling techniques were used to test for the persistence of cerebrocerebellar projections. In some animals, labeled pyramidal tract axons either ended abruptly at the lesion site or coursed around the lesion and continued through the brainstem and into the spinal cord. There were labeled projections to brainstem nuclei, both rostral and caudal to the level of the pyramidotomy, but none were ever observed projecting into the cerebellum. Injections of Fast Blue were made into the spinal cord to determine if the pyramidotomy caused the death of the axotomized cortical neurons. Fast Blue labeled cells were not present in the cerebral cortex ipsilateral to the pyramidotomy when the lesions were made in neonates, although in older animals (> 33 PND) retrogradely labeled cortical neurons remained after pyramidotomy. These findings suggest that the transience of cerebrocerebellar projections is not due to the inability of cortical neurons to sustain both cerebellar and brainstem/spinal collaterals but rather their transience is determined by axon-axon or axon-target interactions in the cerebellum. Furthermore, cortical projections to the cerebellar nuclei will persist if the nuclei are partially deafferented prior to the elimination of the cerebrocerebellar pathway (Anat. Rec., 211:146A, 1985). Ongoing experiments will determine whether cerebrocerebellar projections persist after combined pyramidotomy and partial deafferentation of the cerebellum.

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32.1 GLUTAMATE-ACTIVATED ION CHANNELS IN CULTURED HIPPOCAMPAL NEURONES. R.B. Clark*, M. O'Beirne* and B.A. MacVicar, Dept. Medical Physiology, University of Calgary, Calgary, Alberta, T2N 4N1, Canada. (Spon: S. Roth).

L-Glutamate is a putative excitatory neurotransmitter in the hippocampus, but little is known concerning the properties of the elementary events underlying the membrane response to glutamate and its analogues.

We have measured single ion channel currents, induced by glutamate, in neurones cultured from embryonic rat hippocampus, using the patch clamp technique of Hamill et al. (Pfugers Arch., 391, 85). "Cell-attached" patch recordings were made from neurones after 1-2 weeks in culture. Both cell bathing saline and patch pipette solution were nominally Mg^{2+}-free, but contained 1 mM Ca^{2+}. L-Glutamate (50-250 μm) was added to the patch pipette solution. Temperature was about 20^{0} C.

With glutamate in the pipette, inward current steps of ~2 pA amplitude were recorded at the resting potential of the neurones. Resting potential, measured with conventional (40-60 MΩ) intracellular microelectrodes, averaged -62 mV (± 7, n=5). Single channel current increased approximately linearly with membrane hyperpolarization up to 100 mV from rest (ie. Vm ~ -160 mV), and decreased with depolarizations of 20-30 mV. The slope conductance of the channel over this potential range was 49-55 pS. The apparent reversal potential of the single channel current, obtained by extrapolation, was 50-55 mV positive to cell resting potential, ie. near 0 mV.

Hyperpolarization appeared to reduce the fraction of time that the channel was open (P_o). For example, in one patch (50 μm Glutamate), P_o was 0.19 at the resting potential and decreased to 0.15 with 50 mV hyperpolarization and 0.08 with 100 mV hyperpolarization.

Channel currents occassionally exhibited transitions to 70% of the mean current level, suggesting the existence of subconductance states.

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32.2 A COMPARISON OF THE EFFECTS OF NMDA AND KAINATE ON CA1 PYRAMIDAL NEURONS IN VITRO. C. Rovira*, M. Gho*, Y. Ben-Ari and E. Chérubini. LPN1, CNRS, 91190 Gif-sur-Yvette, France.

The excitatory amino acids N-methyl-aspartic acid (NMDA) and kainic acid (KA) produce a long lasting increase of the excitability of hippocampal neurons. The aim of the present study was to compare their actions on CA1 pyramidal cells recorded intracellularly from the slice preparation.

When CA1 pyramidal cells are excited by a long intracellular depolarizing pulse the increase in the firing rate is not maintained (accommodation) perhaps as a result of the activation of K^+ channels (notably $gKCa^{2+}$ and the M-current, e.g. Madison and Nicoll, 1984). Application of kainate (50-200 nM) produces a small depolarization ($<$10 mV) and an increase in the firing rate. At the resting membrane potential, the accommodation and the after-hyperpolarization (AHP) which followed the depolarizing pulse were reduced. NMDA (AHP) induced a small depolarization ($<$5 mV), bursts of spikes but, in contrast to kainate, a clear cut enhancement in the amplitude and duration of the AHP.

In the presence of TEA (5-10 mM) and TTX (1 μM), the depolarizing pulse produced Ca^{2+} spikes (blocked by Co^{2+}). NMDA augmented and kainate reduced the number of these spikes. When an AHP was still present (lower doses of TEA), it was increased in amplitude and duration by NMDA and decreased or abolished by KA. Barium spikes (produced in 0 Ca^{2+}, TTX (1 μM) and Ba^{2+} : 2 mM) were completely blocked by kainate but not by NMDA. Finally, in Mg^{2+} free medium, the effects of NMDA were completely abolished.

In summary, NMDA and KA induce epileptiform activity but have opposite effects on Ca^{2+} spikes and AHP mediated events. The enhancement by NMDA of Ca^{2+} spikes and AHP and its blockade in the absence of Mg^{2+} is in keeping with the suggested Mg^{2+} associated region of negative slope conductance (Nowak et al., 1984). The mechanisms of action of kainate are still unclear ; our results however suggest that the blockade by kainate of the accommodation and AHP may play a more direct role in the epileptogenic properties of this compound.

Madison D.V. and Nicoll R.A. J. Physiol., 354, 319-331, 1984.
Nowak L., Bregestovski P., Asher P., Herbert A. & Prodiantz A. Nature, 307, 462-465, 1984.

32.3 MICROMOLAR AND NANOMOLAR CONCENTRATIONS OF D-2-AMINO-5-PHOSPHONOVALERATE HAVE OPPOSITE EFFECTS ON EPILEPTIFORM BURST-FIRING IN THE RAT HIPPOCAMPAL SLICE. G.L. King and R. Dingledine. Dept. of Pharmacology, Univ. North Carolina, Chapel Hill, NC 27514.

2-Amino-5-phosphonovalerate (APV) is thought to be a selective antagonist for N-methyl-D-aspartate (NMDA) receptors, which when activated by N-methyl-aspartate turn on a voltage- and calcium-dependent cation conductance in hippocampal pyramidal cells. We have previously reported (Hynes & Dingledine, 1984, Neurosci. Abstr. 10: 229) that 10-100 μM DL-APV reduces the duration of epileptiform bursting induced in the CA1 region by bicuculline. This action appears specific as micromolar concentrations of DL-APV do not affect: 1) excitatory synaptic transmission, 2) spike-frequency adaptation, 3) voltage-dependent calcium spikes or 4) cell membrane potential and input resistance. We have further characterized the action of the more potent D isomer of APV on epileptiform burst-firing induced in the CA1 region by 100 μM picrotoxin. These studies were performed in both an interface and submersion chamber and compounds were applied either in the superfusate or by pressure ejection.

In extracellular recordings of population spike bursts evoked by stratum radiatum stimulation, 1-100 μM concentrations of D-APV, like DL-APV, selectively reduced burst duration without affecting the amplitude of the first population spike; the latter was reduced by 1000 μM concentrations. Half-maximal inhibition of bursting occurred at 1-10 μM APV. Intracellular recordings showed that, in a fashion also similar to DL-APV, 10-100 μM concentrations of DAPV reduced the number of population spikes in an evoked burst and decreased the duration of the depolarizing wave underlying the burst, with no effect on either input-resistance or membrane potential. Furthermore, at current intensities below threshold for evoking an action potential in disinhibited slices, D-APV reduced the amplitude of the excitatory postsynaptic potential (EPSP) in a weakly voltage-dependent manner. These results are consistent with D-APV blocking a synaptically-activated NMDA receptor, and also suggest that the postsynaptic receptor responsible for the epileptiform burst is distinct from that which mediates the conventional EPSP.

In contrast, and quite unexpectedly, nanomolar concentrations (10-100) of D-APV prolonged the extracellularly recorded epileptiform burst. Such burst prolongation was also seen under low-magnesium conditions. These results raise the possibility that D-APV, like nicotine action on ACh receptors, may activate and then block NMDA receptors. We are presently investigating whether this effect of D-APV at lower concentrations is mediated by the NMDA receptor. Supported by NS-06953, NS17771 and DA-12360.

32.4 EXCITATORY ACTIONS OF L-PROLINE IN THE RAT HIPPOCAMPAL FORMATION: PHARMACOLOGY, EXCITOTOXICITY AND SODIUM-DEPENDENT UPTAKE. J. Victor Nadler, Brian Ault, Abraham Hakim* and Debra Pistorino*. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

L-Proline blocks the response of hippocampal pyramidal cells to orthodromic and antidromic stimulation, as judged by recording of field potentials in the rat hippocampal slice (Ault and Nadler, Soc. Neurosci. Abstr., 10, 229 (1984)). The block appears attributable mainly to excessive postsynaptic depolarization, because (1) enhancement of cell firing preceded the block, (2) acidic amino acid excitants produced the same effects and (3) the effects of L-proline on antidromically-evoked firing were only partially reduced by superfusion with Ca^{2+}-free medium. Because few neutral amino acids appear to possess neuroexcitatory activity, we studied the pharmacology, excitotoxicity and sodium-dependent uptake of L-proline for comparison with the well-characterized acidic amino acid excitants.

The pharmacology of excitatory responses to L-proline was tested on the antidromic population spike in area CA1 of the rat hippocampal slice. Antidromically-evoked pyramidal cell firing was completely blocked by 8 mM bath-applied L-proline, but was unaffected by D-proline. The suppressive effect of L-proline was reversibly abolished by 500 μM γ-D-glutamylglycine or kynurenate. 25 μM 2-amino-5-phosphonovalerate (2-APV, AP5), which totally prevented any response to N-methyl-D-aspartate, did not alter responses to L-proline. Removing Mg^{2+} from the superfusion medium increased the potency of L-proline about 4-fold. These results suggest that L-proline acts at some type of excitatory amino acid receptor, although its pharmacology does not appear to coincide with that of any excitatory amino acid receptor yet described. Interestingly, all treatments that affected the action of L-proline similarly affected transmission at Schaffer collateral-commissural synapses.

L-Proline produced axon-sparing lesions of the hippocampus upon focal injection into that region. These lesions closely resembled those produced by equal doses of L-aspartate.

Crude synaptosomal fractions of hippocampal formation took up L-[^{3}H]proline in a sodium-dependent manner. Among hippocampal regions, regio superior exhibited the greatest uptake and fascia dentata the least. Preliminary lesion studies indicate an association of proline uptake with projections of CA3 pyramidal cells.

These results provide further evidence for an excitatory action of L-proline and suggest that this amino acid may play a role in the neuroexcitation produced by projections of CA3 pyramidal cells. (Supported by NIH grant NS 16064.)

32.5 **DICARBOXYLIC AMINO ACIDS BLOCK EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL SLICE.** J. ffrench-Mullen* & R.S. Fisher. Neurology, Johns Hopkins Hospital, Baltimore, Md. 21205.

A recent study (Bernstein & Fisher, 1985) has shown that L-GLUT perfusion over hippocampal slices produces blockade of excitatory transmission, relatively preserving function of parallel inhibitory pathways. Utilizing field and intracellular recordings in the in vitro rat hippocampal slice we show that exposure to dicarboxylic amino acids can block epileptiform activity. Penicillin (PEN),1.7-3.4 mM, was used to produce epileptiform potentials. Subsequent perfusion with 1-2 mM L- and D-GLUT, and L-ASP, blunted epileptiform activity. N-methyl-aspartate (NMA) at 5-15 micromolar enhanced epileptiform field activity produced by PEN, but depressed it at higher doses.

Intracellular recordings were obtained from 16 CA1 pyramidal cells. A transient depolarization averaging 4.0±0.9 mV occurred with 2 mM GLUT. Steady-state RMP at 5 minutes after start of perfusion was 57±1.6 mV in control versus 57±1.9 mV in PEN + L-GLUT (n=10, N.S.). Over-depolarization of the post-synaptic neuron did not appear to be a major factor in the decline of penicillin-induced epileptiform activity. Changes in input resistance were also of small magnitude (megohms): 36.0±3.3 in control vs. 37.6±3.7.

The major action of 2 mM L-GLUT perfusion (in the presence of PEN) was blockade of evoked EPSPs and depolarization shifts. On the average, s. radiatum test shocks produced depolarizations of 9.5±1.4 mV in control perfusate, 14.6±1.4 mV in penicillin and 1.9±0.8 mV in 2 mM L-GLUT (n=8, p<.01 CONTROL vs PEN+GLUT and PEN vs PEN+GLUT, paired t-tests). Changes reversed with wash to control perfusate. The decrease with 1 mM L-GLUT was less severe, but still statistically significant. Intracellular effects of D-GLUT and L-ASP paralleled those reported above.

This blockade of synaptic transmission and epileptiform activity by exposure to dicarboxylic amino acids suggests a mechanism for physiological "down-regulation" of neurotransmission during periods of excessive excitation.

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32.6 INHIBITION OF HIPPOCAMPAL EXCITATORY TRANSMISSION AND DISPLACEMENT OF DL-[^{3}H]-2-AMINO-4-PHOSPHONOBUTANOIC ACID (DL-APB) BINDING BY CYCLIC ANALOGUES OF APB AND GLUTAMATE. M.B Robinson, S.L. Crooks*, R.K. Freund, J.F. Koerner and R.L. Johnson*. Depts. of Biochemistry and Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455.

L-APB selectively inhibits excitatory lateral entorhinal inputs to hippocampal granule cells [Koerner & Cotman, Brain Res., 216 (1981),192]. A class of [^{3}H]-glutamate binding sites distinguished by Ca^{++}/Cl^- dependence [Fagg et al., J. Neurosci., 2 (1982) 958] is displaced by L-APB in the same concentration range (1-10 µM) as that observed for inhibition of synaptic transmission. A DL-[^{3}H]-APB binding site shows similar ligand specificity and ion dependence [Monaghan et al., Brain Res., 278 (1983) 137; Robinson et al., Biochemistry, 24 (1985) in press].

We have synthesized the following cyclic analogues of glutamate and APB to identify structural properties necessary for inhibition of the perforant path and to compare these properties to those for displacement of APB binding: (±)-trans-1-amino-1,3-dicarboxycyclopentane (trans-ADCP), (±)-parf-1-amino-3-phosphonocyclohexanecarboxylic acid (trans-cyclohexyl APB), (±)-pref-1-amino-3-phosphonocyclohexanecarboxylic acid (cis-cyclohexyl APB), and one of the racemic diastereoisomers of 1-amino-3-phosphonocyclopentanecarboxylic acid (cyclopentyl APB). These compounds were bath-applied to the submerged rat hippocampal slice. Evoked synaptic field potentials were recorded with an extracellular electrode placed in the terminal field of lateral or medial entorhinal projections to dentate granule cells. They were also tested for displacement of DL-[^{3}H]-APB binding from synaptic plasma membranes prepared from rat forebrain. Except for trans-ADCP, they all required concentrations greater than 1 mM to inhibit synaptic responses by 50%, and they did not induce population spiking (indicative of postsynaptic depolarization). Trans-ADCP inhibited lateral perforant path responses with an IC_{50} = 400 µM. The inhibition curve for medial perforant path responses spanned a wide range of concentrations suggesting heterogeneity in the response with IC_{50}'s in the range of 30 µM to 1.5 mM. Population spiking occurred at the higher concentrations. Trans- and cis-cyclohexyl APB inhibited 50% of the DL-[^{3}H]-APB binding at 1 mM while cyclopentyl APB and trans-ADCP inhibited 50% of this binding at 3-6 µM.

To summarize, trans- and cis-cyclohexyl APB showed weak potency in both assay systems. Cyclopentyl APB was also a weak inhibitor of lateral perforant path responses, but in contrast to the cyclohexyl derivatives of APB, was 200-fold more potent as a displacer of DL-[^{3}H]-APB binding than as an inhibitor of synaptic responses. These data support our previous suggestion that this binding site is a different or altered receptor from that identified by electrophysiology. [Supported by NIH NS17944].

32.7 KAINIC ACID-EVOKED SEIZURES AND BINDING SITES IN EPILEPTIC AND NON-EPILEPTIC FOWL. D.D. Johnson, J. Tuchek*, R. Wilcox* and R.D. Crawford*. Depts. of Pharmacology and Animal and Poultry Science, Univ. of Saskatchewan, Saskatoon, Canada S7N 0W0.

Epileptic fowl have a high seizure susceptibility as the result of an autosomal recessive mutation. Homozygotes (epileptics) seize spontaneously and in response to photic stimulation or hyperthermia. The heterozygote hatchmates (carriers) do not respond to photic stimulation. In order to begin to test the hypothesis that the high seizure susceptibility may be due to abnormalities in the function of excitatory amino acids we have compared kainic acid (KA) binding parameters and the dose-response relationships for kainic acid-evoked seizures in epileptic and carrier fowl. Binding studies were performed using [^{3}H]-kainic acid and a synaptosomal membrane preparation from adult chickens in a filtration assay system. As in rodents, saturable high affinity binding of [^{3}H]-kainic acid at two sites could be demonstrated in adult chicken brain. Specific binding was always greater than 70% of total binding. In both carriers and epileptics two binding sites for KA were detected. The K_D values (mean ±S.D.) were 2.49±0.96 nM and 66.5±7.8 nM for epileptics; 5.69± 0.64 nM and 67.9±7.5 nM for carriers. The K_D of the high affinity site in epileptics was significantly lower than that in carriers (2.49 vs 5.69, n=6). No differences were detected in B_{max} values (21.3±11.4 and 405.6 fmols/mg protein in epileptics; 42.1±9 and 429.3 fmoles/mg (40 nM) protein for carriers). In displacement studies of bound KA, IC_{50} values were 81.7 nM for kainic acid, 3.5 µM for glutamate and 1.1 µM for quisqualate. No differences between carriers and epileptics was detected. GABA, flurazepam, glycine, metrazol and phenobarbital in the concentration range 10^{-3}-10^{-5}M had no effect on KA binding.

In order to determine dose-response relationships for the induction of seizures by KA, groups of ten one-day-old epileptic or carrier chicks were given i.p. injections. KA evoked ataxia, and repeated clonic seizures. Epileptic chicks were more sensitive to the convulsant effects of KA than their heterozygote hatchmates. The percentage incidence of seizures was 70, 70, and 90 in epileptics and 10, 50, 50 in carriers for doses of 4, 6 and 8 mg/kg respectively. In addition the mean time to onset of the first seizure was significantly less in epileptic than in carrier chicks. These data suggest that the high seizure susceptibility of epileptic chickens may be associated with abnormalities in the function of excitatory amino acids or their receptors.
(Supported by the Medical Research Council of Canada MA-5893).

32.8 A PUTATIVE G_1 EXCITATORY AMINO ACID RECEPTOR MEDIATES VESTIBULAR AFFERENT TRANSMISSION IN RATS. M. R. Lewis*, J. P. Gallagher and P. Shinnick-Gallagher. Dept. of Pharmacology and Toxicology, Univ. Tex. Medical Branch, Galveston TX 77550.

The neurotransmitter and receptor mediating afferent transmission in the medial vestibular nucleus (MVN) was studied using intracellular recording in an in vitro rat brain slice preparation. The preparation consists of a transverse slice of the rostral medulla, containing the major portions of the MVN and entering tracts of the VIIIth nerve. Excitatory postsynaptic potentials (EPSPs) in second-order neurons with latencies of 1mSec were evoked by focal stimulation of VIIIth nerve tracts within the slice. Drugs were applied by drop or superfusion.

EPSPs in second-order neurons were not affected by the cholinergic antagonists hexamethonium or atropine, the H_1-antagonist diphenhydramine or the P_1-antagonist caffeine. However, the excitatory amino acid antagonists kynurenic acid, DL-α-aminoadipate and DL-aminophosphonobutyrate (APB) reduced the EPSPs while D-aminophosphonovalerate and γ-D-glutamylglycine had no effect. These reductions in EPSP size occurred in the absence of nonspecific membrane effects. However, the D- and L- forms of α-aminoadipate separately caused depolarizing or hyperpolarizing effects. APB was the most potent antagonist of the EPSP.

Additionally, the second-order neurons demonstrated non-desensitizing depolarization to DL-homocysteate, and desensitizing depolarization to other agonists such as quisqualate and N-methyl-D-aspartate (NMDA). During desensitization to quisqualate, the EPSP was not reduced.

EPSPs and depolarizations induced by DL-homocysteate and L-aspartate consistently involved increases in conductance while responses to quisqualate, NMDA, and kainate were associated with decreases in conductance. Conductance changes associated with glutamate were highly variable.

These results support our hypothesis that the EPSPs recorded from rat MVN are mediated by an excitatory amino acid (possibly aspartate). The receptor appears to be of the classification "G_1" as defined by Fagni, Baudry and Lynch (J. Neurosci., 3:1538, 1983). We are currently investigating the ionic mechanism of the synaptic response. (Supported by NASA NAG 2-260)

32.9 DIFFERENTIATION BETWEEN GLUTAMATE RECEPTORS BASED ON DESENSITIZATION C.F. Zorumski, G.D. Fischbach Depts. of Psychiatry and Anatomy and Neurobiology, Washington U. Med. School, St. Louis, MO 63110

Glutamate produces excitation by activating two conductances. One, G_1, exhibits a non-linear current-voltage relation with decreasing inward current at hyperpolarized membrane potentials, is activated by N-methyl-D-aspartate and is blocked by 2-amino-5-phosphonovalerate (APV). The other conductance, G_2, exhibits a linear current-voltage relation, is activated by kainate or quisqualate and is insensitive to APV (Mayer & Westbrook, 1984). By voltage-clamping chick spinal cord neurons, we have obtained evidence that these conductances can also be distinguished on the basis of desensitization.

Spinal cord neurons were dissociated from six-day chick embryos and studied between 3 to 10 days after plating with gigaseal whole-cell recording techniques. For recording, growth media was replaced with a solution containing 140 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 10 mM glucose, 12.5 mM HEPES, pH=7.2. Patch pipettes contained 140 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, 11 mM EGTA·KOH, 1 mM $CaCl_2$, pH=7.2. Glutamate was applied by pressure ejection from pipettes positioned 10-50μ from the cell body.

Prolonged (10 second) glutamate applications led to a 27% decline in the glutamate current (N=16). Repeated, 500 msec glutamate applications, at 30 second to 1 minute intervals, also led to a decline in the peak inward current. The desensitization was completely reversible in five minutes. The effect was glutamate concentration dependent, being most reliably produced by concentrations above 100 μM and was critically dependent on extracellular calcium. At an external calcium of 10 mM, the glutamate current showed a 41% decline on repeated administration (N=35).

Two lines of evidence indicate that this desensitization involves the G_1 conductance. First, in the presence of saturating concentrations of APV (1 mM), no rundown of the glutamate current was seen. Furthermore, $CoCl_2$ (1 mM), which also blocked G_1 responses, eliminated the desensitization. Second, very little desensitization was observed when the membrane was clamped to -85 mV, a potential at which the G_1 conductance is markedly decreased. The decrease in G_1 current at hyperpolarized potentials is due to a voltage dependent channel block by Mg^{++} (Nowak et al, 1984). In the absence of Mg^{++}, the voltage dependence of glutamate desensitization was eliminated.

In summary, cultured chick spinal neurons exhibit desensitization to prolonged or repeated glutamate applications. This desensitization is calcium dependent and primarily involves the G_1 conductance.

32.10 KYNURENIC ACID DISTINGUISHES BETWEEN QUISQUALATE AND KAINIC ACID RECEPTORS IN THE MUDPUPPY RETINA. R.F. Miller and P.A. Coleman*. Department of Ophthalmology, Washington University School of Medicine, St. Louis, MO 63110.

We have discovered that kynurenic acid (KynA) distinguishes kainic acid (KA) and quisqualate (QQ) responses among third order neurons of the mudpuppy retina. This raises the possibility that different receptors mediate KA and QQ actions.

We used bath application of all agents in a perfused retina-eyecup preparation. Kynurenate was applied in concentrations of 2.5 to 10 mM and KA and QQ were used at 10 to 100 μM.

In the outer retina KynA has an antagonist action similar to that of cis, 2,3 piperadine dicarboxylic acid (PDA)[1]. Horizontal cell and off-bipolar light responses are blocked by KynA, while on-bipolars are comparatively unaffected.

Among second order neurons QQ appears to be a somewhat weaker agonist when compared to KA: The action of KA is entirely blocked by continuous 5 mM KynA, but an attenuated effect of QQ (100 μM) persists.

Among third order neurons (amacrine and ganglion cells) QQ is generally a more effective agonist compared to KA and evokes strong excitation at 10 μM levels. At a concentration of 5 mM, KynA significantly reduces light-evoked responses and entirely blocks the depolarization of exogenously applied KA (100 μM); at this concentration of KynA, QQ still produced significant depolarizations. Strong QQ and KA effects are seen after synaptic transmission is blocked with Co^{++} suggesting that receptors for these agonists are intrinsic to third order cells.

These findings raise the possibility that QQ receptors may be distinct from KA receptors. However, a single receptor type with differential affinity for QQ and KA cannot be eliminated.

Further evidence in support of separate QQ and KA receptors has come from some selective ganglion cell recordings. Most recordings show QQ and KA cause a depolarization. In a small percentage of ganglion cell recordings KA depolarizes, whereas QQ hyperpolarizes. At higher concentrations both KA and QQ inhibit. This inhibitory effect is converted to excitation by blocking inhibition with a picrotoxin/strychnine cocktail (10 μM each). Thus the QQ inhibition appears to be mediated by inhibitory amacrine cells in these special cases. This observation raises the possibility that inhibitory amacrine cells may contain receptors which are comparatively more QQ sensitive than those of ganglion cells.

KynA is also an effective NMDA antagonist and is, therefore, a broad spectrum antagonist. As far as we know this is the first report of a separate KA vs. QQ antagonism for kynurenate.

Supported by NEI grant EY03014 and EY07057

1. Slaughter, M.M. and R.F. Miller (1983) Science 219:1230-1232.

32.11 THE APPEARANCE OF CHEMOSENSITIVITY TO EXCITATORY AMINO ACIDS IN CULTURES OF MOUSE SPINAL CORD NEURONS. C.L. Mitchell and G.L. Westbrook. Lab. of Developmental Neurobiology, NICHD, IRP, NIH, Bethesda, Md. 20205

L-glutamate (GLU) and L-aspartate (ASP) are neurotransmitter candidates at primary afferent and intraspinal synapses in the mammalian spinal cord. Several receptor types exist for the excitatory amino acids based on selective activation by kainate (KA), quisqualate (QA) and N-methyl-D-aspartate (NMDA), whereas GLU can activate both NMDA and non-NMDA (i.e. either QA or KA) receptors. Recent evidence in culture suggests that a non-NMDA receptor mediates monosynaptic EPSPs formed between dorsal root ganglion and dorsal horn neurons (Jahr & Jessell, J.Neurosci., In press), as well as between spinal cord neurons (Nelson, Pun & Westbrook, submitted). To study the development of these receptors and their relationship to synaptic activity, we have used whole-cell patch recording to test the chemosensitivity of spinal cord neurons during the first week in culture.

Neurons were dissociated from 13 day embryonic mice and plated on collagen-coated 35 mm dishes at 6 X 10^5 cells per plate. Growth medium contained 5 % horse serum and a nutrient supplement. Whole-cell patch recordings were made in physiological saline containing 2 mM Ca^{2+} and 1 mM Mg^{2+}. Agonists were dissolved in recording solution and pressure ejected from pipettes near the soma. Under these culture conditions, neurite outgrowth begins within 24 hours and Na^+-dependent action potentials can be evoked with intracellular stimulation. However no detectable spontaneous EPSPs or IPSPs (> 200 μV) are present for the first 2-3 days, then spontaneous synaptic activity develops rapidly between days 4 and 7 (Westbrook, et al., Soc. Neurosci Abs. 9, 505, 1983).

At day 2-3, most spinal cord neurons were sensitive to QA (10 μM) and GLU (100 μM) as well as γ-aminobutyric acid (GABA, 100 μM). Responses to KA (10 μM) were small or absent. QA and GABA responses were associated with conductance increases while GLU responses resulted in little or no apparent conductance change. Under voltage clamp GLU-activated currents had a region of zero or negative slope conductance consistent with a mixed agonist action on both NMDA and non-NMDA receptors. Both QA and GLU-activated currents had reversals potentials near 0 mV. GABA responses reversed at -50 mV with $KMeSO_4$ and at 0 mV with CsCl solutions in the patch electrode, consistent with a chloride conductance. By day 7, spinal cord neurons were highly sensitive to all agonists tested.

These results suggest that both NMDA and non-NMDA receptors are present before detectable synaptic activity, but that sensitivity increases during a period of rapid synapse formation.

32.12 CONTINUOUSLY INFUSED 2-AMINO-7-PHOSPHONOHEPTANOIC ACID ANTAGONIZES N-METHYL-D-ASPARTATE MEDIATED INCREASES OF CYCLIC GMP IN SEVERAL BRAIN REGIONS. P.P. McCaslin* and W.W. Morgan. Dept. Cellular and Structural Biology, Univ. Texas Hlth. Sci. Ctr. at San Antonio, TX 78284.

2-amino-7-phosphonoheptanoic acid (APH) has been shown to be a comparatively selective antagonist of N-methyl-d-aspartate (NMDA)-mediated excitation as well as a potent anticonvulsant in some species. The effects of the continuous intracerebroventricular (icv) administration of this compound were examined as a prelude to the use of APH in investigations of the role of central excitatory dicarboxylic acidic amino acid neurotransmitter pathways in the development of barbiturate dependence. A 0.023 inch internal diameter polyethylene cannula was stereotaxically implanted into the lateral cerebroventricle of ovariectomized Sprague-Dawley rats and connected to a subcutaneously implanted Alzet minipump. The minipump delivered saline or 54, 27, 2.7 or 0.27 microgram (μg) of APH icv/day for 48 hours before further experimentation. The 27 μg/day dosage was the highest which by itself did not produce behavioral depression. The continuous icv administration of APH produced a dose-related suppression of cyclic guanosine monophosphate (cGMP) levels in the cerebellum but not the cerebral cortex, thalamus or hippocampus. The acute icv injection of 1000 nanograms (ng) of NMDA produced a marked elevation of cGMP in all the above brain regions and induced overt wild running behavior. Pretreatment by the continuous icv infusion of 27 μg of APH for 48 hours completely suppressed the effects of NMDA on cGMP and on behavior. However, this same treatment with APH was totally ineffective in suppressing convulsions induced by acute subcutaneously administered pentylenetetrazol (PTZ, 30 mg/kg). These results suggest that NMDA receptor-related pathways may play a tonic role in the regulation of cerebellar cGMP levels and that there is an NMDA receptor mechanism with input to similar cGMP-related pathways in other brain regions. On the other hand, this mechanism in these other brain regions does not appear to be tonically active. In the rat, APH does not appear to be effective against PTZ-induced convulsions. These data call into question whether an antagonism of NMDA receptors is responsible for the anticonvulsant effects of this drug observed in other species. Supported by NIDA grants DA00755 and DA00083 and by USAF Contract F33615-83-C-0624.

32.13 MODELS OF N-METHYL-D-ASPARTATE ANTAGONISM: A COMPARISON OF 2-AMINO-7-PHOSPHONOHEPTANOIC ACID WITH MUSCLE RELAXANT DRUGS. P.S. Bernard,* G. Pastor* and J.M. Liebman. Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit NJ 07901

It has been proposed that antagonists of N-methyl-D-aspartate-type excitatory amino acid receptors may have various useful therapeutic properties (Meldrum, Clinical Science 68:113, 1985). The reliable in vivo detection of novel NMDA antagonists therefore constitutes an important research problem. These substances have been shown to antagonize audiogenic seizures in DBA/2 mice (Meldrum, Epilepsia 25:Suppl. 2, S140, 1984) but since a wide variety of other substances are also effective, this test procedure lacks selectivity. The present experiments have characterized two effects of systemically administered NMDA (hindlimb scratching and seizures) in mice and their relative responsiveness to a putative NMDA-type receptor antagonist, 2-amino-7-phosphonoheptanoic acid (AP-7). Because the muscle relaxant propeties of AP-7 pose potential difficulties of interpretation, other muscle relaxants, including baclofen and diazepam, have also been assessed for comparison.

Male CF-1 mice were treated with 54 mg/kg i.p. to induce hindlimb scratching behavior, and with 154 mg/kg to induce seizures. Audiogenic seizures were assessed in DBA/2 mice, a seizure-prone strain following exposure to a 110 db bell. Animals received all drugs intraperitoneally 45 min prior to treatment. AP-7 reduced both NMDA-induced hindlimb scratching (ED_{50} = 48 mg/kg) and NMDA-induced clonic seizures (ED_{50} = 36 mg/kg). These ED_{50} values were only slightly higher than that for AP-7 to inhibit audiogenic seizures (ED_{50} = 22 mg/kg) and were considerably lower than that for blockade of the traction reflex (ED_{50} = 110 mg/kg), an indication of neuromuscular impairment. All three models, therefore, appeared to reflect the pharmacological action of AP-7. Baclofen antagonized both NMDA-induced scratching (ED_{50} = 0.7 mg/kg) and audiogenic seizures (ED_{50} = 4 mg/kg) at doses lower than those blocking the traction reflex (ED_{50} = 10 mg/kg). However, baclofen did not block NMDA-induced seizures. Diazepam also blocked audiogenic seizures and NMDA-induced scratching but not NMDA-induced seizures.

In conclusion, the NMDA seizure model discriminates between AP-7 and at least two other muscle relaxants to a greater extent than does the audiogenic seizure model or the NMDA hindlimb scratching model. The in vivo detection and characterization of NMDA-like receptor antagonists should be facilitated by the use of this simple rodent model.

32.14 THE EXCITATORY AMINO ACID RECEPTOR ANTAGONIST, 2-AMINO-7-PHOSPHONOHEPTANOIC ACID (AP7), PRODUCES ANTICONFLICT ACTIVITY, D.A. Bennett, C.L. Corradi and J. Lehmann. Neuroscience Res., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

2-Amino-7-phosphonoheptanoic acid (AP7) has been identified as an antagonist of the cortical excitatory amino acid receptor sensitive to N-methyl-D-aspartate (NMDA) (Perkins et al., Neurosci. Lett., 23:333,1981). As the excitatory amino acid system functions in opposition to the inhibitory amino acid system, it is not unrealistic to propose that an excitatory amino acid receptor antagonist would produce effects comparable to an inhibitory amino acid agonist (i.e. GABA). AP7 has been reported effective in antagonizing seizure behavior (Czuczwar et al., Eur. J. Pharmacol., 83:335, 1982; Croucher et al., Science, 216:899,1982) and effective as a muscle relaxant in genetically spastic rats (Turski et al., Neurosci. Lett., 37:75,1983). These effects are produced after either i.c.v. or i.p. administration. HPLC analysis of mouse brain indicates that AP7 crosses the blood brain barrier with a peak concentration at 30 min (Chapman et al., Neurosci. Lett., 37:75,1983).

We now report that AP7 produced a significant anticonflict effect in rats. At doses of 10 and 30 mg/kg i.p., increases in punished responding (FR-10) of 191 and 130% were seen as compared with baseline controls. No significant decrease in non-punished (VI-30 sec) responding was noted, although at 30 mg/kg i.p. this response was reduced by 11%. At 3 mg/kg i.p. AP7 produced an insignificant increase (34%) in punished responding, whereas 100 mg/kg i.p. produced severe response suppression. In rats trained to discriminate diazepam (10 mg/kg p.o.) from vehicle, AP7 produced dose-dependent (ED_{50} approx. 54 mg/kg i.p.) generalization. This finding might be construed as further evidence of anxiolytic activity. An alternative explanation is that the diazepam cue may be based partly upon muscle relaxant activity. This latter interpretation is consistent with rotorod deficit (60-70%) seen in rats after doses of 100 and 173 mg/kg i.p. of AP7. When the benzodiazepine antagonist, CGS 8216, was given in combination with a dose of AP7 (173 mg/kg i.p.) that induced 90% rotorod deficit in mice, no antagonism was seen. This is in contrast to the complete antagonism of the diazepam-induced rotorod deficit seen in mice following CGS 8216 (ED_{50} = 3.9 mg/kg p.o.), and suggests that the muscle relaxant effect of AP7 is not mediated through benzodiazepine mechanisms.

In summary, like diazepam, AP7 produced anticonflict activity, generalized to diazepam stimuli and produced rotorod deficit. These effects are thought to be directly or indirectly mediated through the excitatory amino acid system without involving the benzodiazepine receptor. The behavioral profile of AP7 reflects activity comparable to what would usually be found with inhibitory amino acid system agonists.

32.15 SYSTEMIC INJECTION OF N-METHYL-ASPARTIC ACID INCREASES ESTROUS CYCLE LENGTH IN FEMALE C57BL/6J MICE. S.G. Kohama,* P.C. May and C.E. Finch. Andrus Gerontology Ctr., USC , Los Angeles, CA 90089.

Mature mice treated as neonates with monosodium glutamate exhibit a variety of neuroendocrine impairments consonant with an excitotoxic lesion of the arcuate nucleus. However, neuroendocrine functions of mice treated as adults with systemic administration of excitotoxins has not been examined. N-Methyl-D,L-aspartic acid (NMA), a neuronal excitotoxin, was administered by s.c. injection (0, 100 or 200 mg NMA/kg body weight; n=13/group) to 8 mo old female C57BL/6J mice and their estrous cycles monitored by analysis of vaginal cytology. Estrous cycle length increased in NMA treated mice compared to their pre-treatment cycle length ($p<0.05$) while vehicle injection had no significant effect on cycle length. The average cycle length one month following treatment was greater in the 200 mg/kg NMA treated mice than controls (6.6 $\pm$ .4 days vs. 5.4 $\pm$ .4 days, $p<.05$). By two months after injection, there were no differences between the groups, in part due to a gradual increase in cycle length of controls and a partial recovery in NMA treated mice. In addition to affecting estrous cycle length, NMA treatment also produced a significant weight gain by one month following injection, but again, a partial recovery of NMA treated mice occurred in the second month. We are continuing to monitor the estrous cycles of these mice to determine if NMA treatment affects the age-related onset of acyclicity. Preliminary results from a separate study indicate that a similar NMA treatment regimen resulted in detectable cell loss within the arcuate nucleus. This presumptive cell loss in the arcuate is consistent with NMA induced alterations in estrous cycle and body weight.

This study was supported by NIA grant AG00117 (C.E.F.), NRSA Fellowship 1F32 AG05329 (P.C.M.) and NIA Training Grant T32-AG00093 (S.G.K.).

32.16 MODIFICATION OF DOSE RELATED RESPONSES TO L-GLUTAMATE MICROINJECTED INTO THE NUCLEUS TRACTUS SOLITARIUS OF RATS AFTER DEGENERATION OF VAGAL AFFERENT NERVES. W.T. Talman. Lab. of Neurobiol. Univ. of Iowa & Vets. Admin. Med Center, Iowa City, IA 52242.

Biochemical, pharmacological, physiological, and receptor binding data support a role for L-glutamate (L-glu) in the mediation of the baroreceptor reflex in the nucleus tractus solitarius (NTS). If L-glu were the neurotransmitter of primary baroreceptor afferents, denervation supersensitivity might be expected to occur with degeneration of the afferent fibers. Thus, we sought to determine whether the dose related changes in arterial pressure (AP) elicited by the microinjection of L-glu into the NTS were significantly altered after removal of the nodose ganglion, the site of cell bodies of baroreceptor and other visceral afferent fibers. Ten adult male Sprague-Dawley rats were used in these experiments. Five were unoperated controls and five were studied 10 days after the removal of the left nodose ganglion. Each rat was anesthetized (halothane 1.5-2%), cannulated for recording AP via the femoral artery, and mounted in a stereotaxic frame. After exposure of the brain stem, microinjections (50 nl) of saline vehicle or increasing doses of L-glu (3 to 1500 pmoles) were made alternately into the right and left dorsomedial NTS. Each dose was usually injected twice on each side. The last injection on each side contained a 2% concentration of pontamine blue in saline for histologic confirmation of injection sites. In control rats the lowest dose of L-glu that elicited a significant fall of AP was 30 pmoles, while the threshold dose in the left NTS of ganglionectomized rats was 3 pmoles. In lesioned, but not in control, rats all doses of L-glu elicited greater responses from the left NTS than from the right NTS. Left NTS dose responses in lesioned rats were also consistently greater than those from the left NTS of control rats. In control rats there was no significant difference between the dose response elicited from the right and left NTS. In lesioned rats the maximal response from the left NTS was 41 $\pm$ 5.4 mmHg fall of AP vs. 34.2 $\pm$ 6.6 mmHg ($p<0.05$) from the right in lesioned rats and 30 $\pm$ 4.3 mmHg ($p<0.05$) from the left in control rats. All injections were confirmed to be symmetrically placed in the right and left NTS for each animal. These data provide evidence suggesting that denervation supersensitivity develops for L-glu after nodose ganglionectomy. If supported by other criteria of denervation supersensitivity and by similar observations after more selective degeneration of baroreceptor afferents, the data would support the hypothesis that L-glu is a neurotransmitter of baroreceptor afferents.

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32.17 SPIDER VENOMS BLOCK SYNAPTIC TRANSMISSION MEDIATED BY NON-N-METHYL-D-ASPARTATE RECEPTORS IN THE AVIAN COCHLEAR NUCLEUS. H. Jackson, M. Urnes*, W.R. Gray*, and T.N. Parks. Depts. of Anatomy and Biology, Univ. of Utah, Salt Lake City, UT 84132.

The study of excitatory amino acid transmission in the CNS would be facilitated by the availability of more specific, potent, and slowly-reversible ligands that act on receptor complexes. We have examined the ability of milked venoms from seven American spider genera (of five families) to suppress synaptic transmission (mediated by receptors of the non-N-methyl-D-aspartate class) between the avian cochlear nerve and cochlear nucleus (nuc. magnocellularis, NM). The ability of bath-applied whole venom, or venom fractions purified by gel filtration and reverse phase HPLC, to depress synaptically evoked responses was assessed by recording field potentials from NM following electrical stimulation of the cochlear nerve in in vitro preparations of the brain stem of hatchling chickens 1-10 days old. In the initial screening experiments, when whole venom was applied at 0.33% (v/v), venoms from Neoscona arabesca and Plectreurys tristes were without effect, while venom from Araneus gemma caused a partial and fully reversible depression of transmission. Whole venom from Argiope aurantia and Peucetia viridens produced potent but fully reversible suppression; the active component of the Argiope venom is positively charged and has a molecular weight of 659 daltons. Venom from the wolf spider (family Lycosidae) produced a rapid and reversible suppression both of postsynaptic currents in NM and the afferent volley, suggesting that this venom contains at least one component that acts presynaptically. In contrast, venom from Hololena curta produced complete but apparently irreversible suppression of transmission without affecting the afferent volley; in repeated experiments, the postsynaptic field potential failed to recover, even after more than 3 hr of washing in venom-free avian Tyrode solution. The active component(s) of this venom resides in a fraction of about 10,000 daltons and appears capable of acting at even lower concentrations than the Argiope venom. The effect of Hololena venom on the survival of cultured chick ciliary ganglion cells and their ability to extend neurites was also assessed. At a concentration that completely suppresses synaptic transmission in NM, the venom did not produce increased mortality of the cultured cells or interfere with the extension of neurites. Further, action potentials could still be evoked from NM neurons by direct electrical stimulation after several hours of transmission blockade. These results indicate that Hololena venom is not generally neurotoxic at the concentrations tested and that the apparent irreversibility of transmission blockade does not result from NM neurons being rendered electrically inexcitable.

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32.18 CORTICAL CHOLINERGIC MARKERS FAIL TO RECOVER FOLLOWING INJECTION OF QUINOLINIC ACID (QUIN) OR IBOTENIC ACID (IBO) INTO THE RAT NUCLEUS BASALIS MAGNOCELLULARIS (nbM). K. Jhamandas*, S. El-Defrawy*, R.J. Boegman, L. Shipton*, and R.J. Beninger. Departments of Pharmacology and Toxicology, and Psychology, Queen's University, Kingston, Ontario, K7L 3N6.

Injections of axon-sparing excitotoxins into the nbM of the rat produce a marked decrease in the neocortical cholinergic markers, choline acetyltransferase (CAT), high affinity choline uptake (HACU) and acetylcholinesterase (AChE). Recently, it was reported that full recovery of CAT occurs 3 months after injection of IBO into the nbM (Wenk and Olton, Br. Res. 293: 184, 1984). In the present study we examined neocortical CAT, HACU, AChE and ^{3}H-acetylcholine release over a 3 month period following a single injection of either QUIN (an endogenous neurotoxin) or IBO into the nbM.

Stereotaxic injections of QUIN (120 nmol) or IBO (25 nmol) in saline were administered as 1 µl infusions into the nbM of halothane-anesthetized rats. The cortical cholinergic markers (CAT, HACU, AChE), as well as spontaneous and potassium evoked (35 mM) ^{3}H-acetylcholine release, were measured 1, 3, 6 and 12 weeks post-injection. Within 1 week, all markers showed a decrease of 40-50% (QUIN) or 35-45% (IBO). No recovery of these markers were observed at 3, 6 and 12 weeks post-injection.

In support of our neurochemical data, light microscopy of cresyl violet stained brain sections 3 months after lesioning showed extensive gliosis at the site of toxin application.

Wenk and Olton (1984) reported significant recovery in cortical CAT activity 3 weeks after an IBO lesion and complete recovery by 12 weeks. In our experiments, we obtained the same maximal degree of cholinergic deficit in the cortex as that reported by Wenk and Olton; however, our results with QUIN or IBO show that cholinergic markers remain depleted following excitotoxin lesions of the nbM.

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32.19 SEPARATE ENZYMATIC ASSAYS FOR CYTOSOLIC AND MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE ACTIVITIES. J.A. Parli*, D.A. Godfrey and C.D. Ross, Dept. Physiol., Oral Roberts Univ., Tulsa, OK 74171

The enzyme aspartate aminotransferase (AAT), which catalyzes the interconversion of glutamate and oxaloacetate with aspartate and α-ketoglutarate, consists of two isoenzymes, cytosolic AAT (cAAT) and mitochondrial AAT (mAAT). Although immunohistochemical procedures have been developed for the determination of both isoenzymes, and most staining results are for the cytosolic isoenzyme, there have not been enzymatic assays which distinguish between their activities. Toward this end, fluorometric assay procedures have been developed for the separate determination of cAAT and mAAT activities. In the cAAT assay, samples are preheated to 70 ^{0} C for 15 minutes, to destroy the mitochondrial isoenzyme. In the mAAT assay, the pH of the incubation medium is 5.0 instead of the usual 7.4, and the aspartate concentration 2mM instead of 40 mM. These parameters were determined from experiments using a commercially prepared cytosolic AAT (cyt. AAT) from pig heart, a mitochondrial preparation produced from mouse liver homogenate (mit. prep.), and whole rat brain homogenate (RBH), which contains both isoenzymes. The apparent K_m values for aspartate and α-ketoglutarate were determined:

	RBH	Heated RBH	Cyt. AAT	Mit. Prep.
K_m Asp (m M)	0.8	2.5	3.3	0.2
K_m αKG (m M)	0.19	0.03	0.05	0.34

The heated RBH and cyt. AAT values are similar, with RBH falling between cyt. AAT and mit. prep. values. When enzyme activity was examined vs. pH, it was found that as pH decreased, both heated RBH and cyt. AAT activity decreased greatly, whereas reduction of the mit. prep. activity was much less. These experiments document that the heated RBH has characteristics similar to those of the cyt.AAT. When the three assay procedures, for total AAT (tAAT), cAAT and mAAT were done for the cyt. AAT, mit. prep., and RBH, the following results were obtained:

	tAAT	cAAT	mAAT	corr mAAT
cyt. AAT (mol/g/hr)	28.5	29.2	0.5	
mit. prep. (mol/kg/hr)	14.5	0.4	6.4	
RBH (mol/kg/hr)	14.7	6.4	3.9	8.8

Since the mit. prep. had virtually no cAAT activity, a correction factor for the mAAT assay was determined by dividing the mit. prep. activity in the tAAT assay by that in the mAAT assay. Evidence that this correction factor is appropriate for rat brain is provided by the finding that using the thereby corrected mAAT activity of RBH, summed with its cAAT activity, gives a value very close to the tAAT activity. The availability of these separate quantitative assays for the AAT isoenzymes, capable of measuring their activities in microgram amounts of tissue, permits more specific comparisons of quantitative with stain histochemical results. (Supported by NIH Grants NS17176 and EY03838)

33.1 FIRST IMMUNOHISTOCHEMICAL DEMONSTRATION OF N-ACETYL-ASPARTYL-GLUTAMATE IN SPECIFIC NEURONS. C.B.Cangro*, D.E.Garrison*, P.A.Luongo*, M.E.Truckenmiller, M.A.A.Namboodiri* and J.H.Neale, Department of Biology, Georgetown University, Washington D.C. 20057

N-Acetyl-aspartyl-glutamate (NAAG) is a nervous system specific dipeptide which is found in millimolar concentrations in certain regions of the central nervous system including the spinal cord. NAAG is reported to displace glutamate binding to the quisqualate subclass of glutamate receptors (Koller and Coyle, Eur. J. Pharm. 98:193, 1984). In order to better understand the role of NAAG in nervous system function, we have developed antisera which recognize NAAG in tissue sections and have applied these immunological reagents initially to study the distribution of the immunoreactivity (IR) of NAAG in spinal sensory neurons.

NAAG was synthesized by the acetylation of aspartylglutamate with acetic anhydride, purified by reverse phase HPLC, characterized by mass spectroscopy and coupled to thyroglobulin using carbodiimide. Rabbit antisera, produced by immunization with this hapten-carrier conjugate, were applied to cryostat sections of dorsal root ganglia which had been fixed by perfusion with carbodiimide and post fixed with paraformaldehyde. The diluted antisera, when used in conjunction with fluoresceinated secondary antiserum, demonstrated a subpopulation of neuronal cell bodies within the spinal ganglia which exhibited immunoreactivity well above other neurons in the ganglia. This differential NAAG-IR was not observed when tissues were fixed with paraformaldehyde alone, suggesting that the IR was due to a small diffusable molecule which was covalently coupled to cellular proteins by carbodiimide mediated peptide bond formation. When antisera were preincubated with 5mM N-acetyl-aspartate, N-acetyl-glutamate, aspartylglutamate, aspartate or glutamate, the immunoreactivity was not affected, whereas it was eliminated by treatment of the antisera with 0.2mM NAAG. Antisera generated against N-acetyl-aspartate (NAA) by a similar procedure failed to demonstrate any differential immunoreactivity within spinal sensory ganglia. Consistent with this observation of NAAG-IR within spinal sensory neuronal cell bodies is our finding that an extract of fresh rat spinal ganglia (H_2O:methanol, 1:9) contains a major absorbance peak which co-migrates with synthetic NAAG during anion exchange HPLC. These data suggest that NAAG is differentially distributed within spinal sensory neurons and that its cellular distribution in other areas of the nervous system can be identified using these antisera.

33.2 DISTRIBUTION OF [^{3}H]N-ACETYL-ASPARTYL-GLUTAMATE BINDING SITES IN RAT BRAIN: A QUANTITATIVE IN VITRO AUTORADIOGRAPHIC STUDY. M.E. Abreu*, R.D. Blakely* and J.T. Coyle. Nova Pharmaceutical Corp., Balto. MD 21224 and Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Balto., MD 21205. (SPON: W.J. Kinnier)

N-Acetyl-aspartyl-glutamate (NAAG) is an endogenous dipeptide that binds to a subpopulation of glutamate receptors in a chloride dependent and calcium enhanced manner. Electrophysiologic studies in pyriform cortex indicate that the potent neuroexcitatory action of NAAG mimics the response of the endogenous transmitter of the lateral olfactory tract. Both responses are selectively blocked by D,L-2-aminophosphonobutyric acid (AP4) suggesting that NAAG interacts specifically with an AP4 sensitive receptor and may serve as a neurotransmitter in this pathway.

A modification of the method described by Greenamyre et al. (J. Neurosci. 4:2133, 1984) was utilized in the current studies. Briefly, slide-mounted rat brain tissue sections (10 micron) were preincubated at 4°C for 1 hr in 50 mM Tris Cl (pH 7.4) containing 2.5 mM $CaCl_2$ followed by a 45 min incubation at 4°C with 200 nM [^{3}H]-NAAG (44.1 Ci/mmol) or 200 nM [^{3}H]L-glutamate (45.9 Ci/mmol). At the end of the incubation, tissue sections were rapidly rinsed in cold buffer, followed by a rinse with 2.5% glutaraldehyde in acetone and immediately dried on a slide warmer (40°C). L-glutamate (10^{-3}M) was used to determine non-specific binding.

Scatchard analysis indicated that [^{3}H]-NAAG binds to a single site with an apparent K_D of approximately 300 nM. The binding of [^{3}H]-NAAG was heterogeneously distributed in a pattern similar to that of [^{3}H]-glutamate binding; and, in addition, the density of NAAG sites represents a substantial fraction of glutamate sites ranging from 50-75%. For both ligands, the highest density of binding was found in the hippocampus in which a distinct laminar pattern was apparent with the greatest binding observed in the stratum moleculare of the dentate gyrus and in the CA_1 region of the stratum radiatum while lower levels of binding were measured in stratum oriens, the granule cell layer of the dentate gyrus and the subiculum. Most regions of the cerebral cortex were heavily labeled, exhibiting a dense band of binding in the external cortical layers. Other forebrain structures including the caudate-putamen, accumbens, anterior olfactory nuclei and olfactory tubercle demonstrated moderate to high levels of binding. An intermediate level of binding was observed in the thalamus and superior colliculus while lower levels were apparent in the cerebellum, substantia nigra and substantia gelatinosa of the spinal cord. Very little specific binding was observed in the mesencephalic and pontine tegmentum. These initial studies demonstrate that [^{3}H]-NAAG binding exhibits a marked regional variation that is co-extensive with sites labelled by [^{3}H]-glutamate.

33.3 A HIGH-AFFINITY SYNAPTOSOMAL UPTAKE SYSTEM INVOLVING N-ACETYL-ASPARTYL-GLUTAMATE. R. Blakely*, L. Ory-Lavollee*, R. Thompson* and J.T. Coyle. Dept. of Neuroscience. Johns Hopkins Univ. School of Medicine, Balto., MD 21205 (SPON: D. Braitman).

N-Acetyl-aspartyl-glutamate (NAAG), an endogenous brain peptide which acts as a competitive inhibitor at a subpopulation of Cl-dependent, Ca^{2+} stimulated [^{3}H]-glutamate ([^{3}H]-Glu) binding sites may be involved in neurotransmission at some synapses generally considered to use glutamate and/or aspartate as transmitters. In the present study, we examined the possibility that NAAG might be inactivated by a high-affinity synaptosomal transport system. Crude rat brain synaptosomes, prepared by differential centrifugation, were washed twice with 0.32M sucrose and resuspended in 50mM Tris Cl buffer (pH 7.4) containing 120mM NaCl, 10mM dextrose, 4.7mM KCl, 4.9mM $MgSO_4$, and 2.5 mM $CaCl_2$. Choline chloride was substituted for NaCl to assess Na^+ dependency. Following a 5 min preincubation at 37°C, aliquots containing ~300ug of synaptosomal protein were transferred to tubes containing uptake buffer, N-acetyl-aspartyl-[^{3}H]-glutamate(46.6Ci/mmol, 4nM), or [C14]-acetyl-aspartyl-glutamate(55uCi/mmol, 10uM) or [^{3}H]-Glu(50 Ci/mmol, 4nM) with or without potential inhibitors. Assays were performed at 37°C in a shaking water bath and terminated by filtration with a modified Brandel Cell-Harvester. Uptake of label from [^{3}H]-NAAG (Km=5uM) was saturable, occurred in an osmotically sensitive compartment, was temperature- and Na^+-dependent and linear with time up to 10 min. Subsequent studies were thus conducted with 5 min incubations. The initial rate of transport of label varied across CNS regions from 1.5 (cerebellum) to 9.5 (hippocampus) pmol/mg/hr while the ratio of uptake of label from [^{3}H]-NAAG to [^{3}H]-Glu varied from 4% (cerebellum) to 10.5% (septum/hypothal). Transport of both compounds was sensitive to ouabain and veratrine treatment. All inhibitors of [^{3}H]-Glu uptake also inhibited transport of [^{3}H]-NAAG label. HPLC analysis of radioactivity transported by synaptosomes from [^{3}H]-NAAG revealed no [^{3}H]-NAAG but rather an increase with time in material which co-eluted with [^{3}H]-Glu. One mechanism which could account for these findings is the action of a peptidase cleaving labeled Glu from NAAG, which is then subject to a high-affinity uptake system. In support of this hypothesis, we found that transport was not detected when [^{14}C]-NAAG, labeled at the N-terminal acetate, was the substrate. Structural specificity of such a protease is suggested by the ability of N-acetyl-glutamyl-glutamate (NAGG)(IC50=8uM) but not N-acetyl-glutamyl-aspartate (IC50>100uM) to compete with this transport process. The unacetylated dipeptides, AG and GG, were potent inhibitors of this process (IC50=10uM). Observation that NAAG, NAGG, AG, GG and quisqualate (IC50=2uM) fail to interfere with [^{3}H]-Glu transport argues that these agents exert inhibition not at the level of the carrier but target another recognition site, possibly a protease.

33.4 EFFECTS OF GLUTAMATE CONTAINING DIPEPTIDES ON THE BINDING OF [3H]-L-GLUTAMATE TO RAT BRAIN MEMBRANES. R. Zaczek*, S. Arlis*, A. Markl*, T. Murphy* and J.T. Coyle. Dept. of Neuroscience. Johns Hopkins Univ. School of Medicine, Balto., MD 21205.

Phenylalanyl-glutamate (Phe-Glu) and other dipeptides containing glutamate at the carboxy terminus have been shown to increase the binding of [3H]-2-amino-7-phosphonoheptanoic acid while inhibiting the binding of [3H]-kainic acid. We have extended the study of these compounds to their effects on the binding of [3H]-L-glutamate to rat brain membranes. Phe-Glu increases glutamate binding in a dose dependent (EC50=50uM) and reversible manner. This effect is robust with the binding increasing 20-fold in the presence of 100 uM Phe-Glu. Saturation isotherms of glutamate binding in the presence and absence of Phe-Glu indicate that the increase in binding is due to an increase in the Bmax of glutamate binding. This increase in binding is temperature dependent displaying a Gaussian temperature curve with an optimal temperature of approximately 37°C. Phe-Glu dependent [3H]-L-glutamate binding has both chloride dependent and independent components. Rate experiments have shown that glutamate binding to Phe-Glu dependent sites is an ordered process, Phe-Glu having to be present at the binding complex before [3H]-L-glutamate is able to bind. Several dipeptides related to Phe-Glu can also increase glutamate binding (Arg-Glu > Phe-Glu > Tyr-Glu > Tryp-Glu). Preincubation experiments suggest that an endogenous functional analogue of Phe-Glu may exist in brain.

The increase in binding with Phe-Glu represents the appearance of novel sites rather than an increase in the number of existing ones since Phe-Glu dependent and independent glutamate binding display several differences. There is a 3-fold difference in the affinity of glutamate for the two sites (Kd=700nM independent; Kd=2.0uM dependent). The pharmacology of the two sites also differs. N-acetyl-aspartyl-glutamate, a potent inhibitor of Phe-Glu independent binding (Ki=300nM), is ineffective at inhibiting the binding of glutamate to the Phe-Glu dependent site (Ki>100uM). While quisqualic acid displays a biphasic displacement of glutamate binding to the Phe-Glu independent site and is more potent than ibotenic acid, it shows monophasic displacement of equal potency to ibotenic acid at the Phe-Glu dependent site. Finally, the ontogenetic profiles of the two sites differ. While postnatal levels of Phe-Glu independent binding increase only 2-fold in forebrain, there is a several-fold increase in Phe-Glu dependent glutamate binding from postnatal day 7 to adult. These results suggest that certain excitatory amino acid processes may be modulated by small peptides containing glutamate.

33.5 GLUTAMATE CONTAINING DIPEPTIDES ENHANCE THE NEUROTOXIC ACTIONS OF KAINIC ACID IN VIVO. J.Ferkany and L. O'Brien* Nova Pharmaceutical Corp., Div. Mol. Pharmacology, Baltimore, MD 21224.

Carboxyterminal L-glutamate (GLU) containing dipeptides (leucyl-GLU; LG: methionyl-GLU; MG) enhance the specific binding of [3H]GLU and [3H]2-amino-7-phosphono heptanoic acid and inhibit the specific binding of [3H]kainic acid (KA) to brain membranes in vitro (Ferkany et al., Neurosci. Letts. 44:281, 1984; Zaczek et al. Tr. Am. Soc. Neurochem. 16:303, 1985). We now report that intra-striatal coinjections of LG or MG with KA but not ibotenate (IBO) enhance the toxic actions of KA on striatal interneurons.

Male Sprague Dawley rats (200-250 g) were anesthetized with Chloropent@ and positioned in a stereotaxic apparatus. Drugs dissolved in saline and titrated to a neutral pH were injected into the striatum over a period of 60 sec in a final volume of 1 ul. Animals allowed to recover for 3 days were sacrificed by decapitation, the striata dissected and assayed for choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD) activity. Striata contralateral to the injection site served as controls.

Injection of KA (7.5 nmole; nm) resulted in a 30±4 and 45±5 (N=8) percent decline in CAT and GAD activities, respectively. Although injections of 300 nm LG or MG alone had no effect on the activity of either enzyme, coinjection of LG or MG (300 nm) with KA (7.5 nm) induced a further 15-20 percent decline in GAD and CAT activities when compared to striata of animals injected with KA alone ($p<0.05$). Like the dipeptides, injections of GLU (1000 nm) or D-GLU (500 nm) had no effect on the activities of striatal GAD or CAT. However, coinjection of GLU (500 nm) but not D-GLU (500 nm) with KA (7.5 nm) produced a decline in the activities of both enzymes comparable to that observed following coninjections of LG or MG with KA. Thus, the possibility exists that the interaction of LG and MG with KA results from the metabolism of these compounds to GLU. Coinjection of the aromatic dipeptide, phenylalanyl-GLU (PG) (300 nm) with KA also caused an additional decline in enzymes activities; however, this effect was not significant ($p = 0.08$). None of the compounds tested increased the toxic actions of IBO (100 nm) on striatal enzyme activities when coinjected with the latter neurotoxin.

LG, MG and PG allosterically modulate the binding of excitatory amino acids (EAA) in vitro; they do not block the uptake of EAAs in vitro. Preliminary investigations indicate that PG is not metabolized by brain slices, homogenates or synaptosomes. Injection of large amounts of these compounds into the hippocampus fail to produce behavioral or EEG-detectable effects suggesting the compounds are not neuroexcitatory (Zaczek, personal comm.). The current results suggest that substances other than EAAs may influence the neurotoxic actions of KA in vivo. Supported in part by NINCDS grant 1 R43 NS21400-01.

33.6 [3H]AMPA BINDS TO TWO SITES IN RAT CRUDE SYNAPTIC MEMBRANES: PHARMACOLOGICAL CHARACTERIZATION. Deborah E. Murphy, Elaine W. Snowhill* and Michael Williams. Neuroscience/Cardiovascular Research, Pharmaceuticals Division, CIBA-GEIGY Corp.,Summit,NJ 07901

The binding of [3H]AMPA (amino-3-hydroxy-5-methylisoxazole-4-propionic acid,α-[5-methyl-^{3}H]) an agonist for the putative quisqualate-type excitatory amino acid receptor (Krosgaard-Larsen et al., Nature,284: 66,1980) was examined in rat brain membranes. Filtration and centrifugation procedures were examined; the latter greatly increased specific binding and was used routinely. Tissues were incubated with [^{3}H]AMPA (27.5 Ci/mmol, NEN, Boston) in 50 mM Tris-HCl, pH 7.4 (4°C). Specific binding observed with freshly prepared crude synaptic membranes (CSMs; 34%) increased two-fold when the tissue was pre-treated with detergent (0.04% Triton-X 100) or when the tissue was frozen and thawed three times. Association studies indicated that binding reached equilibrium within 20 min and remained stable for nearly 2 hr: binding was reversed by the addition of 1mM l-glutamate. Scatchard analysis on the frozen/thawed CSMs indicated the presence of two distinct binding sites: one with high affinity (Kd= 17±6 nM) and one with low affinity (Kd=3.8±0.4 µM). Apparent Bmax values were 0.2±0.3 and 22.9±0.6 pmol/mg protein respectively. The biphasic inhibition curves seen with quisqualate and unlabeled AMPA further supported the presence of two separate binding components. The inhibition of [^{3}H]AMPA binding was consistent with the labeling of a quisqualate-type receptor being stereoselective for the isomers of glutamate. The order of potency of various amino acids studied was: quisqualate>AMPA>l-glutamate>kainate, l-glutamic acid diethylester> d-glutamate. N-methyl-D-aspartate, d&l-aspartate and d&l-amino adipate were inactive at 100 uM. These data show considerable agreement with those reported previously (Honoré et al., J. Neurochem. 38:187, 1982). However, in contrast, the use in the present study of higher specific radioactivity AMPA showed clear evidence for two binding sites in tissue that had been frozen and thawed three times. Binding was also weakly sensitive to kainate in contrast to the lack of effect of this amino acid in mouse brain membranes at pH 5.5 (Tsai and Lehmann, this volume).

33.7 QUISQUALATE-TYPE RECEPTOR SITES LABELED BY [^{3}H]AMPA - LACK OF INTERACTION WITH KAINATE. C. Tsai* and J. Lehmann (SPON: D.L. Cheney). Neurosci. Res., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

The previous description of conditions for the specific binding of [^{3}H]α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) showed that the profile of [^{3}H]AMPA binding was largely in agreement with the labeling of a quisqualate-type receptor (Honore et al., J. Neurochem. 38:173, 1982). However, under these conditions, kainate also inhibited binding of [^{3}H]AMPA. We report here the development of conditions for [^{3}H]AMPA binding which do not show this interaction with kainate, and hence provide a more specific assay for quisqualate-type receptor binding sites. While the present conditions yield a pharmacological profile which is more in agreement with the labeling of a quisqualate-type receptor, they also yield a very unusual pH maximum (5.5). The results shown below (performed at 24°C in mouse whole brain minus cerebellum and brain stem) support the hypothesis that [^{3}H]AMPA labels a quisqualate-type receptor.

Test Compound	IC_{50} values (uM)		
	centrifugation	filtration	Honore et al.
AMPA	12	38	0.3
Bromowillardiine	14	25	-
L-GLU	43	55	1.3
Quis	52	55	0.3
IBO	500	400	>100
L-Asp	700	320	>100
D-Asp	1000	>1000	>100
D-GLU	>1000	875	100
Kainate	5000	>12500	6
NMDA	>1000	>1000	-
Muscimol	>1000	>1000	-
GABA	>1000	>1000	>100
GDEE	76% ↑ @1000	78% ↑ @1000	>100

All of the following compounds showed no inhibition when tested at 1 mM in the centrifugation assay: AP3, AP4, D-AP5, L-AP5, AP7, γ-D-GLU-GLY, kynurenic acid, L-cysteic acid, streptomycin, NAAG, (+/-)cis-2,3-piperidine dicarboxylic acid, N-α-aspartic acid-L-glutamate, DL-2-amino-6-hexanoic acid, and γ-D-glutamylaminomethyl-phosphonic acid.

33.8 N-METHYL-D-ASPARTATE (NMDA) RECEPTORS: PHARMACOLOGICAL AND BIOCHEMICAL CHARACTERIZATION. G.E. Fagg and J. Baud. Friedrich Miescher Institute, P.O. Box 2543, Basel, Switzerland.

The A1 (NMDA-preferring) sub-type of acidic amino acid receptor is being implicated increasingly in a wide range of brain mechanisms, including excitatory synaptic transmission, long-term potentiation, seizure activity and neurodegeneration. Recently, we demonstrated that this receptor may be labeled in vitro using L-^{3}H-glutamate (Glu) and that it is enriched in postsynaptic densities (PSDs) isolated from the rat brain (Fagg & Matus, Proc. Nat. Acad. Sci.USA, 81, 6876-6880; 1984). Here we describe the general ligand selectivity and basic biochemical properties of this receptor.

L-^{3}H-Glu bound reversibly to PSD fractions with Kd 0.3 µM. Specific binding at 50 nM ^{3}H-Glu (non-specific determined using 0.5 mM L-Glu) represented 87% of total binding, of which 80% was sensitive to inhibition by A1 receptor ligands such as NMDA (Ki 6.3 µM) and 2-amino-5-phosphonopentanoate (Ki 1.0 µM). This site could also be labeled with ^{3}H-NMDA, but the percentage specific binding (29%) was less favourable for routine analyses. Structure-activity studies showed that L-Glu was the most potent inhibitor of NMDA-sensitive L-^{3}H-Glu binding examined (Ki 0.2 µM), while the higher and lower homologues (L-α-aminoadipate, L-asp) were weaker. Replacement of the ω-carboxyl group of Glu with other acidic groups decreased inhibitory potency in the order carboxyl > sulphonic > phosphonous >> phosphonic, while replacement of the α-carboxyl function with phosphonous or phosphonic moieties abolished activity. In the case of D-asp, inhibitory potency declined as N-substituents increased in size (methyl > ethyl > propyl), while lack of an amino group (succinate, glutarate) destroyed activity. A number of peptides were moderately effective inhibitors of ^{3}H-Glu binding (e.g., Phe-Glu, Ki 7 µM), while substances such as phencyclidine and Mg^{2+} were without effect.

Receptor binding activity was retained after freezing or lyophilizing PSD fractions, but was abolished by heating at 60°C for 15 min. Treatment of PSDs with trypsin or chymotrypsin (1% of PSD protein) markedly decreased L-^{3}H-Glu binding, while the Ca^{2+}-activated proteases calpain I and II had little effect. The thiol reagent p-hydroxymercuribenzoate (0.1 mM) similarly did not modify binding to a marked extent.

These data indicate that the A1 receptor is a proteinaceous component of the postjunctional membrane. The receptor recognition site exhibits a high degree of ligand selectivity and is distinct from those sites regulated by Mg^{2+} and the 'sigma opiates'.

We thank Drs. J. Dingwall, L. Maier and P. Diel for gifts of compounds, and Dr. T. Murachi for samples of calpain I and II.

33.9 **EXCITATORY AMINO ACID BINDING SITES: CORRESPONDENCE BETWEEN AUTORADIOGRAPHIC AND MEMBRANE FRACTION PREPARATIONS.** D.T. Monaghan, D. Yao*, L. Nguyen* and C.W. Cotman. Dept. of Psychobiology, Univ. Cal., Irvine, CA 92717.

The excitatory amino acids, especially L-glutamate, are thought to exert their neurotransmitter action through the activation of at least four receptor classes. Previously, we have used quantitative autoradiographic techniques to describe four pharmacologically distinct L-[^{3}H]-glutamate binding sites, three of which appeared to correspond to physiologically defined excitatory amino acid receptors. There are few studies of these particular L-[^{3}H]-glutamate binding sites in membrane preparations. Consequently, the following experiments were performed to determine the correspondence of the autoradiographically determined binding sites to those observed in membrane preparations.

1)N-Methyl-D-aspartate (NMDA) receptors appear to be labelled by L-[^{3}H]-glutamate in autoradiographic preparations. To assess the similarity of NMDA-sensitive L-[^{3}H]-glutamate binding sites in membrane fragments to those we have described in autoradiography we have determined their pharmacological profile in rat brain synaptic plasma membranes (SPMs) (J. Neurosci. 1 (1981) 620-625). Following the incubation of SPMs with 10 nM L-[^{3}H]-glutamate (44-50 Ci/mmol, ICN or NEN), 50 mM Tris-acetate, pH 7.0, for 2.5 minutes at 0-4°C, 80% of the specific binding is displaced by NMDA with a Ki value of 4 ± 1μM. The pharmacological profile of these sites correspond to both the NMDA receptor and to the autoradiographically determined binding site For example, the NMDA antagonists D-α-aminoadipate and D-2-amino-5-phosphonopentanoate exhibited Ki values of 10.1 ± 0.6 and 2.3 ± 0.4μM. Furthermore, binding to both autoradiographic and membrane fraction NMDA sites was inhibited by 100μM GTP. Nucleotide inhibition exhibited the same specificity as that found for other GTP-sensitive receptors.

2)Kainate (KA) receptors labelled in autoradiography by L-[^{3}H]-glutamate are readily displaced by Ca^{++} ions, but [^{3}H]KA sites show partial displacement. We have used EGTA (5mM) washed SPMs to assess the effect of Ca^{++} upon the affinity and Bmax of [^{3}H]KA binding sites. [^{3}H]KA (60 Ci/mmol, NEN, 0°C, 30 min., in 50 mM Tris acetate, pH 7.0) exhibited two affinities, 6.6 ± 0.8 nM and 25.6 ± 4.9 nM. However, in the presence of 5mM Ca^{++} only a 23.1 ± 1.9 nM site was detected. Thus, the previously described L-[^{3}H]-glutamate binding site which was both KA and Ca^{++}- sensitive may represent high affinity KA binding sites. (Ca^{++}-sensitive [^{3}H]KA binding sites were not equivalent to slow-dissociating sites.)

3)We previously reported that the Cl^-/Ca^{++}-enhanced binding sites observed in autoradiography were distinct from those determined in membrane preparations. Using previously described methods (Nature 306 (1983) 176-179) we find that the autoradiographically observed sites are potently inhibited by 4-acetamido-4'-isothiocyano-2,2'-disulfonic acid stilbene (SITS, Ki value 10 ± 3μM), moderately by 100μM L-aspartate, and insignificantly by 5mM Na^+ or 100 μM D-aspartate. Consequently, these sites may represent binding at Cl^--dependent L-[^{3}H]-glutamate uptake carrier sites in astrocytes. (Supported by DAAG 29-82-K-0194)

33.10 IMMUNOCHEMICAL CHARACTERIZATION AND IMMUNOHISTOCHEMISTRY OF THE GLUTAMATE BINDING PROTEIN IN RAT BRAIN, S. Roy, T. Stormann*, L. Vacca, M. Cunningham*, and E. Michaelis. Depts. of Biochemistry, Anatomy, and Human Development, U. of Kansas, Lawrence, KS 66045.

A glutamate-binding protein [GBP] has been purified from rat and bovine brain synaptic membranes. Based on the selectivity of the GBP binding sites for L-glutamate analogs and its lack of glutamate metabolizing enzymatic activity, it was proposed that the GBP is the recognition macromolecule of an L-glutamate receptor complex [Michaelis et al., J. Neurochem. 42, 397, 1984]. The GBP has been reconstituted in liposomes where it functioned both as a glutamate-recognition and ion channel-forming protein [Stormann et al., Neurosci. Abstr. 10, 958, 1984]. Antibodies (Ab's) against the bovine brain GBP raised in rabbits show very high immunological selectivity for this protein. They also inhibit the binding activity of GBP from either bovine or rat brain and block the activation of ion channels by glutamate in rat brain synaptic membranes [Roy and Michaelis, J. Neurochem. 42, 838, 1984; Roy et al., ibid., 44, in press]. These Ab's bound to denatured bovine and rat GBP that had been subjected to SDS-electrophoresis and blotted onto nitrocellulose paper. The Ab's that were raised in rabbits were also used to map the glutamate binding protein distribution in brain sections. The distribution of GBP antigenic sites in neurons was examined by the peroxidase-anti-peroxidase (PAP) immunohistochemical procedure in vibratome sections from the hippocampus and cerebellum. The highest immunoreactivity was localized in the dentate gyrus, hilar regions, and the CA_3 region of the hippocampus. The distribution was frequently seen as doublets or singlets on the dentrites of the pyramidal and granule cells in this region. The protein A-gold particle staining procedure was used to examine the distribution of antigenic sites at the electron microscopic level. Gold particle clustering in synaptosomal preparations was associated with membrane sites, particularly sites on dendritic fragments. The synaptic localization of this protein in intact brain tissue at the electron microscopic level is currently being explored by the immunogold and PAP staining procedures. (Supported by grants KS-84-G-21 from AHA, AA04732 from NIAAA, and DAAG-29-83-0065 from ARO.)

33.11 **STEREOSPECIFIC BINDING OF L-GLUTAMATE TO ASTROCYTE MEMBRANES** R.J. Bridges*, M. Nieto-Sampedro, and C.W. Cotman. (SPON: R. Vicedomini, Univ. of Kentucky) Department of Psychobiology, University of California, Irvine, CA 92717.

Glutamate appears to be a major excitatory transmitter in the mammalian central nervous system. Multiple types of glutamate binding sites have been identified. These binding sites may represent any number of functions (e.g., transmitter receptor, modulatory sites, transport systems) and may be present on either neurons or their associated glia.

Recently we have identified an L-glutamate binding site on membranes prepared from cultures of primary astrocytes. The binding of glutamate was determined using ^{3}H-L-glutamate and separating bound ligand, from that in solution, by rapid centrifugation. Assays were performed in 50 mM Tris-acetate, pH 6.9, for 60 minutes at 30° C. Non-specific binding was corrected for by subtracting the amount of ^{3}H-L-glutamate bound in the presence of 500μM L-glutamate. Sodium was omitted from the assay buffers to prevent binding to the Na^+-dependent transport site. Under these conditions the specific binding of glutamate was difficult to discern from non-specific binding. However, when Cl^- (Tris-Cl^-) was included in the buffer, specific binding increased 10-fold. This Cl^--enhanced L-glutamate binding is stereoselective and has a relatively high affinity (K_D~ 0.5μM). The specificity of the binding was examined by determining the extent to which ^{3}H-L-glutamate (100 nM) is displaced by various analogues (100 μM). Little or no inhibition is observed with kainic acid, N-methyl-D-aspartate, or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). A small amount of inhibition (25% or less) is seen with D-aspartate or L-2-amino-4-phosphonobutyric acid. Substantial inhibition is observed with quisqualic acid (50%) and L-aspartic acid (95%).

The pharmacological properties of the astrocyte binding site are different from the Cl^- dependent glutamate binding site observed in synaptic membrane preparations (SPMs) (Fagg, G.E., Mena, E.E., and Cotman, C.W.(1983) Adv. in Biochem. Psychopharm. 37,199). Importantly, the binding site on the astrocyte membranes is insensitive to freezing, unlike the site in SPMs. The specificity is, however, quite similar to that of a Cl^- dependent glutamate transport system recently described in glioma cells (Waniewski, R.A. and Martin, D.L. (1984) J. Neuro. 4, 2237). The pharmacological profile of the site also suggests that it may be present in brain slice autoradiographs of ^{3}H-glutamate binding when Cl^- is included in the binding medium.This presence of a class of glutamate binding sites on astrocytes demonstrates the difficulty in assigning a transmitter receptor function to a binding site in a heterogenous preparation, such as a brain homogenates or a brain slice. A detailed characterization of the binding site specificity and ionic requirement should allow this astrocytic site to be studied independently from the other glutamate binding sites and provide insight into its physiological role. (Supported by DAAG 29-82-K-0194)

33.12 AUTORADIOGRAPHIC LOCALIZATION OF CEREBELLAR GLUTAMATE RECEPTOR SUBTYPES. J.M.M. Olson*, J.T. Greenamyre, J.B. Penney and A.B. Young. Neuroscience Lab. Bldg., 1103 E. Huron, University of Michigan, Ann Arbor, MI 48104.

Glutamate is believed to be an excitatory neurotransmitter of the central nervous system and it has been implicated in the pathogenesis of several neurodegenerative diseases including Huntington's disease and olivopontocerebellar atrophy. Its actions are mediated by 3 distinct receptors named for the specific agonists N-methyl-D-aspartate (NMDA), kainate (KA) and quisqualate (QA). Certain glutamate analogues which interact with these receptors produce excitotoxic lesions resembling those seen in human disease.

In order to determine the synaptic location of these receptors, [^{3}H]glutamate and [^{3}H]kainate were used to label receptors autoradiographically in cerebella of control mice, mice lacking presynaptic glutamatergic granule cells due to treatment with the cytotoxic agent methylazoxymethanol acetate, and nervous mutant mice which lack the postsynaptic Purkinje cells. In control mice, [^{3}H]glutamate binding was found throughout cerebellar cortex. The majority of the molecular layer glutamate binding sites had a high affnity for QA, whereas most of the granule cell layer sites were sensitive to NMDA. This indicates that QA and NMDA receptors are located predominantly in the molecular and granule cell layer, respectively. In addition, [^{3}H]kainate binding sites are more abundant in the granule cell layer than in the molecular layer in control mice.

There was minimal [^{3}H]glutamate binding in the granule cell layer of granuloprival mice but the adjacent molecular layer showed "hot spots" of increased QA-sensitive binding, suggesting up-regulation of QA receptors in this layer. In these mice, NMDA-sensitive binding and [^{3}H]kainate binding were decreased in the granule cell layer but were normal in the molecular layer. In Purkinje cell-deficient mice, QA-sensitive sites were almost entirely absent from the molecular layer but NMDA-sensitive binding was normal.

This study indicates that QA sites are postsynaptic to granule cells and are located on dendrites of Purkinje cells. These sites can apparently up-regulate in response to denervation. NMDA sites are associated with granule cell perikarya but not with Purkinje cell dendrites. Finally, KA sites are associated with both granule cell perikarya and non-granule cell elements in the molecular layer.

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33.13 L-GLUTAMATE BINDING SITES IN RAT SPINAL CORD MEMBRANES: EVIDENCE FOR TWO CHLORIDE ION DEPENDENT SITES. E. Edward Mena, Central Research Division, Pfizer Inc., Groton, CT 06340.

Membrane preparations from cortex and cerebelum are routinely employed in L-Glu binding studies. However, in vitro preparations of spinal cord frequently have been used to characterize the electrophysiological responses elicited by L-Glu. Thus, the inability to equate the binding sites of L-Glu with spinal cord receptors may be due, in part, to differences in L-Glu binding sites in cortex and spinal cord.

The effects of various ions on the major classes of ionically regulated L-Glu binding sites (Na^+-dependent, Cl^--dependent and Cl^--independent) (Mena et al., Brain Research, 243, 378, 1982) in synaptic plasma membranes (SPM) from rat spinal cord and forebrain were examined. Chloride ion dependent binding sites were nearly 1.7-fold higher in spinal cord SPM (Bmax values of 108 + 18 pMol/mg protein) as compared to forebrain SPM (Bmax values of 62 + 12 pMol/mg protein). Sodium ion dependent binding, on the other hand, was nearly 6-fold less in spinal cord (Bmax = 74 + 10 pMol/mg protein) compared to forebrain SPM (408 + 26 pMol/mg protein). Uptake of L-Glu (Na^+-dependent) was also 8-fold less in the P2 fraction from spinal cord relative to forebrain (Vmax of 2.89 and 22.3 pMol/mg protein/min, respectively). The regulation of L-Glu binding sites by Na^+, K^+, $NH4^+$ and Ca^{++} was similar in both regions of the central nervous system. In addition, in spinal cord membranes, Br^-, I^-, NO_3^- were equivalent to Cl^- in their ability to stimulate L-Glu binding. The effect of each of these ions was increased in the presence of Ca^{++}. F^- and CO_3^{--} did not significantly enhance binding in the absence of Ca^{++}. However, in the presence of Ca^{++} (0.5 mM) these ions were capable of increasing L-Glu binding over the levels in acetate buffer alone. Phosphate and sulfate were ineffective in the presence or absence of Ca^{++}.

2-Amino-4-phosphonobutyric acid (AP4) (100 uM) was able to inhibit only 80% of the total Cl^- ion dependent L-Glu binding in spinal cord SPM. However, the remaining Cl^- ion dependent binding could be blocked by quisqualic acid (QUIS) (100 uM). More detailed analysis showed that the predominant Cl^- ion dependent site had characteristics similar to the Cl^--dependent binding site in forebrain membranes (i.e. IC_{50} values of 5.7 + 1.4 uM and 119 + 24 nM for AP4 and QUIS, respectively). The other Cl^- ion dependent site was unaffected by 100 uM AP4 but was blocked by QUIS (IC_{50} = 6.7 + 1.4 uM). The results show that, in spinal cord membranes, Cl ion stimulation of L-Glu binding consists of two components, only one of which can be equated with the AP4-sensitive L-Glu binding site.

33.14 AUTORADIOGRAPHIC LOCALIZATION OF AMINO ACID RECEPTORS IN RAT AND HUMAN SPINAL CORD. J.T. Greenamyre, R. Albin*, J.B. Penney and A.B. Young. Neuroscience Lab. Bldg., 1103 E. Huron, Univ. of Michigan, Ann Arbor, MI 48104.

The inhibitory amino acids, glycine and GABA, and the excitatory amino acids, glutamate and aspartate, have all been proposed as major neurotransmitters in the mammalian spinal cord. Each of these amino acid transmitters acts through specific receptor mechanisms to produce its physiologic response(s). Furthermore, it has been shown that GABA, glutamate and aspartate each act at multiple receptor subtypes. Using quantitative receptor autoradiography, we have mapped the anatomical location of the various receptors with which these amino acids interact in rat and human spinal cord.

Glycine receptors were labelled with [^{3}H]strychnine as described previously (Penney et al., Eur.J.Pharmacol. 72:421, 1981). Glycine receptors were found in all laminae of the rat spinal cord and at all levels. The receptors were rather uniformly distributed throughout the laminae with a slightly higher density in substantia gelatinosa. A similar distribution was found in human thoracic spinal cord sections.

$GABA_A$ receptors were labelled with [^{3}H]GABA in Tris-citrate buffer and $GABA_B$ receptors were labelled with [^{3}H]GABA in Tris-HCl buffer with 2.5 mM $CaCl_2$ in the presence of 40 µM isoguvacine. $GABA_A$ receptors were found in both dorsal and ventral horns of rat spinal cord with the highest levels being in the substantia gelatinosa. In contrast, $GABA_B$ receptors were found almost exclusively in substantia gelatinosa. Similar patterns of $GABA_A$ and $GABA_B$ binding were seen in human spinal cord.

Excitatory amino acid receptors were labelled with [^{3}H]glutamate and subtypes of receptors distinguished pharmacologically as described previously (Greenamyre et al., J. Pharmacol. Exp. Ther. 233:1, 1985). Glutamate binding was highest in substantia gelatinosa in both rat and human spinal cords and was otherwise uniformly distributed in the dorsal and ventral horns. Most of the sites in substantia gelatinosa were of the high affinity quisqualate type; about 50% of the sites in the ventral horn were sensitive to N-methyl-D-aspartate.

The distribution and pharmacology of the receptors labelled autoradiographically corresponds well with the receptors described in electrophysiological studies.

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33.15 CHANGES IN AMINO ACID LEVELS IN THE SPINAL GRAY MATTER AFTER AXOTOMY OF PRIMARY SENSORY AND DESCENDING FIBERS. S.J. Potashner and L. Dymczyk*. Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT, 06032.

L-glutamate and/or L-aspartate (L-GLU/L-ASP) are proposed as putative transmitters of dorsal sensory neurons and of one or more of the pathways descending from the brain to the spinal cord, because axotomy of primary sensory and descending tracts depressed the high affinity uptake and the release of D-ASP in the guinea pig spinal cord (Potashner and Tran, J.Neurochem. 42: 1135 1984); J.Neurochem. in press (1985)). D-ASP is a nonmetabolizable marker taken up and released by glutamatergic and/or aspartatergic neurons. In this study, we determined if these lesions altered the stores of endogenous GLU, ASP, and other amino acids in the guinea pig spinal gray matter.

Bilateral dorsal rhizotomy (DR; roots C5-T1) and hemicordotomy (HC; at segment C5) were used in separate studies to interrupt primary sensory and descending tracts. The resulting preterminal degeneration of fibers in the spinal cord was visualized with the Nauta-Rasmussen silver stain in light microscopic preparations. In biochemical studies, performed 2 days after surgery, spinal segments C5-C8 were rapidly excised from anesthetized animals, quick-frozen, cut in a cryostat into transverse sections (40um), and lyophilized at -40°C. Using typical silver-stained sections as guides, areas of the spinal gray that receive heavy projections from the primary sensory and the descending tracts were microdissected from the lyophilized sections and weighed on a quartz fiber microbalance. The levels of amino acids in acid extracts of the microdissected pieces were determined using an HPLC amino acid analyzer.

After DR, the heaviest preterminal degeneration in the spinal gray appeared in laminae III-IV and medially in laminae IV-VI. DR reduced the levels of GLU, ASP, and GABA by 21, 34, and 26% in laminae III-IV and by 33, 28, and 23% in the medial parts of laminae IV-VI. The levels of other amino acids were increased. After HC, the heaviest preterminal degeneration in the spinal gray appeared in the lateral parts of laminae IV-VI and the dorsolateral part of lamina VII. HC reduced the levels of GLU and ASP by 28 and 22% in the lateral parts of laminae IV-VI and by 28 and 26% in dorsolateral lamina VII. Levels of other amino acids were unchanged or raised. These findings suggest that the axonal endings of the primary sensory and of one or more of the descending tracts contain stores of L-GLU and L-ASP. Together with previous findings, these results are consistent with a transmitter role for L-GLU/L-ASP in primary sensory and in one or more descending pathways. Possible reasons for the reductions in GABA levels are under investigation. (Supported by NS17219 from NINCDS)

33.16 PHARMACOLOGICAL STUDIES OF EXCITATORY AMINO ACID RECEPTORS USING A NEW TECHNIQUE OF GLUTAMATE INDUCED TRANSMITTER RELEASE FROM CULTURED NEURONS. J. Drejer*[1], T. Honoré*[1] and A. Schousboe*[2]. Ferrosan Res. Div., Soeborg, DK-2860 (1) and Panum Institute, Dept. Biochem. A, Copenhagen, DK-2200 (2), Denmark. (SPON: ENA)

Using a simple and sensitive method for the study of transmitter release from cultured neurons stimulated with excitatory amino acids, different receptor populations for excitatory amino acids were found in two different primary neuronal cultures. Neuronal cultures prepared from 7-day-old mouse cerebella have been shown to be an almost pure culture of glutamatergic granule cells. Cultured cerebral cortex neurons obtained from 16-day-embryo mice contain mainly GABA-ergic interneurons. For release studies cerebellar granule cells were preloaded with ^{3}H-D-aspartate as a tracer for the proposed transmitter L-glutamate and cerebral cortex neurons were preloaded with ^{3}H-GABA. The release of radioactivity from the cultures was studied in a continuous superfusion system and neurons were intermittently stimulated by the addition of excitatory amino acid agonists to the superfusion medium in the presence or absence of antagonists.

L-glu, L-asp, NMDA, quisqualate, kainate and AMPA were found to induce ^{3}H-GABA release from cerebral cortex neurons. The stimulated release was shown to be calcium-dependent and all stimulations by agonists could be blocked by one or more of the antagonists D-APV, D-APH, GDEE, D-α-AA and γ-DGG according to their known pharmacological selectivities. In granule cells only L-glu and L- and D-asp induced ^{3}H-D-asp release from the neurons. From the relative potencies of antagonists on L-glu induced ^{3}H-D-asp release from granule cells it is suggested that a unique population of excitatory amino acid receptors are present on granule cells and that such receptors might mediate the stimulation of granule cells in vivo via mossy fiber terminals. Binding experiments supported the existence of different types of receptors for excitatory amino acids on the two neuronal cultures; thus quisqualate was found to be a potent displacer of ^{3}H-L-glu binding to cultured cortex neurons whereas in granule cells only L-glu and L- and D-asp were potent displacers of ^{3}H-L-glu binding. The results are summarized in table I.

Table I.

	Displacement of ^{3}H-L-glu binding. IC_{50} (µM)		Induced transmitter-release. EC_{50} (µM)	
	cortex neurons	granule cells	cort. neuron (^{3}H-GABA)	granule cells (^{3}H-D-asp)
L-glutamate	0.05	0.41	6	30
L-aspartate	0.26	0.65	16	33
D-aspartate	-	21	-	140
quisqualate	0.50	36	0.06	>1000
kainate	>10	>60	30	>1000
NMDA	>70	>70	4	>1000

33.17 STIMULATION OF $^{22}Na+$ EFFLUX FROM RAT FOREBRAIN MEMBRANES BY GLUTAMIC ACID AND KAINIC ACID. John C. Matthews* (SPON: W. Marvin Davis). Dept. of Pharmacol., Univ. of Miss., Sch. of Pharm., University, MS 38677.

Glutamic acid, kainic acid and quisqualic acid stimulate $^{22}Na+$ efflux from membranes prepared from rat forebrain in a glass-fiber filter assay. Glutamic acid showed the greatest efficacy with an ED_{50} of ∿ 3μM. Kainic acid produced ∿ 28% of the maximal efflux seen with glutamic acid with an ED_{50} of ∿ 1μM. Quisqualic acid never showed statistically significant increases in $^{22}Na+$ efflux over control experiments. However, values with quisqualic acid were consistently lower than control at concentrations above 0.5 μM reaching ∿ 12% of the maximal efflux seen with glutamic acid. N-methyl-D-aspartic acid showed no detectable efflux activity in this preparation. 2-Amino-phosphonobutyric acid (APB) blocked glutamate or kainate stimulated efflux with IC_{50} values of ∿ 0.1 μM. Calcium was required for the inhibitory action of APB, but not for the stimulatory actions of glutamic or kainic acids. Glutamic and kainic acids at concentrations above 100 μM were found to inhibit rather than stimulate $^{22}Na+$ efflux. Veratridine and tetrodotoxin had no influence on efflux stimulated by glutamic or kainic acids.

33.18 **A COMPARISON OF POTASSIUM RELEASE EVOKED BY STIMULATING AFFERENT FIBERS OF DIFFERENT DIAMETER AND BY APPLICATION OF PUTATIVE NEUROTRANSMITTERS.** **R.A.Davidoff, J.C.Hackman, and C.J. Wohlberg. Neurophysiology Laboratory, VA Medical Center, and Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, Fl. 33101.**

The excitatory amino acid, glutamate, has been proposed as the neurotransmitter released by large diameter afferent fibers and the peptide, substance P, has been postulated to be one of the transmitters released by unmyelinated afferents. In addition, several other putative peptide transmitters have been identified in dorsal root ganglion cells. The present experiments investigated the amount of K^+ released by afferent fibers of different diameter and by the application of possible primary afferent transmitters.

Frogs (Rana pipiens) were decapitated, the spinal cord removed, hemisected and placed in a sucrose gap recording chamber. The cords were superfused with HCO_3^--Ringer's solution bubbled with 95%:5% O_2:CO_2 and maintained at 15°C. DC membrane potentials were recorded from the ninth dorsal and ventral root. K^+-sensitive microelectrodes were used to measure changes in extracellular potassium concentrations in the dorsal horn and intermediate gray matter, produced by tetani (5Hz,10sec) applied to the sciatic nerve with various voltages.

The stimulus voltages required to elicit Aα,Aγ and C fiber compound action potentials were recorded in the dorsal root in response to sciatic nerve stimulation. The largest diameter afferents (Aα, 0.43V+/-0.09,n=3) were responsible for 73.9%+/-6.5% (n=5) of the amount of potassium released by supramaximal stimulation of the sciatic nerve. Aγ fibers (1.03V+/-0.09,n=3) were responsible for an additional increment in the amount of potassium released to 84.0%+/-5.4% (n=5). Maximal release was produced by C-fiber stimulation (100%,11.7V+/-1.7,n=3).

The superfusion of L-glutamate and L-aspartate (1mM, 30sec) resulted in the release of potassium in the dorsal horn. The change of potassium activity above baseline levels was 1.3+/-0.4mM for L-glutamate and 1.1+/-0.2mM for L-aspartate (n=4). Application of N-methyl-DL-aspartate (100uM,30sec), kainate (20uM,30sec), and quisqualate (10uM, 30sec) also evoked the release of potassium; the increase was 1.3+/-0.2, 1.5+/-0.2, and 1.2+/-0.3 mM, respectively. In contrast, the application of several putative peptide neurotransmitters (substance P, cholecystokinin, vasoactive intestinal peptide, somatostatin, neuromedin K, bombesin, substance K, and physalaemin; 1uM,60sec) produced slow depolarizations of the ventral root and an increase in spontaneous activity but did not cause any change in K^+ activity. The only peptide tested which released small amounts of K^+ was eledoisin.

In summary, it appears that the largest diameter afferent fibers are responsible for the majority of the K^+ released as a result of sensory stimulation. **(Supported by VAMC MRIS #1769 and 3369 and USPHS #NS17577 and #HL07188).**

PSYCHOTHERAPEUTIC DRUGS: NEUROLEPTICS

34.1 POSSIBLE ANTI-DYSKINETIC EFFECTS OF CANNABIDIOL L. H. Conti*, L. Stein*, M. Cogen*, P. Consroe* and R.E. Musty (SPON.D. Lorenz, Department of Psychology, University of Vermont, Burlington, VT 05405 and Department of Pharmacology, College of Pharmacy, University of Arizona, Tucson, AZ 85712

Cannabidiol (CBD) is a natural cannabinoid which does not produce a psychological high, but has recently been shown to have anti-epileptic properties in animals and man (Cunha, et al., Pharmacol., (1980),21,175) anti-anxiety properties in animals (Musty, The Cannabinoids, Academic, (1984), 795). In addition, data in humans suggest that CBD suppresses dyskinesia (Snider, S.R. and Consroe, P. Neurology, (1984), 34, 147). The present experiments were designed to test this possibility, using drug induced circling behavior in rats with unlilateral lesions of the substantia nigra produced by injection of 6-hydroxydopamine.

Rats were prepared with unilateral substantia nigra (SN) lesions by injection of 8 ug of 6-OHDA (in 2% ascorbic acid + saline) in 4 ul via cannula at Pellegrino coordinates: 3 mm posterior to and 2 mm lateral from bregma and 8.5 mm ventral from dura.

Six to 8 days after surgery, rats were injected with 5mg/kg meth-amphetamine (MEA), i.p., or 5 mg/kg MEA plus 10 mg/kg CBD. Turning behavior was assessed in a stainless steel bowl 32.5 cm in diameter in a sound attenuated chamber for 15 min. CBD blocked the MEA induced contralateral turning behavior which was observed in the MEA group alone.

In a second experiment, SN lesions were produced, as before. A 35 day period was allowed to elapse in order to test for denervation supersensitivity following apomorphine challenge. On the 35th day, 0.5-1.0 mg/kg of apomorphine hydrochloride (APO) i.p., was administered to each rat. Ipsilateral turning behavior was induced. At two day intervals, the following sequence of drug tests were conducted: APO (as before) + 10 mg/kg CBD, i.v.; APO alone; APO + 40 mg/kg CBD, i.v.; APO alone; APO + 20 mg/kg CBD,i.v.; and APO alone. At 10 mg/kg, CBD produced slight reductions in ipsilateral turning; at 20 mg/kg, CBD produced moderate reductions; and at 40 mg/kg, CBD completely blocked turning.

These results suggest that CBD may block both contralateral turning induced by MEA and ipsilateral turning produced by APO. It may be that CBD blocks changes in turning behavior regardless of its source, i.e., presynaptic or postsynaptic. In addition, the SN lesion technique may serve as a model for testing anti-dyskinetic drugs. Given these conclusions it seems reasonable to propose that CBD has anti-dyskinetic effects.

34.2 SULPIRIDE: EFFECTS ON APOMORPHINE- AND AMPHETAMINE-INDUCED BEHAVIORS SUGGEST A PREFERENTIAL ACTION ON PRESYNAPTIC DOPAMINE RECEPTORS. A. Robertson, J. Rudski*, and C. MacDonald*. Dept. of Psychology, McGill Univ., Montreal, Quebec, Canada H3A 1B1.

We recently reported that the atypical neuroleptic sulpiride enhanced amphetamine-induced stereotypy (Robertson & MacDonald, Eu. J. Pharm., 1985, 109, 81). This might be explained by its greater affinity for presynaptic vs. postsynaptic dopamine receptors (Jenner & Marsden, Acta Psych. Scand., 1984, Suppl. 211, 109). The present experiments examined this possibility in two ways. In the first experiment, the effects of sulpiride (5 & 20 mg/kg SC) on behaviors induced by apomorphine were tested. A low dose of apomorphine (0.1 mg/kg SC) produced hypomotility, an effect believed to represent a presynaptic action (DiChiara et al. Nature, 1976, 264, 564). Amongst a number of atypical and typical neuroleptics tested, only sulpiride antagonized this hypomotility. Apomorphine in higher doses (0.2-1.0 mg/kg, SC) produced stereotypy (sniffing down and licking or gnawing), which has generally been attributed to its postsynaptic activity (Ernst, Psychopharmacol., 1967, 10, 316). Sulpiride did antagonize stereotypy, although its effects were weaker than those of other neuroleptics tested. In the lower dose, sulpiride acted primarily to antagonize stereotyped sniffing down whereas in the higher dose, it blocked stereotyped sniffing down and licking or gnawing. The effects of sulpiride on apomorphine-induced hypomotility and stereotypy are consistent with the notion that this drug has strong presynaptic and weak postsynaptic blocking abilities.

In the second experiment, the effects of sulpiride on amphetamine-induced turning behavior in rats with small electrolytic lesions of the nigrostriatal dopamine bundle were examined. Sulpiride was ineffective in blocking turning; in fact, it had a tendency to enhance the number of turns produced by amphetamine. This effect cannot be accounted for by general increases in motoric behavior as sulpiride itself does not change locomotion nor does it interact with amphetamine-induced locomotion (Robertson & MacDonald, op. cit.). Thus sulpiride has similar enhancing effects on both amphetamine-induced turning and amphetamine-induced stereotypy. The data further suggest that the locus of action of this neuroleptic might be on the nigrostriatal dopamine system.

34.3 **DIFFERENT PROFILES FOR CLOZAPINE, CHLORPROMAZINE, AND PIMOZIDE ON THREE ACTIVITY MEASURES DURING CHRONIC DOSING IN RATS.** H.B. Freese*, J.H. Porter, C.A. Bjornsen*, & G.L. Kaempf* (SPON: M.J. Kallman). Psychology Dept., VA Commonwealth Univ., Richmond, VA 23284.

The classic antipsychotic drugs are effective in the clinical treatment of schizophrenia; however, these "typical" neuroleptics produce extrapyramidal motor side effects. Other "atypical" neuroleptics also have been shown to have antipsychotic activity, but have a much smaller liability for producing motor side effects. To date, there are no preclinical screening procedures which both identify drugs as potential antipsychotic agents and which separate typical from atypical neuroleptics. The present study examined the typical neuroleptics, chlorpromazine (CPZ) and pimozide (PMZ), and the atypical neuroleptic, clozapine (CLOZ), on three different activity measures with chronic dosing regimens.

In Exp., 1 the effects of CPZ (4mg/kg,IP) and CLOZ (10mg/kg,IP) on photocell activity and wheel running in adult male rats were assessed over a 22 day chronic dosing regimen. As shown in the table below, different profiles were evident for CPZ and CLOZ on photocell activity. Over the first 10 days, photocell activity declined for CPZ; whereas, CLOZ produced an initial suppression of photocell activity which diminished over the next 10 days and remained fairly constant until the end of the 22 days. Wheel running was suppressed equally by both drugs over the 22 days.

In Exp. 2, PMZ (1mg/kg,IP) and CLOZ (10mg/kg,IP) were administered to adult male rats for a total of 38 days and spontaneous motor activity (Automec Activity Monitors) was measured on days 1, 8, and 38. Over the first 8 days, the profile for CLOZ was very similar to that for photocell activity. PMZ also suppressed activity, but no recovery was evident over the first 8 days. By day 38, however, activity for CLOZ and PMZ recovered to Vehicle levels.

These preliminary findings suggest that photocell and spontaneous motor activity measures yeild different profiles for typical and atypical antipsychotic drugs during chronic dosing regimens and may be useful for screening potential antipsychotic agents.

PHOTOCELL ACTIVITY

DRUG	DAY 1	DAY 10	DAY 22
VEH(N=6)	157.8	165.6	149.2
CLOZ(N=7)	36.3[a]	75.8[a]	71.5[a]
CPZ(N=6)	62.1[a]	9.8[a,b]	25.5[a]

WHEEL RUNNING

DRUG	DAY 1	DAY 10	DAY 22
VEH(N=6)	86.1	77.3	78.5
CLOZ(N=7)	35.3[a]	22.2[a]	14.9[a]
CPZ(N=7)	36.6[a]	20.1[a]	23.5[a]

SPONTANEOUS MOTOR ACTIVITY

DRUG	DAY 1	DAY 8	Day 38
VEH(N=12)	314.2	296.0	254.8
CLOZ(N=6)	28.2[a]	120.2[a]	209.3
PMZ(N=6)	205.0[a,b]	175.0[a]	262.3

a = sign. diff. from VEH (p<.05) b = sign. diff. from CLOZ(p<.05)

34.4 **THE EFFECTS OF PIMOZIDE AND EXTINCTION ON OPERANT BEHAVIOR AND WHEEL RUNNING ACTIVITY IN RATS.** J. M. Willis* & J. H. Porter. Psychology Dept., Va. Commonwealth Univ., Richmond, VA 23284.

The suppression of operant responding by antipsychotic drugs in rats and other laboratory animals has often been attributed to the motor side effects of these drugs; however, Wise (The Behavioral and Brain Sciences,5:39,1982) has proposed that neuroleptic drugs interfere with operant behavior by decreasing the hedonic value of rewards (i.e.,anhedonia) before producing any significant impairment of performance capability. The fact that neuroleptic drugs produce an extinction-like pattern of response cessation has been offered as support for this theory. However, these studies primarily have used only continuous reinforcement schedules and have not obtained any independent measure of motor activity during the operant test sessions.

In the present study, six adult male rats maintained at 80% body weight were tested on a fixed-interval (FI) 1-min food reinforcement schedule and given simultaneous access to a running wheel attached to the operant chamber. After stable FI responding and wheel running were obtained, the rats were tested for four days under extinction (EXT). This was followed by four days on the FI schedule, and then four days of testing with pimozide (PMZ) while the FI schedule was still in effect. Three rats were tested with .5 mg/kg PMZ and three rats with 1.0 mg/kg PMZ.

A typical extinction curve was evident for barpressing during the four day test periods for EXT and for both doses of PMZ. As shown in the table below, both EXT and PMZ produced a significant suppresion of barpressing (responses/min) and the number of food pellets earned (no pellets were actually delivered during EXT); however, the rats continued to earn most of their pellets during PMZ testing in spite of the reduced rate of barpressing. Examination of the pattern of responding revealed that EXT disrupted the FI scallop, but that PMZ did not. Wheel running (revolutions/min) increased significantly during EXT testing, but PMZ produced no significant change in the rate of running.

These data demonstrate that while both EXT and PMZ produced a similar pattern of response cessation over the four day test periods, their effects on the pattern of responding, number of pellets earned, and wheel running differed. Also, the lack of suppression of wheel running by PMZ argues against a motor impairment interpretation of PMZ's suppression of operant behavior.

MEANS FOR THE LAST DAY OF EACH TEST CONDITION

	FI	EXT	FI	.5 mg/kg PMZ	1.0 mg/kg PMZ
BARPRESSING	16.7	0.4	17.3	3.3	2.2
NO. OF PELLETS	58.4	8.3	58.6	53.3	50.0
WHEEL RUNNING	4.6	8.0	2.8	1.6	2.1

34.5 THE EFFECT OF ATYPICAL NEUROLEPTICS ON HORMONE SECRETION IN THE RAT. H.Y. Meltzer, M. Simonovic, T. Koyama, G.A. Gudelsky, and J.I. Koenig, Dept. of Psychiatry, University of Chicago, Chicago, IL. 60637

It has recently been shown that neuroleptics can elevate plasma levels of beta-endorphin (END) as well as PRL in the rat (Endocrinology, 110:657). The current studies were designed to compare the effects of the atypical neuroleptics, clozapine (CL) and fluperlapine (FL), with those of the typical neuroleptics haloperidol (HA), and spiroperidol (SP) on PRL, END and corticosterone (B) secretion in the rat. All drugs and their respective vehicles were injected i.p. The adult male Sprague-Dawley rats were sacrificed by decapitation and trunk bloods were collected into chilled plastic tubes containing EDTA. Concentrations of PRL, B and END in plasma were determined by RIA. The rate of dopamine (DA) synthesis in tuberoinfundibular neurons was determined by measuring the accumulation of DOPA in the median eminence following treatment with NSD 1015 (100 mg/kg). DOPA levels were determined by HPLC with electrochemical detection. As previously reported, the administration of CL, FL, HA and SP elevated plasma levels of PRL. However, the time course of this effect differed: CL (20 mg/kg) and FL (20 mg/kg) elevated PRL levels for 2 hrs, while the PRL levels after HA (1 mg/kg) were elevated for more than 4 hrs. In contrast to PRL, HA and SP did not stimulate the secretion of either B or END, whereas CL and FL both elevated the plasma concentrations of B and END in a dose-related manner. FL was more potent than CL in stimulating PRL secretion while the reverse was true for B and END. The shortened stimulatory effect of CL and FL on PRL secretion prompted us to study the effect of these agents on the activity of the tuberoinfundibular DA neurons. Both CL and FL increased the accumulation of DOPA in the rat median eminence; HA was without effect. Therefore, these atypical neuroleptic agents not only block DA receptors but also stimulate the synthesis and release of DA from the median eminence while typical neuroleptics are devoid of the latter effect. These actions could account for the abbreviated PRL response and the elevation of both serum B and END levels.

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34.6 EFFECTS OF A POTENTIAL ANTIPSYCHOTIC COMPOUND, BMY14802, ON SINGLE-UNIT ACTIVITY OF DOPAMINE NEURONS OF THE RAT SUBSTANTIA NIGRA ZONA COMPACTA. R.T. Matthews, D. Blair*, and R. Sallis*, Dept. of Anatomy, Texas A&M Univ. Sch. of Med., College Station, TX 77843.

BMY14802 [α-(4-fluorophenyl) - (5-fluoro-2-pyrimidinyl)-1-pipirazine butanol] has several behavioral and biochemical effects in rats suggesting it may influence dopaminergic (DA) neurotransmission in the CNS. Thus BMY14802 inhibits conditioned avoidance responding, reverses apomorphine (APO)-induced stereotyped behaviors and increases striatal and frontal cortex levels of the DA metabolite, DOPAC. However, BMY14802 has little affinity for ^{3}H-spiperone binding sites (IC_{50} >10^{-6} M) and reverses haloperidol-induced catalepsy. The present experiments were designed to measure the effects of BMY14802 on the activity of DA neurons in vivo using electrophysiological techniques in chloral hydrate anesthetized rats. Extracellular single-unit activities of DA neurons in the substantia nigra zona compacta (SNC) were recorded with tungsten electrodes (Bunney et al., J.P.E.T., 185:560, 1973; McMillen et al., J. Neurosci., 3:733, 1983). BMY14802 increased the firing rates of DA neurons (effective dose range 0.5-12.5 mg/kg, i.v., n=7). BMY14802 at similar doses also (1) reversed APO-induced inhibition of firing rate (10 µg/kg, i.v., n=8), (2) blocked the inhibitory effect of a second 10 µg/kg of APO on these same neurons (n=4), (3) reversed D-amphetamine-induced inhibition of firing rate (1.0-3.0 mg/kg, i.v., n=5), and (4) attenuated the effect of a second dose of d-amphetamine. From these data, the effect of BMY14802 alone or after DA agonists resembles the effect of neuroleptics such as haloperidol on the firing rate of SNC DA neurons. Further experiments were designed to test whether the effects of BMY14802 may be mediated by blockade of DA receptors. BMY14802 at 10 mg/kg, i.v. increased DA neuronal impulse flow after blockade of most or all DA receptors by haloperidol (0.5 mg/kg, i.v.). This suggests a non-DA receptor mechanism of action of BMY14802 on SNC DA neuronal impulse flow which may account for the reversal of haloperidol-induced catalepsy by this compound. Preliminary microiontophoretic experiments showed that neither systemic (10 mg/kg, i.v.) nor locally applied BMY14802 (50 mM, co-microiontophoresed) blocked the inhibitory effect of microiontophoresed DA on SNC DA neuronal firing rates. This suggests that BMY14802 does not block DA autoreceptors in the SNC.

In summary, BMY14802 appears to increase SNC DA neuronal impulse flow and reverse the impulse flow depressant effects of DA agonists through a non-DA receptor mechanism. (Supported by Bristol-Myers Co., Evansville, Indiana)

34.7 COMPARATIVE EFFECTS OF BMY 14802, A POTENTIAL ANTIPSYCHOTIC DRUG, AND HALOPERIDOL ON RAT BRAIN DOPAMINERGIC TRANSMISSION. Brian A. McMillen and Helen L. Williams*, Dept. of Pharmacology, School of Medicine, East Carolina Univ., Greenville, NC 27834.

The potential antipsychotic drug, BMY 14802 [α-(4-fluorophenyl)-5-fluoro-2-pyrimidinyl)-1-piperazinebutanol], is a moderately potent inhibitor of conditioned avoidance responding and apomorphine stereotyped behavior. This drug does not cause catalepsy and has no affinity for D2 receptors. At 3.0 mg/kg s.c., BMY reduced haloperidol (HALO) catalepsy. BMY exhibited an IC50 > 10^{-5}M against striatal membrane binding of 0.1 nM 3H-spiperone. From 1.0-30 mg/kg, BMY exhibited a flat dose-response effect on striatal and frontal cortical (FCTx) dopamine (DA) metabolism (DOPAC concentrations). After 10 or 30 mg/kg BMY, striatal DOPAC levels increased 55% or 109% compared to a 283% increase after 0.3 mg/kg HALO. When this dose of HALO was combined with 2.5 mg/kg amfonelic acid (AFA), AFA-induced behavioral stimulation was blocked and DOPAC concentrations increased 8 fold. When AFA was combined with BMY, DOPAC concentrations were less than after BMY alone including doses that blocked AFA-stimulated behavior. This result indicated that the inhibition of stimulated behavior was not due to a strong blockade of postsynatpic D2 receptors. Data from FCTx were qualitatively similar. To test whether BMY altered in vivo DA synthesis, BMY was injected 30 min before 100 mg/kg NSD-1015 and L-DOPA allowed to accumulate for 30 min. Doses of BMY that did not alter striatal DOPAC levels markedly increased L-DOPA accumulation. This effect was not due to inhibition of DA autoreceptors because BMY failed to block apomorphine's inhibition of GBL-activated tyrosine hydroxylation.

The subchronic effects of BMY on the ability of DA metabolism to respond to HALO challenge and on striatal D2 receptor Bmax and K_D were used to assess risk of extrapyramidal dysfunction. Two week osmotic minipumps were used for administration of BMY at either 3 or 15 mg/kg/day s.c. The larger dose of BMY significantly reduced FCTx DOPAC levels and reduced the response of striatal DOPAC levels to acute challenge by HALO. Despite the reduced response to HALO, D2 receptor sensitivity was unchanged when determined by 3H-spiperone binding 4 days after stopping the 15 mg/kg/day infusion. The lack of altered postsynaptic DA receptor sensitivity by BMY suggests this drug may have fewer extrapyramidal side-effects than classical antipsychotic drugs. The data indicate that the pharmacological effects of BMY to inhibit stimulated behavior and conditioned avoidance responding may have a nondopaminergic mechanism. Should clinical trials of BMY demonstrate antipsychotic efficacy, then this drug would truly be an atypical drug since it has no affinity for DA receptors.

(Supported by Bristol-Myers Co., Evansville, IN).

34.8 IN VITRO AUTORADIOGRAPHY OF ^{3}H-BUSPIRONE AND ^{3}H-2-DEOXYGLUCOSE AFTER BUSPIRONE ADMINISTRATION. S.L. Moon [1,2] and Duncan P. Taylor[1]. [1]Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, IN 47721 and [2]Indiana University School of Medicine, Evansville, IN 47732.

Buspirone (BuSpar®) is a new, effective, anxiolytic drug. Structurally distinct from the benzodiazepines widely prescribed to treat anxiety, buspirone lacks the untoward side effects associated with benzodiazepine use. While the benzodiazepines bind with high affinity to gamma-aminobutyrate sites in brain, buspirone interacts with serotonin (5-HT) and dopamine sites. Previously, we demonstrated ^{3}H-buspirone in the rat is nonuniformly distributed in brain after an anxiolytically-relevant dose of 10 mg/kg (Soc. Neurosci. Abstr. 10:17, 1984). Many of the regions preferentially labeled in vivo were limbic-related structures and regions rich in 5-HT receptors or dopamine. To evaluate further the sites that interact with buspirone, we have used in vitro autoradiography to visualize the distribution of: (1) ^{3}H-buspirone and receptors for (2) dopamine and (3) 5-HT on alternate sections of rat and human brain. Moreover, we have analyzed the effect of buspirone administration on glucose uptake in rat through the use of ^{3}H-2-deoxyglucose autoradiography.

Our in vitro autoradiography in the rat shows that ^{3}H-buspirone densely labels medial frontal and entorhinal cortices, lateral septum, and the dentate gyrus. Moderate to high amounts of ^{3}H-buspirone are found in all fields of the hippocampus, the interpeduncular, dorsal raphe, facial, trigeminal, and oculomotor nuclei. The striatum shows a gradient of label with moderately high levels rostrally and laterally, but diminishing in the medial and caudal portions. A sparse distribution of ^{3}H-buspirone in the thalamus contrasts with heavier labeling of the hypothalamus. The labeling in substantia nigra is slightly enhanced. There is a strong resemblance in the pattern of ^{3}H-buspirone and ^{3}H-5-HT labeling; however, both ligands concentrate in regions where the other does not. For example, in rat and human, ^{3}H-5-HT not ^{3}H-buspirone, binds to the globus pallidus and substantia nigra, pars reticulata. This suggests that buspirone has a greater preference for 5-HT 1A rather than 1B receptors and is consistent with binding data from homogenates (Riblet et al., Psychopathol. 17: Suppl. 3, 69, 1984). However, that buspirone labels the facial and trigeminal nuclei may reflect an interaction with another 5-HT receptor as well.

Buspirone (15-40 mg/kg, p.o. or i.p.) increased glucose uptake in the medial portion of nucleus accumbens and substantia nigra, pars compacta. Decreased metabolism occurred in the lateral habenula, hippocampus, interpeduncular nucleus, and layers IV-V of cortex. Higher doses may alter metabolism in other brain regions. Thus, the metabolic pattern also suggests a strong interaction for buspirone with the limbic system.

34.9 BMY 14802: A POTENTIAL ANTIPSYCHOTIC AGENT THAT DOES NOT BIND TO D-2 DOPAMINE SITES. Duncan P. Taylor, Michael S. Eison, W. G. Lobeck, Jr.*, L. A. Riblet, D. L. Temple, Jr. and J. P. Yevich, CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, IN 47721.

The potential antipsychotic agent BMY 14802, known chemically as α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazinebutanol, was first identified by its ability to block the conditioned avoidance response in rats with potency comparable to clozapine (ED_{50} values of 26.4 and 24.0 mg/kg, p.o., respectively). BMY 14802 also inhibits apomorphine-induced stereotypy (ED_{50}=33.0 mg/kg, p.o.) and has a clozapine-like profile in the Sidman avoidance test. Unlike currently-marketed antipsychotics, BMY 14802 does not produce catalepsy; indeed, BMY 14802 can reverse a previously-established trifluoperazine-induced catalepsy (ED_{50}= 16.9 mg/kg, p.o.). Chronic administration of 15 or 30 mg/kg, p.o., for 29 days had no effect on the maximal number of [^{3}H]-spiperone binding sites in rat striata. On the other hand, the antipsychotic haloperidol (3 mg/kg, p.o.), whose long-term clinical use is associated with the occurrence of tardive dyskinesia, produced a significant increase in the number of binding sites. These findings suggest that the clinical use of BMY 14802 would not be associated with extrapyramidal side effects. In contrast to clozapine, BMY 14802 does not interact with muscarinic cholinergic receptor binding sites in vitro and does not protect against the lethal effects of physostigmine in rodents. This suggests that cholinergic mechanisms are not directly involved in BMY 14802's ability to reverse neuroleptic-induced catalepsy. In further contrast to clozapine, BMY 14802 has minimal interaction with α_1-adrenergic or H_1-histaminergic binding sites in vitro. It is likely that this may account for the absence of α_1-adrenergic blockade in vivo and sedation as measured by reduction in spontaneous motor activity, production of incoordination on the rotarod and potentiation of ethanol in the production of hypnosis. In vitro receptor binding studies have not provided insight into the mechanism of action of BMY 14802 as it does not block any sites at concentrations less than 500 nM (among them α_2-adrenergic, β-adrenergic, type 1 and 2 serotonergic, benzodiazepine, opiate, glycine, glutamate, imipramine, or nitrendipine). Significantly, BMY 14802 does not block [^{3}H]spiperone binding to rat striatal sites in vitro (IC_{50}>1000 nM). Furthermore, metabolites which might interact with dopamine binding sites are not formed in vivo as no such activity is present in serum or directly in the striatum using ex vivo techniques. Thus, BMY 14802 appears to be a potential antipsychotic drug which lacks the side effect potential of conventional agents (movement disorders) or atypical agents (sedation, cardiotoxicity). (Further data on BMY 14802 will be presented by McMillen and Williams, this meeting).

34.10 ANTAGONISM OF AMPHETAMINE-INDUCED BEHAVIORAL CHANGES BY BMY13859-1 IN A PRIMATE MODEL OF PSYCHOSIS. R.F. Schlemmer, Jr. & J.M. Davis. Ill. St. Psychiat. Inst., Chicago, IL 60612

BMY13859-1,8-(4-(4-(1,2-benzisothiazol-3-YL)-piperazinyl)butyl)-8-azaspiro(4.5)decane-7,9-dione HCl, is a compound which demonstrates an antipsychotic profile in rodent screening models for these agents. The present study was designed to determine the effect of BMY13859-1 on the amphetamine (Amphet) model of psychosis in a primate social colony. This model is of particular relevance because Amphet induces several behavioral changes in this paradigm which resemble characteristics of amphetamine psychosis and schizophrenia. Also, these changes can be antagonized by known antipsychotic agents. The subjects for the study were members of a stable social colony of 5 adult Stumptail macaques (Macaca arctoides), 1 male and 4 females. The expt was designed as a cross-over study where 2 females received drug treatment at one time followed by similar treatment of the other 2 females. The male remained untreated throughout the study. The expt began with observation of undrugged behavior (Base-1) followed by administration of d-amphet, 1.6 mg (base)/kg in time-release form, n.g. every 12 hr for 12 consecutive days. Five months later after Base-2 observation, the monkeys received BMY13859-1, 5 mg (salt)/kg, n.g. every 12 hr for 7 days, then BMY13859-1 + Amphet, as before, for the next 12 days. During the expt, "blind", experienced primate observers conducted two 60-min observation sessions daily where the behavior of each animal in the colony was recorded on a computer according to a 40 item checklist using the focal-sampling technique. Chronic Amphet treatment induced several behavioral changes of particular interest for the study of psychosis including: 1) intense scratching (which has been associated with tactile hallucinations in humans), 2) increased submissive gestures 3) increased checking (visual scanning), 4) the induction of stereotypies, and 5) social withdrawal characterized by increased distancing from other colony members and decreased social grooming. BMY13859-1 significantly antagonized the amphet-induced intense scratching and increase in submissive gestures to Base-1 & Base-2 levels in those monkeys who developed these responses when treated with Amphet alone. Amphet-induced increase in checking & stereotyped behavior was prevented by BMY13859-1 in all monkeys throughout treatment. Increased distancing scores induced by Amphet were significantly antagonized & returned to Base-1 & Base-2 levels in 2 monkeys during the later stages of Amphet treatment. At the same time, severely reduced social grooming scores in Amphet-treated monkeys were significantly increased by concomitant treatment with BMY13859-1 in 2 monkeys. It is unlikely that the antagonistic effects of BMY13859-1 can be attributed to sedation since resting scores for BMY13859-1 alone or concomitantly with Amphet did not exceed Base-2 levels for any monkey. The results of this study demonstrate that BMY13859-1 is a good antagonist of Amphet-induced behavior in primates. Importantly, the activity of BMY13859-1 in this model is consistent with known antipsychotic drugs. (Supported through a grant from Bristol-Myers).

34.11 THE NOVEL ANTIPSYCHOTIC BW 234U ANTAGONIZES ENVIRONMENTALLY INDUCED ELEVATION OF DOPAMINE METABOLISM IN PREFRONTAL CORTEX. D.M. Vaughn, K. Viik and B.R. Cooper. Dept. of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

BW 234U, a new drug with antipsychotic effects in open clinical trials, represents a potentially new generation of novel non-neuroleptic antipsychotic agents. Prior work in animals has shown that BW 234U blocks apomorphine-induced aggression but does not block apomorphine-induced or amphetamine-induced stereotyped behaviors, and high doses do not cause catalepsy. BW 234U does not block dopamine receptors nor elevate dopamine metabolism in whole brain over a behaviorally relevant dose range (Vaughn et al., Neurosci. Abstr. 77.16, 1984; Ferris et al., Neurosci. Abstr. 77.13, 1984). Since BW 234U does not interact directly with dopamine neurons in a manner similar to neuroleptic antipsychotic drugs, the present experiments were conducted to explore the possibility that BW 234U might affect "psychological" activation of dopaminergic neurons. Male Long Evans rats (Blue Spruce Farms, Altamont, NY) were injected with 12.5 mg/kg i.p. and 25 mg/kg i.p. BW 234U and compared with two control groups, a pH adjusted (pH = 4) saline injected, handled group brought into the lab environment and treated in every way similar to the BW 234U injected animals, and a group of rats sacrificed at the same time of day (10:00-11:00 a.m. EST) immediately upon removal from the animal quarters. Dopamine, DOPAC, HVA, 5HT, 5HIAA and NE were measured in slices of prefrontal cortex or striatum cut approximately 2 mm thick, using a brain mold (Wheeler, S.C. and R.H. Roth, Naunyn-Schmiedeberg's Arch. Pharmacol., 312:151-159, 1980). Relative to the uninjected controls, the saline injected group had a 27% increase in DOPAC and 50% increase in HVA in prefrontal cortex but not striatum, and the elevation of dopamine metabolism was significantly reduced by prior treatment with BW 234U. 5HIAA levels were also elevated by saline injection, but BW 234U did not reduce this environmentally induced change in serotonin metabolism. Norepinephrine levels were not significantly altered by any treatments in these tissues. Results show that the novel antipsychotic BW 234U can reduce the activation of dopamine neurons in prefrontal cortex produced by an environmental change. The mechanism of this effect at the cellular level is not known.

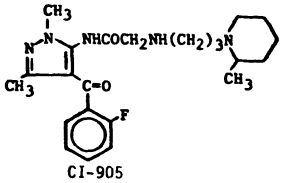
34.12 NEUROCHEMICAL PROPERTIES OF CI-905 AND CI-913, AGENTS WHICH EXHIBIT ANTIPSYCHOTIC-LIKE EFFECTS IN ANIMALS. T. A. Pugsley*, Y. H. Shih*, S. F. Stewart*, L. L. Coughenour*, (SPON: B. P. Poschel). Warner-Lambert/Parke-Davis Pharm. Res., Ann Arbor, MI 48105.

CI-905 (N-[4-(2-fluorobenzoyl)-1,3-dimethyl-1H-pyrazol-5-yl]-2-[[3-(2-methyl-1-piperidinyl)propyl]amino]acetamide (Z)-2-butandioate (1:2)) and CI-913 (2-(diethylamino)-N-[4-(2-fluorobenzoyl)-1,3-dimethyl-1H-pyrazol-5-yl]acetamide monohydrochloride) are novel agents which have been shown to have an antipsychotic-like profile in animal tests and to exhibit no propensity to cause extrapyramidal side effects (EPS). The present study examined the in vitro and in vivo biochemical properties of behaviorally active doses of CI-905 and CI-913.

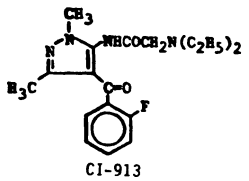
CI-905 and CI-913 did not cause any significant changes in rat striatal dopamine (DA) or homovanillic acid levels, did not effect the α-methyl-p-tyrosine-induced disappearance of DA in rat brain and did not alter the γ-butyrolactone-induced increase in rat striatal DA indicating no effect on DA turnover. In addition neither agent affected brain norepinephrine turnover. Both agents elevated rat brain 5-hydroxyindoleacetic acid levels without altering serotonin (5-HT) levels suggesting that CI-905 and CI-913 increased brain 5-HT turnover. Unlike known antipsychotics which elevate rat serum prolactin levels, CI-905 and CI-913 caused a decrease in prolactin. CI-905 and CI-913 did not exhibit any significant in vitro affinity for DA, muscarinic, adrenergic, serotonergic, adenosine A_1, benzodiazepine, GABA or slow-calcium channel receptors. Nor did CI-905 and CI-913 affect rat striatal DA-stimulated adenylate cyclase activity. The lack of affect of CI-905 and CI-913 on rat brain Da turnover indicates that these agents would have little or no propensity to cause EPS and is in agreement with behavioral studies in monkeys. Although studies with these agents have been terminated because of animal toxicity, these findings demonstrate together with the behavioral results, that it is possible at the preclinical level to discover non-dopaminergic antipsychotic-like candidates.

34.13 ANTIPSYCHOTIC-LIKE EFFECTS OF CI-905 & CI-913 IN PRECLINICAL TESTS. T.G. Heffner, D.A. Downs, H.A. DeWald*, S.E. Harrigan*, K.L. Sledge*, J.N. Wiley*, and L.D. Wise*. Departments of Pharmacology and Chemistry, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The preclinical and clincial efficacy of available antipsychotic (AP) drugs is commonly attributed to their blockade of brain dopamine (DA) receptors. However, available AP drugs have incomplete efficacy in man and produce neurological side effects. In an attempt to discover AP drugs with improved efficacy and lesser propensity for extrapyramidal dysfunction (EPS) and tardive dyskinesia, we have sought to identify agents which display the behavioral profile of AP drugs in preclinical tests but which do not interact with brain DA receptors.



CI-905



CI-913

CI-905 and CI-913 are novel fluorobenzoylaminopyrazoles which are not brain DA receptor antagonists; they lack affinity for brain DA receptors in vitro and do not elevate serum prolactin in rats in vivo. Like known AP drugs, CI-905 and CI-913 reduce spontaneous locomotion in mice at doses that do not produce ataxia. Neither agent reduces cage climbing in mice induced by the DA agonist apomorphine. CI-905 and CI-913 display AP-like selective inhibition of one-way conditioned avoidance behavior in rats; each attenuates shelf-jump avoidance at doses that do not impair escape responding. Each agent also inhibits continuous (Sidman) avoidance responding in rats and squirrel monkeys at doses that do not impair shock termination responding. Both CI-905 and CI-913 attenuate rewarding intracranial self-stimulation in rats. Unlike known AP drugs, neither CI-905 nor CI-913 elicits dystonic movements in Cebus monkeys sensitized to haloperidol, a primate model of EPS. The preclinical profiles of CI-905 and CI-913 would predict efficacy in the treatment of schizophrenia without liability for EPS. Although adverse toxicological findings in animals precludes clinical evaluation of these agents, the present results indicate that many of the behavioral effects of AP drugs in animals do not require blockade of brain DA receptors.

34.14 D1 AND D2 DOPAMINE RECEPTOR ANTAGONIST CHARACTER OF SELECTED NEUROLEPTICS. B. K. Koe, R. Sarges* and W. M. Welch*. Pfizer Central Research, Groton, CT 06340.

Recently, in a study of the pyrroloisoquinoline neuroleptic, Ro 22-1319, a selective D2 DA receptor antagonist, interactions of neuroleptics at this receptor are envisioned to involve "pi" interactions with the two aryl rings of tricyclics, diphenylpiperidines and dexclamol/butaclamol, or aryl ring plus adjacent C=O of haloperidol, sulpiride, molindone and Ro 22-1319, as well as ionic interactions with the basic nitrogen. An "auxiliary binding site" was also proposed, interaction with which confers D1 antagonist characteristics (Olson *et al.*, in Dopamine Receptors, ACS Symposium Series, 1983, p. 251). Thus, sulpiride, molindone and Ro 22-1319, predominantly D2 antagonists, lack a lipophilic group at the basic N for binding at this auxiliary site. We describe below the relative D1 and D2 characteristics of several new neuroleptics based on inhibition of [3H]spiperone binding and DA stimulated adenylate cyclase activity of rat striatal preparations. The potent activity of these DA antagonists is shown by the low doses for half-maximal increase in striatal dopa accumulation. Flutroline, a potent 5-aryltetrahydro-γ-carboline neuroleptic (Koe *et al., The Pharmacologist* 21:180, 1979; Harbert *et al., Mol. Pharmacol.* 17:381, 1980), exhibits less D1 character than its hexahydro-γ-carboline analog, CP-52,215 (Sarges *et al., J. Med. Chem.*, in press), even though they contain the same lipophilic group on basic N. The corresponding 5-arylhexahydro-γ-carbolines with only H (CP-52,344) or Me (CP-47,276) on basic N potently inhibit both DA stimulated adenylate cyclase and [3H]spiperone binding. Two 2-aminomethyl-8-chloro-3,4-dihydro-5-methoxynaphthalene neuroleptics, CP-34,771 and CP-35,988 (Welch *et al., J. Med. Chem.* 21:257, 1978), with lipophilic 4-hydroxy-4-phenylpiperidinomethyl residues that could extend into the auxiliary binding site, are virtually inactive on D1 adenylate cyclase. These findings suggest that the auxiliary binding site proposed by Olson *et al.* (1983) may not be a major determinant of D1 or D2 receptor antagonist character.

Drug	Dopa Acc. ED_{50} μmol/kg	[3H]Spip. Bind. IC_{50} μM	DA-Stim. Aden. Cyclase IC_{50} μM	D1/D2
Chlorprothixene	8.0	0.014	0.04	3
CP-52,215	1.9	0.025	0.33	13
Flutroline	2.3	0.012	1.8	150
Haloperidol	0.17	0.009	0.31	34
CP-52,344	0.28	0.0067	0.30	45
CP-47,276	n.t	0.0075	0.31	41
Molindone	2.5	0.140	103	736
CP-35,988	1.7	0.0088	100	11364
CP-34,771	0.20	0.0025	>100	>40000

34.15 ELECTROPHYSIOLOGICAL EFFECTS OF REPEATED ADMINISTRATION OF APOMORPHINE, EMD 23 448 AND (+)3-PPP ON A9 DOPAMINE AUTORECEPTORS. Jeffrey M. Goldstein, Linda C. Litwin* and Jeffrey B. Malick. Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Delaware 19897.

Recent data suggest that dopamine (DA) neurons possess autoreceptors which inhibit neuron function. Administration of low doses of the DA agonist apomorphine inhibits the activity of DA neurons through selective activation of DA autoreceptors. This effect mimics the functional blockade of DA neurons obtained after neuroleptic administration and may explain the clinical efficacy of low dose apomorphine in schizophrenia. However, there have been several reports that have demonstrated subsensitivity of DA autoreceptors after repeated stimulation by DA agonists (e.g., apomorphine, bromocriptine). These findings may compromise the DA autoreceptor-stimulation strategy in the treatment of schizophrenia. In the present studies, we have addressed the issue of DA autoreceptor subsensitivity by determining the effects of repeated administration of apomorphine, EMD 23 448 (3-(4-(4-phenyl-1,2,3,6-tetrahydropyridyl-(1))-butyl)-indole, hydrochloride), and (+)3-PPP ((+)-3-(3-hydroxyphenyl)-N-n-propylpiperidine) on A9 DA cell firing using conventional extracellular recording techniques.

Repeated administration of apomorphine (3x daily for 9 days), at a dose selective for the autoreceptor (0.05 mg/kg, sc; Rebec and Lee, Brain Res 250, 188, 1982) caused a 51-fold shift in the median effective dose (MED50) of apomorphine required to suppress A9 DA cell firing (0.0032 mg/kg, iv in vehicle group compared to 0.16 mg/kg, iv in subacute apomorphine group). Similarly, EMD 23 448 (3x daily for 9 days), at a dose selective for the autoreceptor (2 mg/kg, ip; determined behaviorally) caused a 5.8-fold shift in the EMD 23 448 MED50 for suppression of cell firing (0.06 mg/kg, iv in vehicle group compared to 0.36 mg/kg, iv in subacute EMD group). In contrast to these findings, (+)3-PPP (3x daily for 9 days), also at a selective dose for the DA autoreceptor (10 mg/kg, ip; determined behaviorally) did not cause a significant change in the (+)3-PPP MED50 for suppression of cell firing. In fact, there was a slight increase in the sensitivity of the autoreceptor following repeated administration of (+)3-PPP (MED50 = 1.42 mg/kg, iv in vehicle group compared to 0.35 mg/kg, iv in subacute (+)3-PPP group).

The results with apomorphine and EMD 23 448 clearly indicate that there is DA autoreceptor subsensitivity after repeated administration. Although the results with (+)3-PPP suggest that DA autoreceptor subsensitivity does not occur following subacute treatment, further studies should be performed before definitive conclusions can be drawn.

34.16 PHARMACOKINETICS OF REDUCED HALOPERIDOL IN RODENTS AND NONHUMAN PRIMATES. J.L. Browning†, C.A. Harrington†† and C.M. Davis*††. Tex. Res. Inst. of Mental Sci., Houston, TX 77030†† and Baylor College of Medicine, Dept. of Neurosurgery, Houston, TX 77030†.

Reduced haloperidol, a major metabolite of haloperidol may have effect on the therapeutic efficacy of haloperidol (Ereshefsky et al J. Clin Psychopharm 4:138). We have investigated some of the pharmacokinetics of haloperidol and reduced haloperidol in several species using a radioimmunoassay developed in our lab (Browning et al J. Immunoassay 6:45). In rodents and non-human primates, reduced haloperidol had a longer half-life than haloperidol in plasma and red blood cells. In rats and non-human primates, no detectable levels of haloperidol or reduced haloperidol were found twenty-four hours after the last dose of acute or chronic dosage given orally or by injection. However, the rabbit exhibited significant quantities of reduced haloperidol twenty-four hours after a single injection (2 mg/kg IM).

Additionally in all species tested, levels of haloperidol and reduced haloperidol had a greater half-life in red blood cells as compared to plasma. After ten days of 4 mg/kg haloperidol IM once per day, the brain levels of haloperidol and reduced haloperidol were almost ten fold the plasma levels in rabbits.

These results indicate that reduced haloperidol may accumulate in brain tissues and that plasma levels may be insufficient as an indicator of brain cells.

34.17 REDUCED HALOPERIDOL AND HALOPERIDOL IN RED BLOOD CELLS AND PLASMA OF PSYCHIATRIC PATIENTS. C.A. Harrington and C.M. Davis*. Tex. Res. Inst. of Mental Sci., Houston, TX 77030.

We have developed a means of measuring the concentration of both reduced haloperidol (RH) and haloperidol (H) in plasma and tissue that utilizes chemical derivatization, liquid chromatography, and radioimmunoassay (J. Immunoassay 6:45). Quantification of H and RH in the plasma of psychiatric patients has demonstrated that high doses of H can result in an increased plasma concentration of RH in excess of the plasma H concentration. In addition Ereshefsky, et al (J. Clin. Psychopharm. 4(3):138) has observed in schizophrenic patients that high plasma concentrations of RH (RH/H>1) are correlated with poor treatment outcome, although in subsequent treatment with Prolixin patients showed demonstrable improvement. Due to these observations we have begun to study the pharmacokinetic of H and RH in animals (Browning, et al, Neurosci. Abstr.) and begun to refine the relationship between plasma and red blood cell drug concentrations and therapeutic effect in patients receiving H.

Plasma levels of H and RH were quantified in a group of over 100 patients. Levels of H exhibited a seeming linear relationship with dose, however, RH levels accumulated in a non-linear fashion at doses exceeding ~30 mg/day. Analysis, in another group of patients, of plasma and whole blood levels of both H and RH allowed the red blood cell level of both compounds to be determined. H exhibited a linear relationship between plasma and red blood cell levels with a ratio of RH-RBC/H-Plasma = .9. RH also exhibited a linear relationship between plasma and red blood cell levels, however, RH-RBC/RH-Plasma = 2.7. RH was sequested approximately 3 fold more effectively in RBC than H when compared to plasma. This relationship was seen at all dosages. If RH does correlate with negative therapeutic outcome, then its measurement seems called for in clinical assessment of patients treated with H. In addition RBC levels, if they simulate CNS levels of drugs more accurately, may be a better index of the relationship between therapeutic effects of neuroleptics and blood levels. Finally, assessment of the pool size in pharmacokinetic analysis of H and RH may have to address the issue of RH's greater affinity for RBC's as well as other cellular stores.

34.18 BEHAVIORAL EFFECTS OF INTRACEREBRAL CALCIUM CHANNEL INHIBITORS (CCI). R. de Beaurepaire, W.J. Freed and J.A. Grebb. (SPON: J.R. Stevens). Preclinical Neurosciences Section, Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

CCIs have recently been tested in the treatment of mania and depression. Signs of these diseases include abnormal locomotor activity and abnormal feeding behavior. We have therefore tested the effects of two CCI's (verapamil and diltiazem) on locomotor activity and feeding behavior after intracerebral injection.

Methods: Male Sprague-Dawley rats weighing 300 to 400 g were bilaterally implanted with 24 gauge cannulae placed above the lateral ventricles, striatum and paraventricular nucleus of the hypothalamus (PVN). One week after recovery animals were infused with vehicle or CCI: 0.5 mg in 5 µl for intraventricular (I CV) injections, 0.5 mg in 2 µl for striatal injection and 0.15 mg in 0.6 µl for PVN injection. Three behavioral tests were conducted: 1) feeding behavior during satiation (amount of wet mash eaten in one hour), 2) amounts of food eaten by 23-hour deprived rats habituated to eat mash one hour per day, 3) locomotor activity measured in an open field during one hour following injection.

Results: There was no change in feeding behavior after ICV or PVH injection of CCI. Feeding behavior after intrastriatal injection has not been tested. Results for locomotion are shown below.

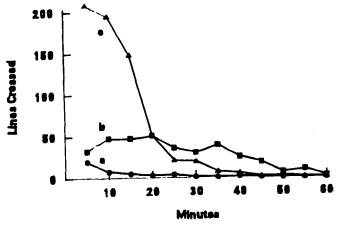


Figure 1: Locomotor activity (mean number of lines crossed during 1 hr) for animals receiving vehicle (n=20; circles), CCI in the ventricle (n=10; squares), or CCI in the PVH (n=10; triangles). Data for verapamil and diltiazem are combined.

Intrastriatal infusion (not represented), produced a decrease in locomotor activity but requires further investigation due to the low baseline activity levels. Verapamil and diltiazem (data are combined in the graph) both produced a similar stimulation of locomotor activity.

Discussion: Thus CCI do not influence feeding behavior even when injected in the PVH, which is known to be involved in the regulation of feeding. Nonetheless, CCI are very active in stimulating locomotor activity, particularly when injected into the PVH. This further supports a role of the hypothalamus and particularly the PVH in the regulation of locomotor activity and contrasts with the inhibition of locomotor activity produced by injections of calcitonin in the PVH (submitted). These results may be relevant to possible clinical applications of CCI's in the treatment of neuropsychiatric disorders.

35.1 ELECTRICAL THRESHOLD STABILITY OF PRIMARY AFFERENT NEURONS IN DOG TOOTH PULP. R. C. Kramis*, A. C. Kloka*, A. C. Brown, W. J. Beeler*, and R. W. Fields*. (SPON: L. Gronke). Dept. Physiol. & Biophysics SD, Oregon Health Sci. U., Portland, OR 97201.

The object of these experiments was to investigate adaptation and/or sensitization of primary afferent fibers in dog incisor and canine teeth upon repeated electrical stimulation. In anesthetized dogs, the superior alveolar nerve was surgically exposed, desheathed, and dissected into small branches. Individual branches were placed upon small hook electrodes for recording unit responses from the test teeth. Electrical stimuli were applied to the teeth at two sites: coronal and apical. Coronal stimuli were delivered through the intact enamel, using a monopolar paste electrode incorporated into a custom-molded acrylic cap to obviate possible inflammation-induced nociceptive ending sensitivity changes, a possible consequence of hard tissue removal. In addition, apical stimulation was delivered using bipolar amalgam electrodes placed in cavities prepared in the hard tissue near the site of nerve exit from the tooth, in order to enable measurement of conduction velocity in the nerve axon outside the tooth. Eighty-one units were tested in six dogs. Conduction velocities determined from apical stimulation ranged from 0.5 to 42 meters/sec. Coronal stimulation resulted in lower mean conduction velocities, presumably due to slower conduction within the tooth pulp. Refractory period averaged about 2 msec when stimulus intensity was just threshold, and decreased to about 1 msec when intensity was raised to twice threshold. The more rapidly conducting fibers showed stable thresholds (±10%) even after 20 minutes of 1 per second repeated stimulation. However, all units with conduction velocities in the C-fiber range required progressively greater stimulus intensity to reach threshold, and some adapted so rapidly that it was impossible to determine the threshold value. It was concluded that myelinated fibers in the A-beta and A-delta conduction velocity range do not adapt upon repeated 1 hz electrical stimulation, but that C-fibers adapt rapidly. Supported by NIDR grant DE-05895.

35.2 MESENCEPHALIC TRIGEMINAL SENSORY AXONS IN CATS FORM UNENCAPSULATED RUFFINI-LIKE MECHANORECEPTORS IN PERIODONTAL LIGAMENT BUT NOT TOOTH PULP. M.R.Byers, R.F.Martin*, T.A.O'Connor*, and W.K.Dong. Depts. of Anesthesiology and Biological Structure. Univ. of Washington, Seattle, WA, 98195.

Mesencephalic trigeminal sensory neurons are involved in dental proprioception as well as reflex sensitivity of masticatory muscles. The location of mesencephalic (mes V) receptors in dental tissue has not been determined previously. Functional studies have suggested that periodontal ligament is the main site of innervation (Jerge, J.Neurophys 26:379, 1963) but tooth pulp has also been suggested. We have injected ^{3}H-proline into the mes V nucleus in order to map the location of mes V receptors in cat dental tissue using autoradiography and to analyse their fine structure using EM-autoradiography. The entire right mes V nucleus was injected stereotaxically in two adult cats with 3 ul of ^{3}H-L-proline (25uCi/ul) divided among 3 sites along the rostral-caudal extent of the nucleus. The ipsilateral trigeminal main sensory and motor nuclei were not labeled. Heavily labeled axons were followed in semi-serial light microscopic autoradiograms from the mes V tract, out the ipsilateral root, past the trigeminal ganglion and along trigeminal dental nerves to endings in apical periodontal ligament of all ipsilateral maxillary and mandibular teeth in both cats. No labeled axons or endings were found in any tooth pulps, or in bundles of axons entering the tooth roots. Instead, labeled endings were confined to a narrow ring in the periodontal ligament around the root apex. Their distribution was much less extensive than for mechanoreceptors originating in the trigeminal ganglion (Byers, J.Comp.Neurol. 231:500, 1985). EM-autoradiography showed that labeled mes V endings are unencapsulated Ruffini-like mechanoreceptors; nearby bundles of unmyelinated axons or rare corpuscular receptors were not labeled.

Our results show that dental proprioceptive information carried by sensory neurons of the mes V nucleus originates in unencapsulated Ruffini-like mechanoreceptors in periodontal ligament. Moreover, those receptors are much less numerous than similar mechanoreceptors in periodontal ligament originating in trigeminal ganglion. Finally, the location of mes V receptors just outside the tooth root apex makes them suitable for monitoring the confinement of neuroanatomical tracers such as HRP to tooth pulp; if mes V neurons label after such injections, then the tracer must have leaked out of the tooth.

Supported by NIH grants DE05159, DE05130, DE00099.

35.3 MORPHOLOGY AND SYNAPTIC CONNECTIONS OF SMALL MYELINATED PRIMARY TRIGEMINAL AXONS ARBORIZING AMONG THE NEURONS IN THE BORDER ZONE OF RAT TRIGEMINAL NUCLEUS ORALIS. W. M. Falls and M. M. Alban*. Department of Anatomy, Michigan State University, East Lansing, MI 48824-1316.

The narrow, 100 to 200 μm wide, border zone (BZ) of rat trigeminal nucleus oralis lies immediately adjacent to the entire dorso-ventral extent of the spinal V tract (SVT). Preliminary Golgi and retrograde horseradish peroxidase (HRP) transport studies indicate that the dendritic arbors of the majority of BZ neurons are oriented in the rostrocaudal axis and are confined, for the most part, to this zone. Some BZ cells project to the cerebellum while others send descending intratrigeminal projections to the medullary dorsal horn. This study examines the morphology and synaptic connections of a population of small diameter primary trigeminal axons which generate bouton bearing terminal strands throughout BZ. These strands parallel and closely approximate the dendrites of BZ neurons and are in a position to provide input directly to these cells. Primary axons were brought into view by injecting 0.05 to 1.0 μl of 30% of HRP in 2% DMSO into the sensory root of the trigeminal nerve and allowing two days for the anterograde transport of the HRP-DMSO into the terminal axonal strands. Portions of these axons were also visualized in Golgi preparations. Thinly myelinated parent branches (0.5-1.5 μm in diameter) descending in SVT leave the tract by making a sharp medial bend and enter BZ directly. Within BZ many of these branches terminate by giving rise to a sparsely branched, long (up to 500 μm), rostrocaudally oriented, unmyelinated terminal strand characterized by several relatively widely spaced axonal endings. Based on the size and morphology of the parent branches in SVT these endings are considered to be from small Aσ primary trigeminal axons. The dome-shaped endings of the primary axons measure 1.0 to 2.0 μm in diameter and contain numerous agranular, spherical synaptic vesicles (40-60 nm) evenly dispersed throughout the terminals. In the BZ neuropil the endings lie in simple triadic arrangements where each forms a single asymmetrical axodendritic synapse on a dendritic shaft. It is at these synapses that this population of primary axons is thought to transfer its input directly to BZ neurons. A small (0.5-1.5 μm) axonal ending filled with flattened synaptic vesicles (29 x 60 nm) forms a symmetrical axoaxonic synapse on the primary ending as well as an axodendritic synapse on the same dendritic shaft receiving the primary input. In view of their symmetrical synaptic contacts terminals containing flattened synaptic vesicles are thought to belong to axons derived from at least one source which can inhibit or diminish the firing of BZ neurons. This would be accomplished either postsynaptically through the axodendritic synapses on the dendritic shafts or presynaptically through the axoaxonic synapses on the primary endings. Supported by N.I.H. Grant DE06725.

35.4 SIZE AND DISTRIBUTION OF IDENTIFIED CAT DORSAL ROOT GANGLION (DRG) NEURONS, R.D. Rose, M.J. Sedivec & L.M. Mendell Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Adult cat spinal ganglion (DRG) somata (in L6-S2) were identified by (i) orthograde labeling with wheat germ agglutinin or horseradish peroxidase, or (ii) a combination of intracellular recording/labeling and physiological characterization techniques (electrodes filled with Lucifer Yellow, LYCh, in 1-2 M LiCl; 8-35 Mohm). Cats were anesthetized deeply throughout surgery and other experimental procedures. Transport experiments labeled sural or triceps surae afferent pools bilaterally. Typically, orthogradely labeled primary afferent pools spanned one or more ganglia. The distributions suggest that afferent pools are unimodally distributed rostro-caudally, bilaterally symmetrical, and variable between animals. Adjacent labeled neurons were frequently observed. LYCh labeled somata were limited to units with fast peripheral fiber conduction velocities (PCV, 77 m/s±4 SE, range 31-113). These units were large (2836 um^2 ±253, cross section through nucleus, range 801-6743). LYCh labeled somata (n=30) included neurons with peripheral fibers supplying muscle spindles, tendon organs, hair follicles, mechanoreceptors, etc. There was no significant difference in size (radius, area, volume) of these LYCh labeled muscle and cutaneous afferents (t-test, $p>0.1$). Size of our LYCh labeled somata did not correlate with PCV (but see Lee et al., Soc. Neurosci. Abstr. 9:257) nor rheobase (Rh, 4nA±0.8 range 1-7) of the soma using simple linear and non-linear regression techniques over the range of PCV and Rh examined. The finding that there is no simple relationship between rheobase and somal size among DRG neurons suggests that differences in membrane properties (active or passive) or the site of spike initiation during intrasomal activation contribute to differences in rheobase. In motoneurons the range in size is not sufficient to account for observed range in Rh and differences in specific membrane resistance for the different motoneuron types have been implicated (Gustafsson and Pinter, J. Physiol. 357:453-83). Although size and PCV are not correlated in our data, combining our data with that from rat (Harper & Lawson, J. Physiol. 359:31-46) and mouse (Yoshida & Matsuda, J. Neurophysiol. 42:1134-45) reveals a good relationship across species as PCV range is extended to smaller values. Thus analysis of means shows: radius = 7.8 $PCV^{0.28}$ ($r=.95$, $p<0.01$); and PCV is linearly related to somal volume ($r=.86$, $p<0.05$). The strength of this correlation derives primarily from the differences between the groups; within each group correlations are much weaker. Supported by NIMH MH08323 (RDR), NS14899 & NS16996 (LMM).

35.5 RHEOBASE OF PHYSIOLOGICALLY IDENTIFIED DORSAL ROOT GANGLION NEURONS. M.J. Sedivec, R.D. Rose, H.R. Koerber & L.M. Mendell, Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

In anesthetized cats, we have characterized properties of intracellularly recorded dorsal root ganglion (DRG) cells with peripheral fibers in sural and triceps sural nerves. The major aims of these studies are (i) to examine correlations between properties of cell bodies and peripheral fiber conduction velocity (PCV), and (ii) to compare these findings with those from similar studies in α-motoneurons (MN). The present studies are limited almost exclusively to units with peripheral A fibers. Recordings were made with 4% Lucifer Yellow in LiCl (1-2 M) filled electrodes (8-35 Mohm). Reconstructions of labeled fibers and somata confirmed the correlation of spike shape and recording site (Ito, Japan J. Physiol. 186:189-212, Sato & Austin, J. Neurophysiol. 24:569-82). We recorded from 107 somata (>60 mV action potential amplitude with resting membrane potential = 61mV±1 SE). These units were characterized electrophysiologically according to PCV and rheobase (Rh); the latter was examined in view of its diagnostic value in categorizing MNs supplying different motor unit types (Fleshman et al., J. Neurophysiol. 46:1326-38). Rh of DRG somata ranged from < 1 to 15 nA with most (78%) under 5 nA. This overlaps the lower end of the MN Rh spectrum (1-40 nA). Cells with high values of PCV uniformly have small values of Rh. As PCV decreases, somata with higher values of Rh are observed: thus, over the range of PCV from 40-125 m/s, there is a small (r= -0.3) but highly significant negative correlation between PCV and Rh ($p<0.01$). For cells with PCV in the Aδ range (n=14) this relationship is reversed ($r=+0.7, p<0.01$). Two cells activated by high threshold mechanical stimulation and with PCV in C fiber range had Rhs of 1 and 6 nA. The lowest mean value of Rh was observed for cells innervating muscle spindles (2.4±0.4nA; n=12) and the highest mean value was noted for cells innervating G2 hairs (5.3±1.4nA; n=11).The finding that Rh increases as PCV decreases from 125-40 m/s is unexpected considering the opposite relationship over this same range for MNs. The relationship for MNs is in the expected direction: cells with fast PCVs have large soma-dendritic surface areas (Burke et al., JCN:17-28), and the highest Rhs (i.e., they require more current to reach threshold). DRG soma size remains roughly constant in the Aβ range of PCV (Rose et al., Soc. Neurosci. Abstr. 11) and rheobase did not correlate with input resistance in DRG somata. Therefore, we suggest that other factors, e.g. differences in somal membrane properties (active and/or passive) or location of spike initiation site, may be responsible for these findings in DRG cells. Supported by NIMH MH08323 (RDR), NS14899 & NS16996 (LMM).

35.6 CENTRAL TERMINAL DISTRIBUTION OF UNMYELINATED AFFERENT FIBERS. Y. Sugiura*, E. Schrank* and E. Perl. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.

In anesthetized guinea pigs, pipette microelectrodes were used to record electrical signs of activity intracellularly from single dorsal root ganglion neurons. After characterization of their responses to dorsal root volleys and to peripheral tissue stimulation, the neurons were stained intracellularly by iontophoresis of horseradish peroxidase (HRP) from the recording electrode. Second cervical ganglion cells with C fibers (ca. 0.5 m/sec conduction velocity) and with nociceptive characteristics, i.e., excited by noxious pinch and either intensive heating or cooling of the ear, were marked with HRP. After histological and histochemical treatment the dorsal root fiber was successfully traced into the spinal cord. The distribution pattern of the central fiber was studied at the light level. Reasonably extensive staining of terminal regions for two nociceptive units was found. These fibers bifurcated within the dorsal rootlet. Upon entering the spinal cord, one branch projected rostrally, and the other caudal-medially. The rostral branch entered into Lissauer's tract, and after several hundred microns left it to pass into the deeper layers. Terminal and en passant enlargements were found in lamina I and in the dorsal part of lamina III. The en passant enlargements were 1 μM in diameter. The end terminals were smaller, about 0.3 μM in diameter. The caudal-medial directed branch passed along with myelinated fibers in the dorsal columns or Lissauer's tract and then passed into the gray matter. In the most completely stained example, the caudal-medial branch took a U-turn laterally and terminated in lamina I.

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35.7 SUBSTANCE P SUPRESSES THE RESPONSE TO BRADYKININ AND TO HEAT OF NON-MYELINATED FIBERS IN EXPERIMENTAL NEUROMA OF THE CAT'S SURAL NERVE

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Substance P (SP) released from the peripheral nerve endings of the somatosensory nerve fibers is vasoactive, it mediates antidromic vasodilatation and neurogenic inflammation. We have studied whether SP is also neuroactive, by testing its effects onto the regenerating nerve sprouts in a neuroma. In addition, we have investigated the release of SP from neuroma sprouts.
In 9 cats, experimental neuroma were produced by transecting and ligating the sural nerve under pentobarbital sodium (40mg/kg) anesthesia. After 2-3 weeks, the animals were again anesthetized and the sural nerve was exposed for electrical stimulation and single fiber recording. The desheathed neuroma was put in a perspex chamber for perfusion with Ringer's solution with or without SP, bradykinin (BK), or Ringer's solution heated to 50°C. Of 81 identified C-fibers, 23 responded to BK (10μg/ml - 100μg/ml). In contrast, no fibers were excited by SP (10pg/ml-100μg/ml). However, when SP was given before BK we observed supression of the BK response to 34% of control with SP (1μg/ml) and to less than 5% of control with SP (10μg/ml). The responses to 50°C heat were completely supressed by SP (1μg/ml).
In 3 experiments we collected the perfusate and measured the SP concentration by radioimmunoassay. We found a significant SP release with BK (100μg/ml) which was 2 - 7 times higher (up to 610pg/5min) than the control Ringer's solution.
From these findings we conclude that SP released from nerve sprouts in a neuroma may decrease chemical excitability of the sprouts.

35.8 POTASSIUM CHANNEL-BLOCKING AGENTS AFFECT SPONTANEOUS DISCHARGES FROM NEUROMAS IN RATS. L. C. Russell* and K. J. Burchiel* (SPON: John Bonica). Neurosurgery Research, Veterans Administration Medical Center (151), 1660 S. Columbian Way, Seattle, WA 98108.

Spontaneous impulse generation originating in the end-bulb neuroma is frequently observed in experimental models of chronic deafferentation. Acute microfilament recordings were performed in the proximal nerve of 35 Sprague-Dawley rats with saphenous neuromas. The effect on observed spontaneous activity in A-fibers terminating in the neuroma of the potassium channel-blocking agents tetraethylammonium bromide (TEA) and 4-aminopyridine (4-AP), as well as the effects of gallamine were tested. TEA reliably increased spontaneous firing in active fibers and initiated spontaneous activity in some fibers with no spontaneous baseline discharge. 4-AP had no effect on baseline activity of either spontaneously-active or quiescent fibers. However, 4-AP inhibited spontaneous activity induced by prior TEA treatment. Gallamine application produced effects similar to TEA in that spontaneous activity was dramatically increased. These results imply that a tonic potassium conductance is present in regenerating fibers in the neuroma and that this conductance moderates the tendency toward hyperexcitability and spontaneous firing. Spontaneous activity in nociceptive afferents may represent the mechanism of chronic pain and paresthesias which often accompany peripheral nerve injury. These results would suggest that agents which either increase potassium conductance or selectively inhibit the sodium current in regenerating axons might be effective in the treatment of these chronic pain syndromes.

36.1 RELATIONS BETWEEN STIMULUS FORCE, SKIN DISPLACEMENT, AND DISCHARGE CHARACTERISTICS OF SLOWLY ADAPTING TYPE I CUTANEOUS MECHANORECEPTORS IN GLABROUS SKIN OF SQUIRREL MONKEY HAND. Benjamin H. Pubols Jr. and Melissa E. Benkich*. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209

The viscoelastic properties of the glabrous skin of the squirrel monkey hand are similar in many ways to those of the raccoon forepaw (B. H. Pubols, 1982). A major difference, however, is that squirrel monkey skin is more compliant, in that a given force applied to either a digital or a palmar skin pad produces a greater displacement in squirrel monkey than in raccoon skin.

The purpose of the present study was to examine the contribution of viscoelastic properties of squirrel monkey glabrous skin to slowly adapting Type I (SAI) mechanoreceptive afferent fiber discharge. Individual fibers of the median and ulnar nerves were isolated by microdissection in six monkeys anesthetized with pentobarbital sodium. Both threshold and suprathreshold responses to punctate mechanical stimuli controlled with respect to either force or displacement were studied.

The median absolute force threshold (N = 21) was 80 mg (range = 48-340 mg), and the median absolute displacement threshold was 17.5 μm (range = 5-30 μm). Both of these sets of values are lower than corresponding absolute thresholds for SAI mechanoreceptors of the raccoon forepaw. Squirrel monkey absolute force and displacement thresholds were significantly positively correlated ($P < .001$).

Application of suprathreshold forces (range = 1-8 gm) and displacements (range = 500-1000 μm) revealed greater interunit variability in response to maintained stimulation than previously found in the raccoon. In 11 out of 14 cases, the rate of adaptation was significantly less during constant force than during constant displacement stimulation. In the most extreme example, a 1 gm stimulus force applied to the distal pad of one of the digits produced a negligible decline in discharge rate over a 10 sec period, while a constant 500 μm displacement resulted in a decline in discharge rate to approximately 1/3 the initial value. In two examples, it was found that adaptation was faster to constant displacement than to constant force stimuli only when relatively high amplitude stimuli were employed.

Because the distribution and morphological features of the SAI mechanoreceptor, the Merkel cell-neurite complex, are similar in glabrous skin of raccoon forepaw and monkey hand (Munger, Pubols, and Pubols, 1971; Halata, 1975; Munger, unpublished observations), we propose that differences in their functional characteristics may be attributable to differences in properties of surrounding tissues, rather than in properties of the receptor organs themselves. (Supported in part by research grant NS-19487, USPHS.)

36.2 PERIPHERAL NEURAL REPRESENTATION OF THE SHAPE OF A STEP STROKED ACROSS THE MONKEY'S FINGERPAD. R.H. LaMotte and M.A. Srinivasan*. Dept. of Anesthesiology, Yale Univ. Sch. Med., New Haven, CT 06510

In the present study, we investigated the peripheral neural representation of small shapes stroked across the finger. Each shape was a step change in height of a plate. The cross-sectional shape of the step from low to high approximated that of a half-cycle of a sinusoid. Step height was maintained at 0.5mm, while the half-cycle wavelength was varied from 0.45mm (steep) to 3.1mm (gradual). A servocontrolled mechanical stimulator stroked the step back and forth over the glabrous skin of the fingertip while maintaining contact force at 20g wt. Stroke distance along the mediolateral axis was 18mm and stroke velocity varied from 1 to 40mm/s. Each step was stroked first from the high to the low side of the step, i.e. step-stroking off the skin (SS-off) and then back again from low to high (step stroking onto the skin, SS-on).

Evoked action potentials in single primary mechanoreceptive afferent fibers innervating the fingerpad of the anesthetized monkey (M. fascicularis) were recorded by the methods of fiber dissection and single unit recording. Ten rapidly adapting fibers (RA) (Meissner type) and eight slowly adapting fibers (SA) (type I) were studied. Each fiber's responses to a step stroked in one direction provided a spatial response profile in which the occurrence of each action potential corresponded to a position of the step on the skin. The SAs, but not RAs, exhibited a base discharge that was interrupted by a sequence of "pause-burst-pause" for SS-on and "burst-pause" for SS-off. RAs exhibited a single burst to a step moved in either direction. Both SAs and RAs had a greater burst frequency for the SS-on than SS-off.

The spatial response profile of SAs and RAs were also altered by changes in step-shape and stroke velocity. For both SAs and RAs, discharge rate during the burst increased with increases in stroke velocity and with steepness of the step. The only response feature that remained more or less invariant over a range of stroke velocities was the spatial width of the burst in SAs which, for SS-on, decreased as step shape became steeper.

These results were interpreted as showing a close relationship between the spatial response profile and the profile of skin deformation expected to occur when a step was stroked across the skin. The base discharge of SAs and their greater sensitivity to changes in skin curvature distinguished the response profiles of SAs from those of RAs.

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36.3 ARE MERKEL CELLS THE MECHANOSENSORY TRANSDUCERS IN RAT TOUCH DOMES AND XENOPUS TOUCH SPOTS? L. Mills*, K. Mearow, B. Visheau and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Since their discovery over a century ago only one function has been firmly established for the Merkel cells of skin, namely to act as targets for growing mechanosensory nerves (Diamond (1982); Curr. Topics in Dvptl. Biol. 17:147). However in the EM synapses are observed between neurites and Merkel cells (reciprocal synapses in salamander and Xenopus), supporting the common assumption that these specialized epidermal cells are mechanosensory transducers. We have now attempted to destroy the Merkel cells in situ by utilizing their ability selectively among epidermal cells to take up and store quinacrine (Nurse et al. (1983); Cell and Tissue Research 228:511-524). In anaesthetised rats pre-injected with the fluorescent dye (10-15 mg/kg) the skin was depilated, and identified touch domes irradiated (330-500 nm) for 2 or more min totally to bleach their population of ca. 90 quinacrine fluorescent Merkel cells (dome cells were unable to be quinacrine labelled for at least the next 8 d). After 1-5 d recordings were made from the cutaneous nerves, and the mechanosensitivity across single domes was mapped with a 16 μm prodder tip, responsive and unresponsive regions being resolved to within about 50 μm (cf. Diamond et al. (1984) Soc. for Neurosci. Abs. Vol.10 #307.15). While some irradiated domes showed a regional (occasionally total) loss of responsiveness, others behaved apparently normally, or with moderately increased thresholds. However, examination in the EM revealed that many of these domes were dramatically affected. In these, the very few Merkel cells that could be identified had no, or very few, of the characteristic cytoplasmic dense-cored granules, and sometimes these were "empty". These presumed Merkel cells were almost all "sick" by EM criteria (although neighbouring basal cells were normal) and none had identifiable neuritic contacts. In these domes the relatively infrequent recognisable nerve endings (with their underlying Schwann cells) occupied their usual position just above the basal lamina, but most of them abutted spaces containing pockets of granular material, presumably glycogen.

Preliminary findings from similar experiments on Xenopus skin resemble those in the rat. Thus both the slowly adapting Merkel cell - neurite complexes of the touch dome, and the rapidly adapting ones of Xenopus (Mearow & Diamond (1983) Soc. for Neurosci. Abs. Vol.9 #228.12) can function when their Merkel cells are absent or grossly abnormal, and these cells seem unlikely therefore to be actively involved in the initiation of the mechanosensory responses. While e.g. receptor adaptation and fatigueability have yet to be quantified, we believe our findings raise the question - could the synapses between Merkel cells and their nerves relate to trophic, rather than to physiological, nerve-target interactions? (Supported by MRC Canada, and PHS Grant NS. 15592.)

36.4 EFFECTS OF SYNTHETIC CAPSAICIN ON THE INNERVATION OF THE SKIN. K. Chung, R.J. Schwen*, D.D. Anderson* and R.E. Coggeshall. (SPON: H.J. Nauta). Marine Biomedical Institute and Departments of Anatomy and Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550; and The Procter and Gamble Company, Cincinnatio, OH 45247.

We have reported (Neuro. Lett., 53: 221, 1985) that subcutaneous injection of synthetic capsaicin (N-vanillylnonanamide) caused loss of unmyelinated ureteral axons even though axons in the dorsal roots seemed to be undamaged. Thus, we suggested that these unmyelinated axons are damaged peripherally but spared more proximally. If these findings are generalizable, we would predict loss of somatic unmyelinated axons near their termination but no such loss in the nerves conveying these axons. To test this, we developed a fine structural assay for the axons in the skin of the posterior leg of the rat. This assay depends on the observation that bundles of 1-8 unmyelinated axons are found with regularity near the basal lamina that underlies the epidermis. We counted these axons in a 1 mm length of subepidermal tissue of the posterior leg as well as in the sural nerve in 4 animals that received 50 mg/kg of N-vanillylnonanamide 24 hours prior to sacrifice and in 4 control animals that received only the vehicle for N-vanillylnonanamide. Routine electron microscopic fixation and embedding procedures were used. The data show that the number of axons in N-vanillylnonanamide-treated animals was only 22% of the number of axons in control animals, which is a significant difference ($p<.01$), whereas there is no significant difference in sural nerve axon numbers. We thus propose that capsaicin injections cause destruction of unmyelinated axons near their site of peripheral termination but these same axons are spared where they are gathered in nervs. Further experiments to extend these observations, to determine the types of axons that are damaged and to see if there is regeneration of these axons over time, are underway.

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36.5 THE ACTION OF CAPSAICIN ON CORNEAL FREE NERVE ENDINGS. R.W. Beuerman, M. Vigo*, B. Dupuy*, and C.E. Crosson*. LSU Eye Center, New Orleans, LA 70112.

Despite a large number of studies examining several aspects of capsaicin's action on the peripheral nervous system, it is unclear how it affects the excitation of free nerve endings. In the cornea, the free nerve endings lie within 50 microns of the surface of the epithelium. Only the barrier function of the epithelium separates the nerve endings and the extracellular environment from the tear layer. In this system, the physiology of the corneal epithelium and of the free nerve endings can be compared in their response to capsaicin.

Albino rabbits (1.5 - 2 kg) were anesthetized with urethane (1.5 g/kg) and tracheotomized. The long ciliary nerve was isolated in the orbit and prepared for action potential recording by conventional techniques. Care was taken not to damage the epithelial surface. Integrated (1.5 sec t.c.) multi-unit records were used to determine the relative effectiveness of the stimuli. Stimuli consisted of sodium chloride in a concentration series which covered the range of osmotic pressures from 20 mOsm to 1800 mOsm. In other experiments, rabbit corneas were isolated and mounted in a Lucite chamber and the epithelial cells impaled with microelectrodes to determine epithelial barrier function prior to and following the addition of capsaicin.

Preparation responsiveness was initially tested to the sodium chloride series (31°C) as well as to cold (20°, 10°C) and warm saline (40°C). Response profiles of sensory afferents to a concentration series of capsaicin (0.01% to 1%) were similar in amplitude. Therefore, in succeeding experiments a .1% capsaicin solution was used. This was subjectively equivalent to a moderately hot sensation when placed on the tongue. The vehicle was not stimulatory and did not affect the responses to sodium chloride or thermal stimuli. However, capsaicin did decrease the response amplitudes to the sodium chloride series. The results indicated that the response inhibition required several minutes to develop but was long lasting. Forty minutes of continuous washing with isotonic saline failed to restore the responsiveness to sodium chloride. In other studies, the corneal innervation was visualized by light microscopy and using the gold chloride stain following topical application of 0.1% capsaicin solution. The free nerve endings within the epithelium appeared normal. An analysis of corneal epithelial component resistances in response to the acute addition of 0.1% capsaicin indicated that it did not alter the apical, basal, or shunt resistances of the epithelium. In summary, it is suggested that capsaicin has a selective action on neural membranes and may be useful in studying transduction processes.

36.6 CHANGES IN CONFORMATION OF EPIDERMAL-DERMAL BORDER REGIONS OF GLABROUS SKIN WITH DIGITAL DEFORMATION. C.J. Vierck, Jr., R.H. Cohen and D.C. Yeomans*. Dept. of Neuroscience, Univ. of Fla. Col. of Med., Gainesville, FL 32610.

Cutaneous deformations of the glabrous skin of deeply anesthetized Macaque monkeys were viewed through a dissecting microscope fitted with a video camera for taping and subsequent analysis of changes in conformation at the surface and in depth. The tip of a digit was amputated, and the proximal end was viewed through a glass plate, producing clear images of the epidermal-dermal border. Vertical and tangential deformations of the skin with probes positioned near the cut end produced consistent patterns of change in conformation of the secondary dermal papillae (containing quickly adapting receptors) and at the tips of intermediate ridges (containing slowly adapting receptors). With vertical deformations, the epidermal ridges shifted as integral units, in contrast to the pattern expected of a corrugated membrane. As a consequence, the tips of intermediate ridges (rete pegs) maintained constant, mediolateral spacings, rather than fanning out. Most of the vertical shifting occurred at the limiting ridges and the superficial portions of the intermediate ridges, producing consistent patterns of change in conformation of the secondary papillary ridges. For example, the far dermal papilla of a pair bordering a limiting ridge was distorted more than the near papilla of that pair. Distortions of the dermal papillae could be discerned at a distance from the indenting probe, but measurable changes in conformation of the intermediate ridges were more confined to the ridge(s) compressed directly by the probe. These patterns of mechanical deformation are consistent with the relatively lower thresholds and larger receptive fields of quickly adapting receptors. (Supported by NS 07261).

36.7 DOES THE EXTERNAL CUNEATE NUCLEUS PROJECT TO NEURONS IN UPPER CERVICAL DORSAL ROOT GANGLIA IN THE CAT? M.T. Stechison* and J.A. Saint-Cyr. Playfair Neuroscience Unit, and Department of Surgery, Division of Neurosurgery, and Department of Anatomy, University of Toronto, Toronto, Canada M5T 2S8.

In a series of 7 cases of cervical dorsal root ganglion injections of WGA-HRP, retrograde labelling of cells in the ipsilateral external cuneate nucleus (ECN) was noted in addition to the expected anterograde labelling of terminals. Labelled cells were clearly noted to be distinct from anterograde labelling making silhouetting of the cells in labelled terminations an unlikely explanation for the phenomenon. In order to distinguish between true retrograde labelling of these cells, and transsynaptic transport of WGA-HRP, injections of a mixture of tritiated leucine, lysine, and proline were placed in the ECN region of the dorsal medulla in three cats. Injection sites were large encompassing the ECN as well as the adjacent reticular formation, main cuneate nucleus and the medial and descending vestibular nuclei. Following 24 hour survival times, transcardial perfusion with 10% formalin was carried out. In one case all 16 cervical dorsal root ganglia were removed, mechanically pulverized, and placed in tissue solubilizer followed by counting fluid. Liquid scintillation counts were obtained, and activity levels in the ipsilateral C1 - 3 dorsal root ganglia (DRG) were 2.7 - 6.6 times those recorded from the contralateral DRG. The two remaining cases were histologically processed for standard autoradiography. Examination of 7um sections in both bright and dark-field illumination revealed clusters of silver grains in association with some cells in the ipsilateral upper cervical DRG. The greatest amount of labelling was present in C2 and 3, followed by C1. Labelling appeared clearly extracellular in location. Approximately 1 in 10 cells on the strongest labelled sections appeared to be associated with a terminal field. This finding suggests that first order cervical afferents are in receipt of input from ECN neurons. Such centrifugal projections to first order neurons have been described in other sensory systems in mammals, but have not previously been reported in the somatosensory system. Previous investigators (Kayahara et al., Brain Res. 216: 277-290, 1981) have detected the presence of chemical type synapses in C5 to C8 DRG in E.M. sections, but the source of these was not identified. The present findings indicate that ECN may be a source of projections to more rostral cervical DRG. Such a connection could function in the modulation of upper cervical afferent inputs to spinal and supraspinal centres.

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36.8 DIFFERENTIAL PATTERNS OF LABELING IN THE RAT DORSAL HORN AND DORSAL COLUMN NUCLEI WITH TRANSGANGLIONIC TRANSPORT OF WGA-HRP AND HRP. J.E. Swett Dept. of Anatomy, Coll. of Med., Univ. of Calif., Irvine, CA 92717

It is widely assumed that the wheat germ conjugate of horseradish peroxidase (WGA-HRP) is a sensitive substitute for free HRP as a neuroanatomical tracer substance. This may be valid for retrograde labeling of motoneurons from cut nerves but not for transganglionic labeling from cutaneous afferents. In 35 experiments 5-10% WGA-HRP (Sigma) was applied for 4 hours to the cut end of the saphenous (or sural) nerve in one leg while 40% free HRP (Sigma VI or Boehringer Grad I) was applied to the homologous nerve in the opposite leg. After survival periods of 24-108 hours, frozen serial sections (40 um) were obtained through appropriate dorsal root ganglia (DRG) in the longitudinal plane and N. gracilis (DCN) and spinal segments L2-L6 in the coronal plane. Tissues were reacted with TMB as the chromogen. No differences were found in comparing numbers, sizes or densities of labeled DRG cells on either side, but the patterns of distribution of label differed in the dorsal horn and DCN. On the WGA-HRP side a dense band of label was found in lamina II (Swett & Woolf, JCN 231:66, 1985) for all survival times. Label was sparse or absent elsewhere in the dorsal horn and DCN. Lissauer's tract was well labeled while axons in the dorsal columns were not. On the HRP side the band of label seen on the WGA-HRP side was present in 24 hr and absent after 48 hr. Laminae I, III and IV contained the bulk of the reaction product. Massive labeling appeared in afferent terminals in DCN as well as axons in the dorsal columns. Lissauer's tract was weakly labeled. A marked decrease in the amount of reaction product in axons proximal to the DRG's on the WGA-HRP side, but not on the HRP side, suggests that the conjugate is poorly transported centrally from large DRG cells. When a cocktail of 5% WGA-HRP and 30% free HRP was applied to cutaneous nerves in control experiments, the individual labeling characteristics of the two tracers were superimposed. WGA-HRP was preferentially, but not exclusively, transported by small diameter primary afferent fibers, presumably mostly by C fibers rich in FRAP. Conversely, HRP appeared to be transported in both large and small diameter fibers with reaction products in the latter being relatively labile. Thus, neither tracer substance is alone capable of revealing the complete distribution of afferent terminations in the CNS. (Supported by NIH, NINCDS Grant NS-17630).

37.1 PHYSIOLOGY AND PHARMACOLOGY OF THE RELEASE OF SUBSTANCE P FROM SPINAL PRIMARY AFFERENTS. T.L. Yaksh* and V.L.W. Go* (SPON: J.P. Whisnant). Laboratories of Neurosurgical Research and Gastroenterology, Mayo Clinic, Rochester, MN 55905.

In spinal cord, substance P (sP) is found in intrinsic neurons, terminals of descending pathways and primary afferents. Superfusion of the spinal cord in halothane (0.8%) anesthetized cats, artificially ventilated under Pavulon with artificial cerebrospinal fluid (CSF) containing albumin (120 µg/ml) and bacitracin (30 µg/ml) reveals the presence of sP-like immunoreactivity (sP-LI) appearing at the rate of 26±3.9 fmol/30 min. (antisera: R4892; assay sensitivity 3 fmol/tube) which co-chromatographs with sP 1-11, (HPLC: C-18 column with acetonitrile 10-60% gradient in 0.1 M acetate at 1 ml/min). The following properties of the release were noted: 1) Electrical stimulation of the sciatic nerves at intensities which evoke an Aß-Aδ-C fiber compound action potential results in a 2.6±0.4-fold increase in sP-LI. 2) The release is frequency dependent over the range of 10 to 100 Hz with frequencies of 100 Hz showing a reduction in the amount of sP released. 3) Double pulse experiments carried out at 20 Hz, reveals that the apparent refractory period(s) of the afferent fibers from which the sP is released are 10 msec $< x <$ 50 msec, suggesting a small axon population. 4) Reversible cold block of the cervical cord did not abolish the release, excluding a contribution from bulbospinal sP pathways. 5) Treatment with intrathecal capsaicin ($5x10^{-4}$) resulted in a 3.4±0.5-fold increase in sP-LI secretion. This treatment blocked the ability of subsequent electrical stimulation to evoke further sP release. 6) The addition of morphine (µ: 10^{-4}M), D-Ala^2-D-Leu^5-enkephalin (δ: 10^{-5}M), but not U50488H (κ: 10^{-3}M) diminished the stimulation evoked release of sP to less than 50% as compared to pretreatment control. 6) ST-91 (α_2-agonist: 10^{-4}M) but not serotonin (10^{-4}M) depressed the stimulation evoked sP-release. 7) GABA (10^{-3}M) had no effect on sP release. 8) Electrical stimulation of the dorsal lateral quadrant at the cervical level did not alter the levels of sP-LI in the spinal superfusate, but the effects of sciatic nerve stimulation on sP release were greatly diminished. Yohimbine ($5x10^{-5}$M), but not naloxone (10^{-4}M) produced a significant reduction in this suppressent effect of dorsolateral funiculus stimulation of evoked sP-LI release. These observations support the concept that sP-LI is released from small primary somatic afferents and its release is under the influence of spinal µ, δ and α_2 receptor systems known to be present in primary afferent terminals. Spinal pathways traveling in the DLF appear to alter spinal terminal activity in part by an α_2, but not opioid receptor. (Funds from NS-16541.)

37.2 HYPERALGESIA TO HEAT IN HUMAN SUBJECTS AND SENSITIZATION OF C-NOCICEPTORS IN MONKEYS DO NOT OCCUR ADJACENT TO A MECHANICAL INJURY OF THE HAIRY SKIN. J.N. Campbell[1], R.A. Meyer[1,2], A.A. Khan*[1], and S.N. Raja[1]. School of Medicine[1] and Applied Physics Lab[2], The Johns Hopkins University, Baltimore, MD 21205.

Secondary hyperalgesia refers to the alteration in pain sensitivity that occurs outside the site of an injury. Sensitization of nociceptors has been shown to account for the primary hyperalgesia at the site of injury. It has been proposed that sensitization also spreads to adjacent uninjured skin to account for secondary hyperalgesia. Fitzgerald (J. Physiol. 297:207, 1979) reported that a cut in the skin adjacent to the receptive field of C-fiber nociceptors in rabbit resulted in a lower heat threshold relative to that of the control population (i.e., spreading sensitization). We were unable to confirm this finding in monkey. Also, we failed to demonstrate hyperalgesia to heat adjacent to a mechanical injury in human subjects.

The mechanical injury consisted of a 1 mm deep scalpel cut. In the psychophysical experiments, two parallel cuts were made in the volar forearm, 1.5 cm apart and 8 mm long. Heat testing was performed in the uninjured area between the cuts. In the neurophysiological experiments, the cut was 1-4 mm from the edge of the receptive field (RF), and was 5-8 mm long on the distal side of the RF (n=5) or was U-shaped (n=4) so as to surround all but the proximal side of the RF. Heat stimuli were 1s in duration, ranged from 39° to 49°C, and were delivered at 30s intervals. The sequence of heat stimuli were repeated with 10 minute inter-run intervals. The injury was delivered between two of the runs once a stable response to heat was established.

Standard teased-fiber techniques were used to record from single C-nociceptors that innervated the hairy skin of the forearm and hand in pentobarbital anesthetized monkeys. A magnitude estimation technique was used by the human subjects to rate the painfulness of the heat stimuli. The total response to the test heat sequence was used as a measure of the responsiveness of the nociceptors (sum of action potentials) and of the human subjects (sum of ratings). For the nine C-nociceptors, the response after the mechanical injury was 101 ± 6% (mean ± S.E.M) of the response immediately before the injury and, therefore, spreading sensitization did not occur. For the six human subjects, the response after the mechanical injury was 80 ± 12% of the response before injury and, therefore, hyperalgesia to heat did not occur. These results for adjacent mechanical injury are similar to those reported for adjacent heat injury where neither spreading sensitization of C-nociceptors nor hyperalgesia to heat of human subjects was observed.

37.3 ANTIDROMIC NERVE STIMULATION IN MONKEY DOES NOT SENSITIZE C-FIBER NOCICEPTORS TO HEAT. R.A. Meyer, J.N. Campbell and S.N. Raja, School of Medicine and Applied Physics Lab, Johns Hopkins Univ., Baltimore, MD 21205.

Spreading sensitization of nociceptors has been proposed as the mechanism for the secondary hyperalgesia that surrounds an injury. Activation of part of the nociceptive receptor by an injury stimulus could lead to antidromically propagated action potentials to the other part of the receptor resulting in the release of sensitizing substances. A similar axon reflex mechanism is thought to account for the flare that surrounds an injury. Fitzgerald (J. Physiol. 297: 207-216, 1979) reported that antidromic electrical stimulation of the peripheral nerve in rabbit resulted in a lower heat threshold (and therefore sensitization) of C-fiber nociceptors relative to that of the control population. We attempted, without success, to confirm this finding in monkey.

Standard teased fiber techniques were used to record from single C-fiber nociceptors in monkey under pentobarbital anesthesia. A stimulating electrode was placed on the parent nerve proximal (PSE) or distal (DSE) to the recording electrode. A laser thermal stimulator provided constant temperature stimuli to the receptive field. Before and after electrical nerve stimulation, the C-fiber nociceptors were stimulated with test heat stimuli of 1s duration ranging from 39° to 49°C. These heat stimuli do not cause sensitization and have proven useful in monitoring receptor sensitivity (Campbell and Meyer, J. Neurophys., 49: 98-110, 1983). Various electrical stimulation parameters were used over the ranges of 50-100 volts, 0.1 ms, 1-50 Hz, for duration of 1-6 mins. Evidence for effective C-fiber stimulation included pilomotor response, goose flesh, decreased skin temperature, and sweating in the distribution of the nerve.

Following PSE stimulation, the heat threshold for 10 C-fiber nociceptors did not change significantly ($\Delta T = -0.3 \pm 0.2$°C, mean ± SEM), the total response to the test heat stimuli did not change (97 ± 5% of control), and spontaneous activity did not develop. Thus no signs of sensitization were observed. Following DSE stimulation, the responses of 6 C-fiber nociceptors were blocked completely for 4.3 ± 0.8 min. Twenty minutes after electrical stimulation, the response was 88 ± 8% of control. Ten similar experiments in halothane anesthetized monkeys also failed to demonstrate sensitization to heat of C-fiber nociceptors following proximal or distal nerve stimulation.

We conclude that antidromic stimulation of C-fiber nociceptors in the monkey does not result in sensitization of their response to heat. Except for differences in species and in general anesthetics used, we cannot account for the differing results reported by Fitzgerald in rabbit.

37.4 NARCOTICS DO NOT ALTER THE RESPONSE OF C-FIBER NOCICEPTIVE AFFERENTS IN MONKEYS, S.N. Raja[1], R.A. Meyer[1,2], J.N. Campbell[1], A.A. Khan*[1], School of Medicine[1] and Applied Physics Lab[2], Johns Hopkins University, Baltimore, Maryland 21205.

We previously reported that the general anesthetic, halothane, sensitizes nociceptors in a reversible, dose-dependent manner. To determine if narcotics alter the response of primary afferents, we studied the effects of fentanyl on the responses of single C-fiber nociceptive afferents to heat stimuli delivered to their cutaneous receptive field. Standard teased-fiber techniques were used to record from 10 C-nociceptors sensitive to mechanical and heat stimuli (CMHs), in pentobarbital anesthetized (4-6 mg/kg/hr), mechanically ventilated monkeys. Blood pressure, heart-rate, end-tidal CO_2 and core temperature were monitored. Three additional CMHs were studied in brain-dead, unanesthetized monkeys. To determine the effects of the narcotic fentanyl, we recorded responses to repeated suprathreshold heat stimuli (46-48°C) before, during and after the administration of incremental i.v. doses (5-30 ug/kg) of fentanyl. Step increases in skin temperature for specified periods were produced by a laser thermal stimulator under radiometer feedback control. Naloxone (0.8 mg i.v.) was given 5 min after the highest dose of fentanyl. Stimuli were of 1 or 3 sec duration and were repeated at 30 or 60 sec intervals. After a stable baseline response was observed, incremental doses of fentanyl were administered at 5 min intervals. For each of the 10 CMHs, the mean response of 5-10 trials before the administration of fentanyl was compared to the mean response of 5-10 trials after fentanyl administration. The responses of the CMHs at the 5,10,20 and 30 ug/kg doses of fentanyl were 93 ± 3%, 96 ± 2%, 99 ± 4% and 102 ± 6% (Mean ± S.E.M.) of control respectively. The mean response after the administration of naloxone (0.8 mg) was 101 ± 5% of control. In 6 of the 13 CMHS we later studied the change in response to heat stimuli after the administration of halothane (2% inspired concentration for 10 min). The response was significantly increased (146 ± 12% of control, $P<0.02$). Fentanyl (up to 30 ug/kg) also did not affect the responses of 3 CMHs recorded in the brain-dead, unanesthetized monkeys, thus excluding a confounding interactive effect of pentobarbital and fentanyl. In conclusion, fentanyl has no significant effect on the response to heat of C-nociceptive afferents. Thus, the effects of spinally or systemically administered opiates are unlikely due to an alteration of nociceptor function.

37.5 SINE WAVE ANALYSIS OF "COLD" THERMORECEPTOR SENSIVITITY. R.D. Wurster, F.K. Pierau* and C.J. Robinson. Rehabilitation R&D Ctr., VA Med. Ctr., Hines, IL 60141; Kerckhoff Inst., Max Planck Gesellschaft, 6350 Bad Nauheim, F.R.G.: and Dept. Physiol., Loyola Univ. Med. Ctr., Maywood, IL 60153.

"Cold"-sensitive thermoreceptors exhibit peak static mean firing rates at a temperature (Tmax) in the 25 to 30 C temperature range. Dynamic sensitivity has been previously assessed using rapid step-like or ramp temperature changes. However, because of the variations of sensitivity at different static temperatures, analysis of dynamic sensitivity is difficult. In the present study, sine wave analysis of thermal sensitivity was utilized. Lingual nerve thermoreceptor units were recorded from cats anesthetized with alpha chloralose. The dorsal surface of the tongue was placed upon a water-perfused thermode which was capable of producing sine wave temperature changes, as well as maintaining various static temperatures. The lingual nerve was isolated, desheathed and then recorded using bipolar electrodes and an AC amplifier. The nerve was subdivided until a single, cold thermosensitive unit, not sensitive to mechanical stimulation, was recorded. Adaptation temperature was changed in steps (3 to 5 C) from 38 to 18 C. Following adaptation for about 5 min at each step, the temperature was modulated by a 0.5C sine wave oscillation at 0.01, 0.05, 0.1 and 0.3 Hz.

The changes in response from static firing rates depended on whether the temperature was rising or falling. During temperature decreases, the maximum change in firing rate above the static rate was nearly linearly related to the sine wave frequency from 0.05 to 0.3 Hz. During temperature increases, the depression of firing rates below static levels was quite sensitive to temperature shifts, in that the activity decreased to minimal levels (>2 IPS) with any 0.5 degree increase regardless of the modulation frequency. The maximum changes in firing rates were much greater to increasing than decreasing temperatures at all frequencies and static temperatures tested; in other words, the rate of change in firing of cold thermoreceptors was much more pronounced during temperature increases than decreases.

At the slowest frequency (0.01 Hz), the response of the cold units all approached that which would be expected from their static sensitivity curves. At temperatures > Tmax, very slow incremental temperature increases resulted in decreases in firing rate; while at temperatures < Tmax, such increases increased the rate. As the frequency of temperature modulation was increased, the phase shift of the response decreased above Tmax while increasing below Tmax.

From these response characteristics, a model can be constructed of cold nociceptor dynamic sensitivity. Supported by the Max Planck Gesellschaft and the Veterans Administration.

37.6 BLOOD PRESSURE EFFECTS PRODUCED BY VENTRAL ROOT AFFERENT FIBERS. H.K. Shin*, J. Kim* and J.M. Chung. (SPON: D.J. McAdoo). Marine Biomedical Institute, Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Although a large number of afferent fibers are found in the mammalian ventral root their functional role is not clear. To reveal the potential physiological role of ventral root afferent fibers in cardiovascular function, changes in arterial blood pressure evoked by ventral root stimulation were observed in anesthetized cats.

Eighteen adult cats were anesthetized with α-chloralose (70 mg/kg) and systemic arterial blood pressure was monitored through a cannula inserted into the carotid artery. To reduce baroreceptor-mediated reflex compensation of blood pressure, animals were vagotomized and the carotid sinuses denervated bilaterally. The lumbosacral spinal cord was exposed by a laminectomy, and a heated mineral oil pool was formed over the exposed spinal cord. Ventral roots of the 7th lumbar and 1st sacral spinal segments were cut near the spinal cord. The peripheral stump of the cut ventral root was placed on a tripolar stimulating electrode; the most distal lead was grounded to prevent current spread to nearby neural structures.

Low intensity electrical stimulation (< 20T; less than 20 times the threshold for motor fiber activation) of the distal stump of the cut ventral root caused a rise in blood pressure. This elevation was abolished by paralyzing the muscles with gallamine. High intensity electrical stimulation (500T) of the distal stump of the cut ventral root caused a second and marked pressor response. This was not affected by muscular paralysis or cutting the sciatic nerve, but it was abolished by cutting the dorsal root. It was found that the threshold intensity for the second component of the pressor response was within the same range as the intensity needed for activation of C fibers in the ventral root, ranging between 200T and 300T. This response was graded with increasing stimulus intensity, and it showed both spatial and temporal summation. No changes in blood pressure have been observed when the proximal stump of the cut ventral root was stimulated, regardless of intensity.

From the above results, we conclude that unmyelinated fibers in feline spinal ventral root course distally to the dorsal root ganglion and then enter the spinal cord via the dorsal root. Activation of these fibers results in a marked elevation of the systemic arterial blood pressure as in other somato-sympathetic reflexes induced by peripheral C fiber activation.

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37.7 ARRANGEMENT OF VENTRAL ROOT AFFERENT FIBERS IN THE CAT. J. Kim*, H. K. Shin* and J.M. Chung. (SPON: J.E. Blankenship). Marine Biomedical Institute and Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Although the existence of a large number of afferent fibers in the mammalian ventral root has been demonstrated, their peripheral arrangement is not clear. The present study is to investigate the peripheral course of these fibers electrophysiologically using the collision method.

Fifteen cats were anesthetized with α-chloralose (70 mg/kg). After a tracheotomy, the animal was ventilated with a respirator and paralyzed with gallamine. The lumbosacral spinal cord was exposed by a laminectomy and the spinal roots of the S1 segment were cut near the spinal cord. The spinal nerve of the same segment was exposed in the hindlimb and cut where it joins the sciatic nerve. Single fiber recordings were made from dorsal root filaments while the peripheral stump of the cut ventral root was being stimulated. If a single fiber was activated by ventral root stimulation, a peripheral spinal nerve was also stimulated to see if the same unit could be activated. Attempts were made to collide the unit activity that was elicited by stimulation of the ventral root with that of the peripheral nerve and vice versa. Then the conduction velocity was calculated from the latency and the conduction distance and also from results of collision data.

Among a total of 33 dorsal root units that were activated by stimulation of the ventral root, the activity of 17 units was collided with peripherally elicited activity. These results suggest that these primary afferent neurons have 3 processes; one in the dorsal root, one in the ventral root and one in the periphery. For another 11 units, we were unable to verify any collision. In the remaining 5 units, peripheral nerve stimulation did not elicit any electrical activity recordable in the dorsal root. The results of collision data indicated the conduction velocity of the process along the length of the ventral root changed so that it seemed to get slower as it approached the spinal cord.

From the above results, we conclude that many of the ventral root unmyelinated afferent fibers in the feline S1 spinal segment have processes both in the dorsal root and in the periphery. The size of each process may be different.

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38.1 THE PHYSIOLOGICAL BASIS FOR SYMPATHETICALLY MAINTAINED PAINS. W. J. Roberts and M. E. Foglesong*. Neurological Sciences Inst., Good Samaritan Hospital & Medical Center, Portland, OR 97209.

In some chronic pain syndromes such as causalgia and sympathetic reflex dystrophy, the continuous pain is most effectively treated by sympathetic block, yet the physiological basis for these disorders is not clearly understood. A review of the clinical findings associated with the family of sensory disorders which can be collectively labeled "sympathetically maintained pains" (SMP) reveals certain features which are common to all. These are: 1) continuous burning pain subsequent to trauma; 2) allodynia (touch-evoked pain); 3) relief from pain when the relevant nerve or sympathetic trunk is blocked or quanethidine is infused (this depletes norepinephrine peripherally). The clinical findings suggest that sympathetic excitation of afferents contributes to these pains and that an abnormal sensitivity of spinal nociceptive neurons is also involved.

It is proposed that these pains are initiated by trauma-induced nociceptor activity which causes prolonged sensitization of spinal wide-dynamic range (WDR) or multireceptive neurons. After sensitization these WDR neurons respond vigorously to activity in low-threshold mechanoreceptors (e.g. slowly-adapting type I's) which can be induced either by gentle mechanical stimulation (allodynia) or by sympathetic efferent activity (leading to SMP).

Slowly-adapting type I afferents are likely to contribute to SMP because they can be activated by sympathetic stimulation in cats (Roberts and Elardo, Somatosens. Res., in press), because they have been shown to have excitatory actions on WDR neurons (Tapper, et al., 1985), and because they respond vigorously to gentle mechanical stimulation. Other classes of afferents may also contribute to the allodynia in these syndromes, but none appear to respond appropriately to sympathetic stimulation.

Testing of this proposed mechanism is presently being done in anesthetized cats. Single unit recordings from functionally identified spinal sensory neurons are used to test responses to both cutaneous and sympathetic stimulation. Preliminary results to date have shown that some WDR neurons give a sustained response to maintained, low-frequency (5 Hz) stimulation of the sympathetic trunk. Nociceptor specific spinal neurons tested under the same conditions are not activated by sympathetic stimulation. The results to date are consistent with the physiological mechanism for SMP described above.

38.2 DEPRESSED SEROTONIN AND DOPAMINE METABOLITES IN CSF OF PAIN PATIENTS. R. Weiner, G. Karp*, and B. Haber. Division of Neurosurgery, Dept. of Surgery, Marine Biomedical Institute, Depts. of Human Biol. Chem. and Genetics, and Neurology, Univ. of Texas Med. Branch, Galveston, TX 77550.

It is widely believed that serotonin (5HT is released as an inhibitory neurotransmitter from descending axons that modulates the processing of afferent noceceptive information. Thus, chronic pain may in part be due to a defficiency of central inhibitory 5HT mechanisms, and such a defficiency may in turn be reflected in the levels of either 5HT or its metabolite, 5HIAA, in the cerebrospinal fluid. We have analyzed a substantial number of cerebrospinal fluid samples obtained by lumbar puncture. The patients in the general pain category had neck pain, head and neck pain, pain in the lumbar or cervical areas, spinal stenosis and cancer pain. The non-pain group included hydrocephalus, aneurysms, spinal cord atrophy, closed head injury and cervical spondylosis. CSF samples in 0.2M perchloric acid were analyzed by high pressure liquid chromatography with electrochemical detection. The mobile phase was 0.2mM SOS (1-octanesulfonate, sodium) in 7.5% Acetonitrile, pH 2.5, containing 0.05mM EDTA, at a flow rate of 1.5ml/minute, using a 3 micron ODS short column. 5HT was not detected under these or other chromatographic conditions, and therefore, CSF needs to be concentrated at least ten fold to permit 5HT detection. In contrast, the 5HT metabolite, 5HIAA was present in all CSF samples, at concentrations ranging from 9-441 ng/ml. The mean value for 5HIAA in eighteen pain patients was 30.8 ± 3.1 ng/ml, and 110.4 ±19.3 ng/ml in 35 non-pain patients. An unidentified compound that chromatographs with Dopamine under these conditions was present at 357 ± 64 ng/ml in the pain group, and at 177 ± 50 ng/ml in the non-pain group. Homovanilic acid (HVA), the dopamine metabolite was consistently higher in the non-pain group (144.3 ± 2.68 ng/ml), than in the pain patients (55.5 ± 7 ng/ml). Comparison of pain patients with lesions vs. those with chronic pain showed the same pattern, with very little scatter in the values, as opposed to the large scatter in both selected (hydrocephalus) and random control CSF. The significance of the pain related depression of HVA is at present unclear, whereas the consistent depression of 5HIAA levels supports the notion of a decreased activity in descending serotonergic pathways in chronic pain.

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38.3 NEUROGENIC SPREAD OF HYPERALGESIA AFTER INTRACUTANEOUS INJECTION OF CAPSAICIN IN HUMANS. D.A. Simone*, J.Y.F. Ngeow* and R.H. LaMotte (SPON: J.G. Collins). Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Injury to the skin often results in an increased sensitivity to pain within the injured area, i.e. primary hyperalgesia ($1^{o}H$), and in the surrounding skin, i.e. secondary hyperalgesia ($2^{o}H$). Although nociceptors can be sensitized by chemical substances within an injury, there is controversy as to whether $2^{o}H$ results from the diffusion of the sensitizing chemical from injured to non-injured skin or by a neurogenic mechanism (e.g. chain of axon reflexes in periphery). In the present study, an intracutaneous injection of capsaicin produced $1^{o}H$ to heat and both $1^{o}H$ and $2^{o}H$ to mechanical stimuli. A neurogenic basis for the $2^{o}H$ is suggested.

Human subjects gave informed consent to a University-approved protocol. Each subject received a superficial injection of 10ul of capsaicin (1%) into the volar forearm. Capsaicin produced a flare surrounding the injection site and spontaneous burning pain. Within a few minutes there was pain to gentle rubbing with a cotton swab and decreased heat pain threshold (from 41.6 to 32.8°C) within a 1cm area centered over the injection site (area of $1^{o}H$). All subjects experienced pain or tenderness to gentle rubbing in a larger area ($2^{o}H$) surrounding the $1^{o}H$ area within which heat pain sensitivity was usually normal. The mean area of $2^{o}H$ was 37 ± 13 cm^2 (S.D.) and extended beyond the flare area which averaged 17 ± 9 cm^2.

Cooling the injection site to 1-2°C (1 cm^2 thermode) eliminated within 1-5 min the background burning pain and greatly reduced or eliminated the area of $2^{o}H$. The $2^{o}H$ returned to its original or slightly larger area upon rewarming the injection site. This sequence could be repeated several times.

In separate experiments, both $1^{o}H$ and $2^{o}H$ were abolished following an injection of 20-50ul of 0.5% xylocaine into the capsaicin injection site. Similarly, a narrow strip of xylocaine injected 1cm proximal to the capsaicin injection site did not alter $1^{o}H$ but blocked the spread of $2^{o}H$ proximally.

Finally, a pressure block of conduction in A fibers did not alter the development of $2^{o}H$, wherein light rubbing evoked pain but not tactile sensations.

Intracutaneous injection of capsaicin produced $1^{o}H$ to heat and $1^{o}H$ and $2^{o}H$ to mechanical stimulation. The $2^{o}H$ spread by a neurogenic mechanism and involved nociceptors with C fibers. It is possible that capsaicin-induced hyperalgesia may be a useful model for the study of clinical cutaneous hyperalgesia. (Supported by NIH Grant NS 14624)

38.4 REGIONAL SPECIFICITY OF VAGINAL STIMULATION SITES PRODUCING ANALGESIA IN WOMEN. Beverly Whipple* and Barry R. Komisaruk, Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

The present findings confirm and extend our earlier report that vaginal stimulation (VS) differentially elevates pain thresholds but not tactile thresholds in humans. In this study we compared the effects of pressure or pleasurable self-stimulation applied to the anterior (ant) or posterior (post) vaginal wall and clitoris in counterbalanced sequence. Pain thresholds were determined by applying a gradually increasing force to each finger using a Ugo Basile Analgesia Meter. The subject reported when finger pain was perceived (threshold for detection: (Td)) and when finger pain became too uncomfortable to continue (threshold for tolerance: (Tt)). Tactile thresholds were determined on the dorsal surface of the hand using force-calibrated von Frey fibers. VS was self-applied using a specially designed force-calibrated plastic cylinder. The 10 subjects, not previously known to the experimenters, were paid volunteers who received medical and psychological screening prior to participation. The study received prior approval by the University IRB. All values are reported as group mean % increase over the non-stimulation reference threshold obtained at the end of each subject's testing session. The increase in Tt during VS pressure (ant) (26.8%) was significantly greater than that of the no-VS control (1.6%) (Duncan test $p < .05$), but the effects of VS (post) (20.8%) or clitoral pressure (12.2%) were not significant. During self-stimulation applied so that it was experienced as pleasurable rather than just pressure, each showed a significant and greater increase over the control condition (VS ant: 36.6%, VS post: 32.7%, and clitoral: 27.5%; Duncan each $p < .05$). As pleasurable stimulation continued to a maximum of 5 minutes, the Tt increased further (55.7%, 43.5%, and 47.3%, Duncan each $p < .05$), respectively. A distraction (reading) control did not elevate the Tt. Td during VS ant pressure (35.7%) was significantly greater than control (5.7%), while those during VS post pressure (28.4%) or clitoral pressure (19.1%) were not (ANOVA t-tests $p < .05$). The highest Td's were reached after 5 minutes of pleasurable stimulation (VS ant: 67.4%, post: 62.8%, or clitoral: 62.5%; each significantly greater than control; Duncan $p < .05$). The distraction (reading) condition (24.9%) did not significantly increase Td. In contrast to the elevations in Tt and Td, tactile thresholds were not significantly affected by any VS or clitoral stimulation condition (range 4.9% to -34.1%), while there was a significant (78.6%) increase in the reading control condition. It is noteworthy that a) the analgesia-producing self-stimulation was reported by the subjects as neither painful nor stressful, and b) the stimulation self-reported as pleasurable produced greater elevations in Td and Tt than did self-applied pressure. Supported by the Research Council, Rutgers-The State University.

38.5 VAGINAL STIMULATION-PRODUCED ANALGESIA IS PROLONGED BY LEUPEPTIN, A PROTEASE INHIBITOR. S.B. Heller, A.R. Gintzler, A. Stracher* and B.R. Komisaruk. Inst. Animal Behavior, Rutgers Univ., Newark, N.J. 07102 and Dept. Biochemistry, Downstate Medical Center, SUNY, Brooklyn, N.Y. 11203

Vagino-cervical mechanical stimulation (VS) in rats reduces behavioral responsivity to a variety of noxious stimuli, including tail-flick in response to noxious heat. VS is more effective in reducing responsivity to noxious stimuli than a standard dose of morphine sulfate (2mg/kg). Recent evidence implicates intra-spinal mechanisms in behavioral withdrawal responses to noxious stimulation. In spinal rats, VS blocks leg flexion in response to foot pinch. Intrathecally administered protease inhibitors potentiate endogenously released or exogenously administered opioids to increase analgesia.

The present study was designed to determine whether intrathecal administration of Leupeptin (L), a naturally occurring, calcium activated, serine protease inhibitor, would prolong the analgesia resulting from VS in the tail-flick test. Following ovariectomy and implantation of an intrathecal catheter into the lumbo-sacral region, animals were placed into one of four groups (N=10 per group); (1) L+VS, (2) saline (S)+VS (S+VS), (3) L+no VS or (4) S+no VS. Following pre-injection baseline (B) testing, Groups L+VS and S+VS were injected and given B, VS and post-VS (PVS) trials 3, 5, 10, 20, 40 and 60 mins. post-injection. Groups L+no VS and S+no VS were given three series of B trials at each of the corresponding time periods.

Results demonstrate that pre-injection tail-flick latencies (TFL's) did not differ among the four groups. Following injection, TFL's of Groups L+no VS and S+no VS did not change significantly over the entire test period; thus, Leupeptin itself did not produce analgesia. During VS trials, TFL's were equivalent for Groups L+VS and S+VS, but were significantly greater than those of Groups L+no VS and S+no VS in the corresponding test period (Duncan Test, $p < .05$). During PVS trials 5, 10 and 20 mins. following injection, Group S+VS TFL's were significantly greater (Duncan Test, $p < .05$) than those of Group S+no VS at the corresponding test period, confirming that analgesia can persist for minutes following termination of VS. Furthermore, during PVS trials 3, 5 and 40 mins. post-injection, Group L+VS TFL's were significantly greater (Duncan Test, $p < .05$) than those of Group S+VS. Thus, L potentiated vaginal stimulation-produced analgesia after cessation of VS.

Our results show that Leupeptin prolongs analgesia resulting from VS. This may be due to Leupeptin inhibiting the enzymatic breakdown of (a) neuropeptide(s) released in the spinal cord by VS.

38.6 THE EFFECT OF HELIUM-NEON LASER ON LATENCY OF SENSORY NERVE, Snyder-Mackler L*, Bork C*, Fernandez J*, (SPON: J. McElligott) Department of Physical Therapy, Temple University College of Allied Health Professions, 3307 North Broad St., Philadelphia, PA 19140.

Helium-Neon (He-Ne) laser has been reported to have pain relieving effects in humans. One possible mechanism of pain control was investigated in this double blind study. The effect of Cold Laser Therapy (CLT) on the distal latency of peripheral nerve in man was studied.

Twenty normal subjects were randomly assigned to one of two groups: placebo (8) and treatment (12). Antidromic distal latencies of the right superficial radial nerve were recorded prior to the laser or placebo (mock laser) treatment. Six, one square cm areas of skin overlying the superficial radial nerve were irradiated with continuous He-Ne (or mock) laser held .5mm from the skin surface. A second latency reading was recorded immediately after the treatment. Both the subjects and the experimenters were prevented from knowing whether the laser or placebo was used by an opaque shield which covered the fiberoptic tip.

A t-test was employed to analyze the data. The treatment group displayed a statistically significant increase (for $p<.05$) in the distal latency of the superficial radial nerve when compared with the placebo group.

An increase in distal latency corresponds to a decrease in nerve conduction velocity. This decrease in nerve conduction velocity could begin to provide an explanation for the pain relieving effect of He-Ne laser.

PAIN MODULATION: PEPTIDES, MONOAMINES

39.1 INTRASPINAL PEPTIDERGIC REARRANGEMENT FOLLOWING SECTION OF RAT SCIATIC NERVE. A.M.Di Giulio, F.Borella*, P.Mantegazza*, M.Parenti, A.Groppetti,* R.Zanoni*† and A.Gorio*†. Dept. of Pharmacol. Univ. of Milano, Milano and † Fidia Res.Labs., Abano Terme, PD, Italy.

Substance P (SP) is considered to be a putative transmitter in the central terminals of primary sensory neurons in the dorsal horn of the spinal cord. Section of the peripheral processes of these neurons produces a dramatic depletion of SP in the lumbar spinal cord. We have measured the levels of SP, met-enkephalin (ME), dynorphin (DY) and serotonin (5-HT) in the rat lumbar cord following sciatic nerve section. Rat sciatic nerve was cut mono or bilaterally a few millimiters distally to the point where L4 and L5 form the sciatic nerve, then the proximal stump was either ligated or sutured intraperitoneally. The former way caused the formation of a small neuroma which conversely is very large in the latter case. The results obtained can be summarized as follows: 1) the emilateral section of sciatic nerve causes a dramatic depletion of both SP and ME in the lumbar cord starting at day 10 and persisting at day 30 postoperatively. These changes are not magnificated nor anticipated by the bilateral lesion. 2) The different extent of neurite outgrowth, does not affect differently SP and ME decline. 3) 5-HT turnover rate increases rapidly after the lesion and is back to control values 10 days later. 4) immunocytochemistry shows that there is a decrease in both SP and ME in the dorsal horn ipsilaterally to the lesion. The extent of the damage and the time-course are similar if the lesions are performed bilaterally. On the other hand, the anatomical loss of both peptides is bilateral but less remarkable than in the case of monolateral lesion, as if the peptide decrease was intraspinally regulated. The loss of FRAP staining from the dorsal horn is, however, bilateral and quantitatively similar to that observed after monolateral lesions. This result would suggest that FRAP staining faithfully reflects nerve lesions, while peptidergic losses may be regulated by more sophisticated intraspinal mechanism. These data suggest that following peripheral nerve lesion the depletion of SP in the dorsal horn of the spinal cord is associated with a dramatic loss of opioid peptides, which is independent on the extent of aberrant neurite outgrowth and could be regulated intraspinally. 5-HT might represent one of the intraspinal mechanisms related to the peptide loss. Preliminary results indicate that dynorphin may not be greatly altered in this experimental paradigm.

39.2 THE NUCLEUS RAPHE MAGNUS; THE RELATIONSHIP BETWEEN THE PEPTIDERGIC AND SEROTONERGIC NEURONS. R.M. Bowker and L.C. Abbott.* Dept. of VCAPP, Washington State University, Pullman, WA 99164

The nucleus raphe magnus and adjacent portions of the medial reticular formation are known to have important roles in several regulatory functions, including nociceptive and cardiovascular inputs to the spinal cord. Several different putative neurotransmitters are believed to be involved in these functions, including the enkephalins (M-ENK), serotonin (5HT) and substance P (SP), as well as several others. We examined several different putative neurotransmitters in the rostral medullary raphe nuclei for their localizations, distributions, microcircuitry, and potential for interactions with other brainstem neurons. Caudal brainstem tissues were obtained from guinea pigs (Cavia porcellus) perfused with 3.5-4% paraformaldehyde in phosphate buffer followed by sectioning (22-28 μm) and incubation of the sections for 24-36 hours in antiserum raised to M-ENK, 5HT, SP and somatostatin (SOMA) (INC) at dilutions ranging from 1:10,000 to 1:15,000. The sections were then processed for routine immunocytochemistry. Control experiments were also performed.

In these portions of the medullary raphe nuclei anterior to the inferior olivary nucleus (rostral parts of the nucleus raphe pallidus and the nucleus raphe magnus) numerous perikarya were found to contain positive immunoreactivity. These immunoreactive neurons appeared to have several different sizes depending upon their locations in the nucleus. Neurons stained for M-ENK, SP and 5HT were not distributed homogeneously throughout these nuclei, but were concentrated caudally in the nucleus raphe magnus. At the level of the trapezoid body, smaller clusters of stained neurons were present. The SOMA immunoreactive cells appeared to extend more laterally in the adjacent parts of the reticular formation. Morphometric analyses indicate differences between the immunoreactive neurons stained for the various antisera. Quantitative studies are currently being performed.

These results indicate the nucleus raphe magnus and the adjacent parts of the medullary brainstem contain several different putative neurotransmitters. These different chemical agents within the rostral medullary nuclei are potentially capable of modulating many of the physiological effects seen in the spinal cord, either directly by their descending projections to the spinal cord, or indirectly via their interactions in the brainstem and/or spinal cord. While the microcircuitry of these four putative neurotransmitters and their pathways remains to be examined, their interrelationship will be critical for understanding the functions of these midline cell groups.
(Supported by NS22321 and RR05465)

39.3 **CONFORMATIONAL STATES OF 5-HT_1 RECEPTORS MODULATING SPINAL PAIN TRANSMISSION: DIVALENT CATIONS.** J. Hirschowitz*, F.P. Zemlan, R.M. Murphy* and M.M. Behbehani (SPON: D.L. Garver). Laboratory of Psychobiology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0559.

Several lines of evidence indicate that the descending bulbospinal serotonin (5-HT) system suppresses incoming noxious input to the spinal cord and mediates narcotic analgesia. The present paper reports our initial studies characterizing multiple spinal cord ^{3}H-5-HT binding sites which is a prelude to the identification of spinal cord 5-HT_1 receptor subtype specific agonists and antagonists. These 5-HT_1 selective compounds will then be tested in our ongoing microiontophoretic recording studies of spinal cord dorsal horn nociceptive neurons. Incubation of dorsal horn membranes with ^{3}H-5-HT concentrations ranging from 0.10 to 50 nM in the absence of divalent cations revealed a complex ligand-receptor interactions. Computer analysis revealed that a two component model identified a higher affinity binding site with an apparent K_D of 0.034 ± 0.023 nM and a B_{max} estimated to be 36.1 ± 7.6 fmoles/mg and a lower affinity binding site with an apparent K_D of 12.1 ± 2.1 nM and a B_{max} of 295 ± 11 fmoles/mg.

As many ligand-receptor interactions are dependent on divalent cation concentration, the effect of divalent cations (1 mM Ca++, 5mM Mg++) on ^{3}H-5-HT binding in spinal cord was examined. Computer analysis revealed that a two component model identified a higher affinity binding site with an apparent K_D of 0.046 ± 0.024 nM and a B_{max} of 124.8 ± 21.2 fmoles/mg protein, and a lower affinity binding site with a K_D of 12.1 ± 2.4 nM and a B_{max} of 137.6 ± 12.5 fmoles/mg. The present study suggests that two forms of spinal cord 5-HT_1 receptors exist. Further, about 75% of the high affinity receptors appear to undergo a conformational change dependent on divalent cations. This conformational change decreases the receptors K_D from about 40 pM to about 12 nM in the absence of cations.

39.4 **SUBSTANCE P/5-HT COEXISTENCE AND PAIN: SUBSTANCE P EFFECT ON SPINAL CORD 5-HT_1 RECEPTORS.** Robert J. Hitzemann, F.P. Zemlan, R.M. Murphy*and M.M. Behbehani (SPON: T. Mandybur). Laboratory of Psychobiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0559.

Substance P (SP) and serotonin (5-HT) have been shown to coexist in the same bulbospinal neurons and are colocalized in the same dense core synaptic vesicles in spinal cord. In a separate presentation at this meeting, we report that SP antagonists block the inhibitory effect of stimulation of ventral medullary 5-HT/SP fibers on spinal cord neurons responsive to noxious stimulation. These data suggest that 5-HT and SP are coreleased and that the functional effect of SP corelease is to amplify the effect of 5-HT on spinal cord nociceptive neurons. The data presented below suggests that this amplification is mediated by the ability of SP to increase the number (B_{max}) of 10-15 nM ^{3}H-5-HT binding sites.

Increasing doses of SP [10 nM to 10 uM] produced a dose-response related increase in specific high affinity ^{3}H-5-HT binding [2 nM] to spinal cord membranes. Maximal ^{3}H-5-HT binding was observed at about 1 to 10 uM SP where binding increased about 30% ($p<0.001$). The specificity of this SP increase in ^{3}H-5-HT binding was assessed by determination of the effect of other peptides on ^{3}H-5-HT binding:

Peptide	Number of Amino Acids	Specific ^{3}H-5-HT Binding (fmoles/mg)
Buffer	--	85.4 ±3.1
Substance P	11	98.3 ±3.6***
Leu-Enkephalin	5	80.6 ±5.2
Met-Enkephalin	5	81.7 ±4.6
LH-RH	10	80.3 ±4.9
Neurotensin	13	89.2 ±7.1
Somatostatin	14	88.3 ±3.9
Bombesin	14	79.5 ±6.1

Preliminary saturation experiments ([^{3}H-5-HT] = 0.1 to 50 nM) conducted in the presence or absence of 1 um SP indicated that SP increases the B_{max} of the 10-15 nM ^{3}H-5-HT binding sites from 169 fmoles/mg protein to 240 fmoles/mg protein.

39.5 **CONFORMATIONAL STATES OF 5-HT_1 RECEPTORS MODULATING SPINAL CORD PAIN TRANSMISSION: GTP SENSITIVITY.** F.P. Zemlan, R.M. Murphy* and M.M. Behbehani, Laboratory of Psychobiology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0559.

It has been clearly established that spinal cord 5-HT_1 receptors mediate the 5-HT induced inhibition of dorsal horn neurons to noxious stimulation. Several studies suggest that cortical 5-HT_1 receptors are coupled to a C subunit (adenylate cyclase) which catalyzes the formation of cyclic AMP from ATP. The coupling of 5-HT_1 receptors to the C subunit appears mediated by a G subunit which binds GTP and Mg++. The purpose of the present study was to explore the relationship between GTP and 5-HT_1 receptors in spinal cord. Such studies are preliminary to an investigaton of a possible cyclic AMP mediated 5-HT induced inhibition of the response of dorsal horn units to noxious stimulation. The present study investigated the ability of GTP to alter the conformational state (K_D) of dorsal horn 5-HT_1 receptors. By determining the percent of 5-HT receptors demonstrating GTP dependent binding; the percent of dorsal horn 5-HT_1 receptors coupled to adenylate cyclase may be estimated by inference. Incubation of dorsal horn membranes in the presence of divalent cations (5 mM Mg++, 1 mM Ca++) with ^{3}H-5-HT concentrations ranging from 0.10 to 50 nM revealed a complex ligand-receptor interaction. Computer analysis revealed that a two component model identified a higher affinity binding site with an apparent K_D of approximately 0.046 nM and a B_{max} of approximately 125 fmoles/mg protein; and a lower affinity binding site with a K_D of approximately 12.1 nM and a B_{max} of approximately 138 fmoles/mg protein. In the presence of 1 mM GTP, fixed affinity computer modeling indicated that the B_{max} of the higher affinity binding site was decreased to approximately 20.4 fmoles/mg protein while the B_{max} of the lower affinity site increased to 261 fmoles/mg. These data indicate that GTP induced a conformational change in about 44% of the 5-HT_1 receptors suggesting that as many as 44% of dorsal horn 5-HT_1 receptors may be coupled to adenylate cyclase. This study suggests the feasibility of examining whether the inhibition of dorsal horn responsiveness to noxious stimulation observed after iontophoretic 5-HT application can be mimiced by iontophoretic application of db cyclic AMP.

39.6 IMMUNOREACTIVE SEROTONERGIC AXONS IN THE DORSOLATERAL FUNICULUS (DLF) OF THE CAT AND RAT. K. Zahs*, S. Lakos* and A.I. Basbaum. (SPON: A. Enjalbert). Dept. of Anatomy, University of California San Francisco, CA 94143

Although there is considerable evidence for a contribution of raphe-spinal (DLF) serotonergic pathways to opiate and stimulation-produced analgesia, there is some question as to the physiological properties of these axons. Furthermore, a recent study in the rat suggested that there are, in fact, few, if any, descending 5HT axons in the DLF. To address this question, we have examined the DLF in the cat and rat for the presence and fiber caliber of immunoreactive 5HT axons.

The PAP technique was used to localize 5HT-labelled DLF axons. Although these could often be seen in the cat without pharmacological or surgical intervention, this was not so in the rat. Thus, rats underwent a lesion of the cervical DLF two days prior to perfusion with a mixed aldehyde fixative. This resulted in damming of 5HT-immunoreactive axons rostral to the lesion. To characterize the distribution and size of the 5HT axons, EM montages of transverse sections of the DLF were constructed.

Consistent with the fluorescent histochemical studies of Dahlstrom and Fuxe ('65), we found considerable labelling of 5HT axons in the DLF of both the cat and the rat. These undoubtedly are the source of most of the dorsal horn 5HT terminals. EM analysis revealed that almost all of the labelled axons in the DLF of the cat are unmyelinated. Unmyelinated 5HT-immunoreactive axons also predominate in the rat, however, patchy immunoreactive material was present in small caliber myelinated fibers as well.

These data confirm earlier studies suggesting that bulbospinal 5HT axons are predominantly unmyelinated and indicate that the previous recordings of raphe-spinal axons and/or raphe neurons with conduction velocities that ranged from 10 to 50m/s probably originated from nonserotonergic cells in the nucleus raphe magnus. The physiology of 5HT-containing cells of the nucleus raphe magnus thus remains to be determined.

Supported by NS 14627 and NS 16033.

39.7 THE ULTRASTRUCTURAL LOCALIZATION OF POLYCLONAL GABA AND MONOCLONAL GLUTAMATE IMMUNOREACTIVITY IN THE RAT MIDBRAIN PERIAQUEDUCTAL GRAY. J.R. Clements, A.J. Beitz, A.A. Larson and J.E. Madl*. Dept. of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

Injection of glutamate into the PAG has been reported to induce a rage reaction in animals and has also been shown to activate components of the descending intrinsic analgesic system. Injection of the gamma-aminobutyric acid (GABA) agonist, muscimol, into the PAG causes ipsiversive rotation. Since both GABA and glutamate appear to play a significant role in the PAG, we chose to examine the distribution and ultrastructural characteristics of GABA and glutamate-immunoreactive processes in the midbrain periaqueductal gray of the rat. Four adult, male Sprague-Dawley rats were perfused with 4% paraformaldehyde and 0.3 - 0.5% glutaraldehyde and used for examination of GABA-immunoreactivity. An additional five rats were perfused with 5% carbodiimide and 0.5% glutaraldehyde, postfixed with 5% glutaraldehyde and utilized for analysis of glutamate immunoreactivity. Midbrain sections from these 9 rats were cut on a vibratome, stained immunohistochemically for either GABA (with rabbit anti-GABA antisera) or glutamate (with a recently developed monoclonal antibody against carbodiimide-fixed glutamate) using the peroxidase-anti-peroxidase and indirect peroxidase procedures, respectively.

Light microscopic examination of PAG sections showed GABAergic cells concentrated dorsally and dorsolaterally, while immunoreactive fibers were dispersed throughout all regions of the PAG at all rostrocaudal levels. Glutamate-immunoreactive cells were identified in all PAG subdivisions, but were concentrated in the ventrolateral and dorsolateral subdivisions.

Electron microscopic analysis showed that GABA-like immunoreactivity was present in myelinated axons, dendrites, unmyelinated axons, axon terminals containing synaptic vesicles and cell bodies. In the tissue analyzed, the majority of reactive profiles in the ventral PAG were myelinated axons, while the majority of profiles in the dorsal PAG were dendrites. Occasional contacts between immunoreactive axons and both immunoreactive and non-immunoreactive dendrites were seen.

Glutamate-like immunoreactivity was also observed in dendrites, myelinated axons, unmyelinated axons and cell bodies. In samples examined, dendrites comprised the majority of immunoreactive profiles. Immunoreactive axon terminals containing synaptic vesicles were also observed in close apposition to non-immunoreactive processes. Our results suggest that both GABAergic and glutamatergic cells and processes are present in the PAG and are consistent with the proposal that these putative transmitters are important in PAG function.

We thank Dr. Bruce Maley for the GABA-antiserum. Supported by NSF grant #BNS83-11214 and NIH grants DE06682 and NS17407.

39.8 PHENCYCLIDINE SELECTIVELY BLOCKS A SPINAL ACTION OF GLUTAMATE ANALOGS. L. M. Aanonsen*and G. L. Wilcox. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455

Several laboratories are investigating the effects of phencyclidine (PCP) and other sigma receptor agonists on responses of the mammalian CNS to glutamate receptor agonists. PCP seems to antagonize many of the actions of glutamate. We have found that l-glutamate (G) and a potent glutamate analog, L-2-amino-4-(5-tetrazoly)butanoic acid (gamma-glutamyl tetrazole, GG) when injected i.t. in mice elicits biting and scratching. This behavior was similar to but more intense than that elicited by i.t. administration of substance P (SP). We have found a conc/response relationship for G (ED50=12.3mM [9.1-16.8]) and GG (ED50=0.54mM [0.49-0.61]). In order to determine if there was a synergistic action of glutamate with SP an ineffective conc of SP (65nM) was co-administered with effective concs of GG. The results did not indicate a significant shift in the conc/response curves of GG + vehicle (ED50=0.54mM [0.49-0.61]) compared with GG + SP (ED50=0.52mM [0.47-0.58]).

PCP has been shown to have antinociceptive properties and to block glutamate responses in the spinal cord and in the striatum. PCP (1mM, i.t.) alone had no effect while PCP administered with GG significantly shifted the GG conc/response curve to the right. PCP, at similar concs had no effect on SP behavior.

By contrast, studies have indicated that [D-Ala-D-Leu] enkephalin (DADL), a potent opioid agonist (delta>mu) and NE (alpha-2) inhibit behaviors elicited by SP. Since the behaviors evoked by glutamate are similar to SP, the effects of DADL and NE were examined with glutamate. The conc of NE required to inhibit GG was 125x higher than that needed to inhibit SP behavior. Inhibition by DADL of GG-induced behaviors required concs 5000x higher than that necessary to block SP, and motor toxicity was observed at these concs.

The studies presented suggest that the action of intrathecal glutamate may be independent of or indirectly related to the action of SP in the spinal cord. DADL inhibits biting and scratching induced by glutamate only at very high concs and NE inhibits at concs 125x higher than those necessary to inhibit SP behavior. PCP appears to have selective inhibitory action on glutamate-induced behavior. The fact that intrathecal glutamate elicits a much more intense behavioral response than SP and that it is pharmacologically different from SP suggests that glutamate may represent a neurotransmitter involved in a unique, spinally-mediated nociceptive pathway. (supported by NIDA grant #1933 and by a grant from Procter and Gamble Co.)

39.9 **NOREPINEPHRINE INHIBITS FIELD STIMULATION-EVOKED RELEASE OF SUBSTANCE P FROM CHICK DORSAL ROOT GANGLION CELLS IN CULTURE.** G.G. Holz, R.M. Kream and K. Dunlap. Depts. Physiology and Anesthesia Research, Tufts Univ. Schl. Med., Boston, MA 02111.

Recordings from the cell bodies of embryonic chick dorsal root ganglion cells in culture have demonstrated a direct inhibitory action of norepinephrine (NEpi) on voltage-dependent calcium currents. This response results in a decrease in action potential duration which is thought to reflect a decrease in depolarization-induced calcium influx. In a previous study of these cells, Fischbach et al. (Adv. Biochem. Psychopharm. vol. 28, pp. 175-188, 1981) reported that NEpi reduced calcium-dependent substance P release triggered by high K^+ depolarization. Our interest is to determine, through pharmacological criteria, whether NEpi is acting through identical amine receptors to inhibit calcium influx and substance P release. We report here a newly developed in vitro system in which transmitter effects on substance P release may be examined using a more physiological form of depolarization: field stimulation.

Embryonic chick dorsal root ganglion cells were dissociated and grown in the absence of non-neuronal cells on 60 mm collagen-coated tissue culture plates. After 2-3 weeks in culture, each dish contained 4,076 + 265 pg (mean + S.E.M., n=19) of substance P-like immunoreactivity (SPLI). SPLI, as determined by RIA, was authenticated by demonstration of co-elution with synthetic substance P by reverse phase HPLC. SPLI release was triggered by field stimulation under sterile conditions using bipolar platinum stimulating electrodes (square wave DC pulses, 110 V, 1 Hz, 3.0 msec for 10 min). In HEPES-buffered saline containing 2.0 mM Ca^{++}, field stimulation increased SPLI by 3.3 + 0.3 fold over baseline to give 95 + 5 pg SPLI per dish, equivalent to 2.3 + 0.1% of the mean total cellular content (n=23). SPLI release was calcium-dependent in that it was completely blocked by solutions containing 0.1 mM Ca^{++} and 5.0 mM Co^{++} (n=6). In contrast, in solutions containing 2.0 mM Ca^{++} and 1.0 mM Ba^{++}, field stimulation increased SPLI by 7.3 + 1.2 fold over baseline to give 356 + 20 pg SPLI per dish equivalent to 8.7 + 0.5% of the mean total cellular content (n=12). The amount of SPLI released in the Ca^{++}/Ba^{++} solution was significantly reduced to 59 + 4% of control by 50 uM NEpi (n=6, $p < 0.001$, 2 tailed Students t-test). Baseline values of SPLI were unaffected. Tests for release of SPLI were repeated at 5 hour intervals with the plates returned to the incubator in between each period of stimulation. This allowed a test for release before, during and after exposure to NEpi. The mean values of SPLI release before and after treatment with NEpi were compared with the value obtained in the presence of NEpi. Intracellular recordings from the same dishes demonstrated a 36 + 12% decrease in spike duration in response to 50 uM NEpi (n=10) when tested in the Ca^{++}/Ba^{++} solution.

Studies are presently underway to characterize the pharmacological properties of this NEpi receptor, as well as to examine the effects of 5-HT, GABA, baclofen and morphine on substance P release. Supported by a grant to K. Dunlap from the Klingenstein foundation, and R. Kream from New Eng. Med. Ctr.

39.10 DISSOCIATION BY DIAZEPAM OF THE ANALGESIC AND AVERSIVE EFFECTS OF PERIAQUEDUCTAL GRAY STIMULATION IN THE RAT. M.M. Morgan*, A. Depaulis, and J.C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024.

Both analgesia and escape behavior can be induced by electrical stimulation of the periaqueductal gray (PAG) in the rat. In a previous study it was shown that pentobarbital anesthesia, by suppressing behavioral reactions, allows for stimulation-produced analgesia (SPA) to be measured in 95% of the PAG stimulation sites in the rat. This suggests that the behavioral effects induced by PAG stimulation prevents the measurement of the underlying analgesia. The aim of the present study was to determine, at sites supporting aversion, whether SPA can be obtained following administration of diazepam which has been shown to suppress the aversive component of PAG stimulation in the rat.

Male Sprague-Dawley rats were chronically implanted with a bipolar electrode directed at the PAG. Stimulation consisted of 50 Hz monophasic pulses of 0.4 msec duration at intensities ranging from 0.01 to 1.0 mA. Each rat was injected with 4 doses of diazepam (0, 1.0, 2.0, 4.0 mg/kg, i.p.) administered in a counterbalanced order with a minimum of 5 days between tests. Baseline pain sensitivity (tail-flick test), SPA threshold if present, and threshold for the first behavioral response (i.e. escape, rotation) were measured before and 10 min after drug injection. Histology was performed to verify electrode placement.

Treatment with diazepam did not alter baseline tail-flick latencies regardless of dose. Similarly, at sites supporting analgesia prior to drug treatment, SPA threshold was not altered by diazepam. Threshold for aversive reactions (e.g. running, jumping) was increased by diazepam and it was thus possible to measure SPA at an intensity previously inducing aversion. These results support the hypothesis that aversive reactions interfere with the measurement of SPA. It is further suggested that concomittant with aversive reactions produced by PAG stimulation, pain inhibitory mechanisms are activated. (Supported by NIH grant NS-07628, Fondation Fyssen, and a gift from the Brotman Foundation).

39.11 REDUCTION OF THE INHIBITORY ACTION OF NUCLEUS RAPHE MAGNUS STIMULATION ON DORSAL HORN NEURONS BY SUBSTANCE P ANTAGONIST. T.A. Vaughn*, M.M. Behbehani, and F.P. Zemlan. Department of Physiology and Biophysics and Department of Psychiatry. University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0576 (sponsored by G. Khodadad).

There is considerable evidence that stimulation of the nucleus raphe magnus (NRM) inhibits the response of dorsal horn neurons to noxious stimulation. Immunohistochemical methods have shown that many terminals of NRM neurons in the dorsal horn contain both serotonin (5-HT) and substance P (SP). The current study was undertaken to determine the effect of SP and its antagonist (D-Pro^{2}, D-Trp ^{7,9})-SP on the response of nociceptive dorsal horn cells to NRM stimulation.

Rats weighing 250-300 grams were used. Each animal was anesthetized with choral hydrate (400 mg/Kg), placed in a spinal stereotaxic unit and the spinal cord between L1 to L4 was exposed. A tungsten stimulating electrode was placed in the NRM and this region was stimulated with constant current of 10 to 100 μA with duration of 400 μ seconds at a rate between 10 to 300 Hz. A five barrel electrode consisting of a recording electrode and drug electrodes filled with 200 mM glutamic acid (GLU), 3 mm SP in 20 mm sodium acetate and 3 mm substance P antagonist (SPA) in 20 mm sodium acetate. One barrel was filled with 3 M NaCl and was used as a balance electrode. Once a cell was isolated, its response to noxious stimulation was tested. Since the majority of nociceptive neurons in the dorsal horn are silent, continuous application of glutamic acid was used to cause spontaneous firing of these neurons. For each cell (n = 40) the effect of NRM stimulation at 10, 100, and 200 Hz for 100 msec repeated every second in the presence and absence of substance P antagonist was tested.

NRM stimulatron at 10 Hz produced a very slight inhibition of glutamate induced firing rate. In contrast, stimulation at 100 and 200 Hz caused a significant decrease in the glutamate induced firing rate. In presence of SPA, the inhibitory effect of NRM stimulation was reduced in a dose-related manner and in some neuron application of SPA totally blocked the inhibitory effect of NRM stimulation. In several cells the effect of 5-HT antagonist, methysergide was also examined. In these cells, methysergide totally abolished the effect of NRM stimulation.

The results of these experiments are consistent with a hypothesis that SP plays a role in the inhibitory effect of NRM stimulation on dorsal horn nociceptive neurons which may be related to corelease of SP and 5-HT from terminals of NRM neurons at the dorsal horn. The mechanism by which SP amplifies the inhibitory effect of 5-HT, as we report elsewhere at this meeting, may be related to a SP induced increase in the number of 5-HT receptors; as SP increased the number of ^{3}H-5-HT binding sites in spinal cord. Supported by grant NS 18326.

PAIN MODULATION: STRESS ANALGESIA

40.1 CONDITIONED STRESS-INDUCED ANALGESIA (CSIA) IN RATS: OPIATE AND NONOPIATE MECHANISMS. H. S. Hagen* and K. F. Green. Psychology Dept., Cal. State Univ., Long Beach, CA 90840.

CSIA produced by pairing the compound CS of handling, lab room and apparatus cues with footshock has been reported to be naloxone-sensitive by some (e.g., Watkins et al., Brain Res., 1982, 243, 119-132) and to be naloxone-insensitive by others (e.g., Chance & Rosecrans, Pharmacol., Biochem. & Behav., 1979, 11, 643-646). The present experiments used hybridized procedures and delineated conditions for producing opiate and nonopiate varieties of CSIA. In both experiments the compound CS was 1) gloved handling followed by 2) placement in smooth white tubes for restraint during 8 tail flick trials (1/min) and 3) placement in a shock chamber. The UCS of 0.0, 2.5 or 5.0 mA of shock was delivered to separate groups of rats for 15 sec. After shock the rats were placed in rough black tubes (lined with hardware cloth) and given 8 tail flick trials.

In Experiment 1 four days of training with shock were given as described. On Day 5 naloxone (10 mg/kg, ip) was injected into half the rats in each shock condition (volume equivalent of saline into the other half). Shock was omitted on Day 5 for evaluation of conditioning to shock apparatus cues. Preshock tail flick latencies showed that CSIA began on Day 2 and grew through Day 4. The latencies of rats in the 2.5 and 5.0 mA groups were equally elevated over those of the 0.0 mA group. On Day 5 naloxone attenuated the "preshock" latencies of the 2.5 mA group but did not affect the other groups. On Day 5 naloxone did not affect the "postshock" latencies of any group, although the "postshock" latencies were lower than the "preshock" latencies in the shocked groups.

In order to examine the development of opiate CSIA, rats in Experiment 2 were injected on alternate days (counterbalanced order) with naloxone (10 mg/kg, ip) and saline, and trained for three pairs of days. Preshock latencies increased steadily over pairs of days, and again the shocked groups were equally elevated over the nonshocked group. Naloxone attenuated latencies in the 2.5 mA group on the second pair of days and in the 5.0 mA group on the third pair of days.

The two experiments indicate an interaction between shock level and amount of training. After minimal training of only one day, CSIA was present in both shocked groups. Nonopiate CSIA was strongest in the 5.0 mA group after moderate training and in the 2.5 mA group after more training. The opiate component was strongest in the 2.5 mA group after moderate training and in the 5.0 mA group after more training.

Thus, the overall level of CSIA increased with number of CS-UCS pairings. The contributions of the opiate and nonopiate components appeared to complement each other, with the exact proportion depending upon the level of shock and the amount of training.

40.2 AN ENDOGENOUS ANXIOGENIC SUBSTANCE IS INVOLVED IN NON-OPIATE STRESS INDUCED ANALGESIA. A.E.Panerai, P.Sacerdote*, and P.Mantegazza. Dept.Pharmacology, Chemotherapy and Medical Toxicology, School of Medicine, Univ. of Milano, Milano, Italy.

Inescapable, but not escapable foot shock can induce either a naloxone reversible or a naloxone non reversible analgesia, according to the modalities of application of the shock. We also showed that at least one kind of naloxone non reversible shock induced analgesia can be reversed by antagonists of the K opiate receptor.

There is evidence, however, that more than one naloxone non reversible stress induced analgesia exist. It is possible that while naloxone reversible analgesia is sustained by beta-endorphin, and the naloxone non reversible by dynorphine, anxiety itself, might play an important role in the development of stress induced analgesia, possibly through the activation of an endogenous factor. In order to evaluate this possibility, we administered to rats benzodiazepines or different substances to be pure benzodiazepine antagonists or inverse antagonists.

Of these substances only benzodiazepines show a slight analgesic effect when administered alone, while the antagonists seem to be devoid of any significant effect.

Benzodiazepines, administered before the administration of inescapable foot shock, yielding a naloxone reversible analgesia, blunted the analgesic response and this no more reversible by naloxone.

When the antagonist drugs were administered before the administration of shock, analgesia was increased and prolonged when compared to diluent treated rats by the pure, but not the inverse antagonist. Again, despite the characteristics of the shock administered, the analgesia obtained was not reversible by naloxone. These observations suggest the presence of an endogenous anxiogenic substance, that might unveil a naloxone non reversible analgesia.

40.3 FACILITATED TAIL-FLICK PERFORMANCE FOLLOWING STRESSFUL FOOTSHOCK. G.D. Coover, C.E. Lints, and W.E. Briner. Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115.

A stress-induced analgesia paradigm (e.g. Exp.5, Maier, Davies, Grau, Jackson, Morrison, Moye, Madden IV, & Barchas. JCPP. 94:1172, 1980) was designed to minimize 4 potential sources of response bias. Footshocks were delivered to male hooded rats in a small chamber (rather than tail shocks in the same restraint tube used for tail-flick testing) to reduce associative effects such as learned helplessness. The pretreatment shocks (2.4 ma no-load intensity; BRS-Foringer SGS-003 shocker scramblers; about 1 ma on most shockers) averaged 2-sec duration since longer ones may facilitate learned immobility. Since motor debilitation produced poor avoidance behavior 30 min after intense footshock (Sutton, Coover, & Lints. Physiol. Psychol. 9:127, 1981), the rats were tested for debilitation 5-10 min after pretreatment using the absence of a forepaw placing response and 10-25 min after using rearing during a 3-min exposure to a 1 m^2 open field. Tail-flick latencies were measured on rats gently restrained in a towel since this procedure produces shorter latencies than restraint in a tube. In general, the rats were assessed on Days 1, 2, & 3 for baseline activity in the open field and tail-flick latencies (IITC tail-flick apparatus, model 33). On Day 4 all rats were tested for motor debilitation and then tail-flick latencies (median of 3 trials presented at 2-3 min intervals) in the 35 min following a 45-min pretreatment session of 90 footshocks or placement in a chamber without shock (Control).

In Exp.1 three groups were pretreated. The escape group (ES) could terminate shock by pressing a lever inserted 1 sec after shock onset (or lever retraction and shock termination were at 4 sec if no response was made). Duration of shock for the inescapable group (IS) was determined by yoked ES rats. Yoked controls received no shock. Debilitation was determined as above. Tail-flick latencies differed, $F(4,60)=13.8$, $p < .001$, with the shocked rats showing longer latencies if they were debilitated. Debilitated IS (DIS) rats showed longer latencies than DES and Controls. Most importantly, the nondebilitated (ES and IS) groups had shorter latencies than the Controls (Means = 2.59, 2.82, and 3.50, respectively).

In Exp.2 no ES condition was used, tail-flick testing was with a more intense light beam, and following testing in gentle restraint the rats were given 3 more trials restrained in Plexiglas tubes. The IS rats had a mean latency of 2.46 $\pm$ .11 ($\pm$SE) sec in gentle restraint compared to 2.90 $\pm$ .15 for the Controls, $t(44)=2.41$, $p < .025$. In the tubes the latencies for the IS and Control groups were 10.2 $\pm$ 1.0 and 9.8 $\pm$ 1.0 sec, respectively.

Under the conditions of these experiments, shock pretreatment only produced longer latencies when the rats were debilitated. Thus response bias may contribute to findings of stress-induced analgesia.

40.4 STRESS-INDUCED ANALGESIA VARIES AS A FUNCTION OF ESTROUS CYCLE AND SEX STEROID REPLACEMENT THERAPY. S. Ryan, H. Goodale*, D. Weiss* and S. Maier. Department of Psychology, University of Colorado, Boulder, CO 80309.

The following studies find significant variation in stress-induced analgesia (SIA) in female rats as a function of stage of the estrous cycle, and implicate estradiol as an important determinant of SIA.

Experiment I examined SIA in four groups of rats; each group was tested in one of the four stages of the estrous cycle. Pain sensitivity was assessed by rear paw lick latency on the hotplate. Baseline latencies did not differ between groups. Subjects were restrained in Plexiglas tubes where ten 1-mA tailshocks (5 sec each) were delivered on a one-minute variable time schedule. Post-shock latencies indicated that all groups were analgesic to some extent. Estrus subjects were most analgesic, metestrus subjects were least analgesic, and these two groups differed significantly ($p < .01$).

Both estradiol and progesterone levels vary regularly over the estrous cycle. Experiment II assessed the effects of these steroid hormones on SIA. Three to four weeks following bilateral ovariectomy, rats were either treated with 5 µg estradiol benzoate (EB) or equivolume oil vehicle (V) administered subcutaneously (sc) for two consecutive days. Forty-eight hours later, rats were administered sc either 5 mg progesterone (P) or equivolume V. Six hours later, subjects were tested before and after 10 tailshocks as in Experiment I. Again, baseline latencies did not differ. Following shock, groups receiving V only or V + P were the least analgesic and the group receiving EB + V was the most analgesic. The EB + V group differed from the V group ($p < .05$), suggesting that estradiol is an important determinant of brief-shock stress-induced analgesia. The EB + P group did not differ from the V or the EB + V groups, suggesting that progesterone may play some role in prevention or suppression of this analgesia.

These experiments suggest that estradiol contributes to the variable expression of SIA over the estrous cycle.

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40.5 EFFECTS OF ADRENERGIC AND SENSORY NEURON BLOCKADE ON HYPERALGESIA PRODUCED BY HEAT INJURY. T.J. Coderre and R. Melzack*. Dept. of Psychology, McGill Univ., Montreal, P.Q., Canada H3A 1B1.

Sensory neuron blockade produced by capsaicin reduces the inflammatory responses to cutaneous injury. Adrenergic neuron blockade produced by guanethidine reduces pain and hyperalgesia following nerve injury. This study reports on the effects of these two treatments on the increase in pain sensitivity which typically follows a heat injury. Rats were given a s.c. injection of 0.05 ml of 1.5% capsaicin or vehicle (10% tween, 10% ethanol in normal saline) into the plantar surface of one hindpaw, and 4 once-daily injections of guanethidine (30 mg/kg s.c.) or saline. Capsaicin was administered 24 h before injury by immersion of the treated hindpaw into 55°C water for 15 s. The last guanethidine injection was given 4 h prior to the injury. Foot-withdrawal latencies of the injured paw from 47°C water were recorded in the 30 min before and after the injury. Latencies of the paw contralateral to the injury were recorded 24 h after the injury.

The results indicate that the injury produces a reduction in the latencies in both the injured paw and the paw contralateral to the injury indicating the presence of hyperalgesia. Capsaicin produced a significant elevation in the pre- and post-injury latencies of the injured paw. In contrast, guanethidine produced a significant elevation in the latencies of the contralateral paw. Since capsaicin reduced the hyperalgesia in the injured region it is suggested that it is acting on a local neurogenic mechanism. However, the fact the guanethidine reduces hyperalgesia in the paw contralateral to the injury suggests that the spreading of hyperalgesia is mediated by a mechanism which involves abnormal activity in sympathetic fibers.

40.6 AN OPIOID-TRYPTAMINERGIC MECHANISM OF HYPOALGESIA PRODUCED BY MILD STRESS. K.B.J. Franklin, F.V. Abbott and B.J. Connell .Dept. of Psychology and School of Nursing, McGill University,Montreal, Quebec, CANADA.

The relationship between endogenous opioids and basal pain sensitivity is unclear. In animal and human studies opioid antagonists are reported to produce hyperalgesia, no effects and even hypoalgesia. It has recently been shown that the hypoalgesia induced by stress can be prevented by naltrexone or by treatments that reduce brain 5-HT activity. Since common laboratory handling procedures are stressful, it is possible that the inconsistancies result from varying degrees of environmental stress in different laboratories. To test this hypothesis we examined effects of testing in a novel environment on basal pain sensitivity using the formalin test which assesses an animals response to minor tissue injury. As predicted, rats that were exposed to the stress of a novel environment showed less pain than rats that had been prehabituated to the environment.

The role of opioids was tested by administering naloxone 82.0 mg/kg) 5 min before a 15 min test period (30-45 min after formalin). Nalaxone had no significant effect on pain in habituated or nonhabituated rats.

Some forms of stress analgesia can be blocked by treating rats with l-valine which prevents the stress-induced rise in brain tryptophan and 5-HT activity. Valine (200 mg/kg), given prior to formalin, also had no signigicant effect on pain sensitivity, although the habituated rats given valine had somewhat lower pain scores ($.05<p<.1$). The combination of naloxone and valine reversed the hypoalgesia ($p<.01$) in rats exposed to a novel environment but had no significant effect on habituated rats. These results suggest that mild stress can activate an opioid-tryptaminergic system through which stress reduces pain sensitivity.

40.7 **TRYPTOPHAN UPTAKE, OPIOIDS, AND SYMPATHETIC ACTIVITY IN ANALGESIA INDUCED BY STRESS OR BY AN INTERACTION OF STRESS AND MORPHINE.** S. J. Kelly and K. B. J. Franklin. Dept. of Psychology, McGill University, Montréal, P.Q., Canada, H3A 1B1.

We have recently shown that an increase in brain tryptophan uptake is critical to analgesia induced by prolonged restraint and to analgesia induced by an interaction of stress and morphine (Kelly & Franklin, Neuropharmacol., 1985). The studies presented here further examine the biochemical aspects of these two forms of analgesia in rats.

Three hours of restraint in wire mesh restrainers induced an increase that lasted for 10 min in the latency of tail withdrawal from 55°C water. We found that this analgesia was unaffected by naltrexone methobromide (10 mg/kg) given before the restraint, indicating that peripheral opioid receptors were not involved in the analgesia. Naltrexone hydrochloride (1 mg/kg) did not reverse the analgesia when given 15 min before the end of the restraint period but did prevent the analgesia when given before the restraint ($p<.005$). Naltrexone hydrochloride might have prevented the analgesia induced by prolonged restraint by preventing the restraint-induced increase in brain tryptophan uptake which is critical to this form of analgesia. To examine this hypothesis, HPLC with fluorescence detection was used to assay tryptophan levels in the brainstem of rats subjected to restraint. Three hours of restraint were found to increase brain tryptophan levels ($p<.001$). Naltrexone hydrochloride (1 mg/kg) given before the restraint did not affect tryptophan levels. Therefore, the prevention of analgesia induced by prolonged restraint by naltrexone hydrochloride was not due to an effect on brain tryptophan uptake.

In order to explore how restraint increases brain tryptophan uptake, we examined the effect of a chemical sympathectomy induced by guanethidine sulphate (30 mg/kg every day for five days) on analgesia and brain tryptophan. Instead of using analgesia induced by 3 hours of restraint, the potentiation of analgesia in the tail withdrawal test which results from an interaction of a short period of restraint and morphine was used. This effect has also been shown to be dependent upon an increase in brain tryptophan uptake and appears to be a more robust, pharmacologically-induced form of the analgesia produced by prolonged restraint. Guanethidine treatment prevented the analgesia induced by an interaction of restraint and morphine sulphate (5 mg/kg) but had no effect on analgesia induced by morphine sulphate (5 mg/kg) in unrestrained rats. Furthermore, guanethidine treatment prevented the increase in brain tryptophan uptake elicited by restraint.

These studies further delineate the physiological systems that can cause analgesia in response to restraint stress. An opioid mechanism critical to the analgesia is in the central nervous system but does not need to be active at the time of the analgesia. The increase in brain tryptophan uptake that is also critical to the analgesia is dependent upon sympathetic activity and not upon the opioid mechanism.

40.8 Inhibition of Acoustic Startle in the Rat by a Noxious Prestimulus: Effects of Morphine and Naloxone. D. S. Leitner and D. D. Kelly. New York State Psychiatric Inst. and Dept. of Psychiatry, Columbia University, New York, NY 10032.

The startle response is elicited by intense stimulation with an abrupt onset. Its amplitude can be reduced if the startle-eliciting stimulus is preceded by a mild stimulus which does not elicit startle (a prestimulus); the prestimulus must lead the startle stimulus by 50 to 500 msec. Startle inhibition is dependent only upon the characteristics of the prestimulus, not those of the startle stimulus.

In the present experiment, a noxious prestimulus was used to inhibit startle and the effects of morphine and naloxone upon this were investigated. Nine rats were assessed for footshock sensitivity with a flinch-jump test. Based upon the mean thresholds obtained from this procedure, intensities of .1, .2, .4, and .8 mA were selected for use in the startle inhibition procedure. Subjects were presented with 50 startle-eliciting bursts of white noise. On 40 of these trials, the startle stimulus was preceded at 400 msec by a 300 msec footshock, the intensity of which varied among the above values. The 5 types of trials (4 on which the startle stimulus was preceded by a footshock and 1 on which it was presented alone) were presented 10 times each in a block-randomized sequence. The intertrial interval was 20 sec.

All subjects were exposed to each of 4 conditions: An injection of morphine sulfate (5 mg/kg, i. p.) followed after 20 min by an injection of naloxone hydrochloride (2 mg/kg, i. p.); morphine followed by a saline injection; a saline injection only; or a naloxone injection only. The sequence in which each subject was exposed to the conditions was determined by a Latin squares matrix. In a given condition, ten min after the last injection subjects were given a brief tail-flick test to assess nociception, and were then tested in the startle inhibition procedure.

Tail-flick latency was reliably increased, and startle amplitude on control trials was reliably decreased, in the morphine/saline condition; the other 3 conditions were not reliably different from each other. Footshock reliably reduced startle amplitude. This effect was maximum at .2 mA; higher intensities did not produce reliably more inhibition. Finally, at each intensity the percent amplitude reduction was reliably attenuated in the morphine/saline condition; no other comparisons were reliably different.

These data demonstrate that mild footshock reliably inhibits startle, that morphine administration interferes with this effect, and that the interference was reversed by naloxone. This supports previous work which has shown that stimuli on the threshold of perception can inhibit startle, and suggests that this procedure may be useful as an analgesimetric test.

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PAIN MODULATION: CENTRAL PATHWAY MECHANISMS

41.1 MIDBRAIN SUPPRESSION OF A NOCICEPTIVE WITHDRAWAL REFLEX IN THE RAT. I. G. Campbell* & E. Carstens (SPON: M. McNamee). Dept. Animal Physiology, Univ. Calif., Davis, CA 95616.

Midbrain periaqueductal gray (PAG) and more lateral reticular formation (LRF) stimulation produces analgesia and inhibits spinal neurons. PAG stimulation reduced the slope of spinal neuronal stimulus-response functions for graded noxious hindfoot heating, while LRF stimulation shifted the functions in a parallel manner to the right. We have investigated whether these midbrain areas also differentially modulate the force of flexion withdrawals to graded noxious heating of hindfoot skin. Hindpaws of lightly anesthetized rats were attached to a transducer to measure force of hindlimb withdrawal elicited by noxious heat stimuli (42-52°C; 10 s) under isometric conditions; this and direct and integrated EMG activity via 0.1 mm teflon-coated steel wires in the biceps femoris were recorded on a penwriter. The upper 3 rows in Fig. 1A show that withdrawal force (upper trace) and integrated (middle) and direct (lower) EMG activity increased with graded increases in temperature (below each column). During 100 uA stimulation (100 msec trains at 100 Hz, 3/s) in PAG, force and EMG at each temperature were reduced (middle 3 rows) such that slopes of curves relating integrated force (B) or EMG (C) to temperature were reduced, whereas 35 uA LRF stimulation shifted the curves in a parallel manner toward the right. Note rebounds in EMG after termination of midbrain stimulation (brackets). These findings suggest that this spinal nociceptive withdrawal reflex is under the same descending controls that modulate dorsal horn neurons.

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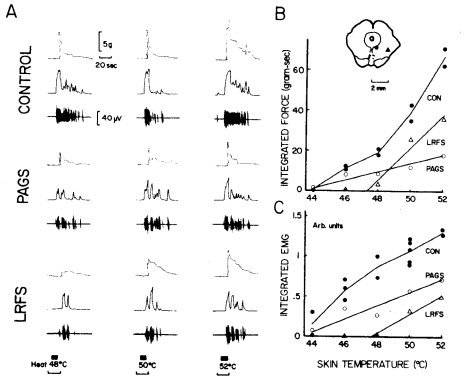

Fig. 1

41.2 CENTRIFUGAL MODULATION OF THE TAIL FLICK REFLEX EVOKED BY GRADED NOXIOUS HEATING OF THE TAIL. T.J. Ness* and G.F. Gebhart (SPON: G.R. Dutton). Dept. Pharmacology, University of Iowa, Iowa City, IA. 52242.

The stimulus-response function (SRF) of spinal dorsal horn neurons to peripherally applied noxious radiant heat has been shown in electrophysiologic studies to be attenuated by supraspinal electrical stimulation in two qualitatively different ways: a reduction in the gain of the SRF, or an increase in response threshold. The purpose of this study was to determine in a simpler, more physiologic preparation whether a SRF characterizing the nociceptive tail-flick (TF) reflex in the rat was similarly modified by stimulation in the brainstem.

Rats were initially deeply anesthetized with pentobarbital (45 mg/kg) for craniotomy and cannulation of the femoral vein and artery. Noxious radiant heat from a projector lamp was focused upon a 1.5 x 11 mm area of the ventral surface of the tail. Heat intensity was varied using a rheostat and five different rates of heat transfer (RHT) were employed to evoke the TF reflex in rats maintained in a lightly anesthetized state by an iv infusion of pentobarbital (3-6 mg/kg/hr). Inhibition of the TF reflex was produced by stimulation in the brainstem (continuous 100 Hz monopolar constant current cathodal pulses of 100 μsec duration) started 10 sec prior to the application of heat to the tail. The inhibitory threshold was defined as the minimum stimulating current which increased the TF latency ≥ 3 times baseline; an intensity, 50% or 80% of this inhibitory threshold, was used subsequently.

The TF latency was established to be linearly related to the RHT. However, the temperature of the tail at which the TF occurred was found to be independent of the RHT. Focal electrical stimulation produced two qualitatively different modifications of the SRFs depending upon the site of and intensity of focal electrical stimulation in the brainstem: Type I, an increase in the thermal threshold of the TF reflex at low RHT's, was produced by stimulation in the rostral medial medulla (n.raphe magnus and n. r. gigantocellularis) and in the lateral midbrain periaqueductal gray (PAG); and Type II, an accelerating increase in the thermal threshold of the TF reflex as a function of the RHT, was produced by stimulation in the medial PAG and the dorsolateral pons. These results are analogous to the centrifugal modulation of spinal dorsal horn SRFs to graded heating of the skin produced by focal electrical stimulation in the brainstem, further establishing the utility of the lightly anesthetized rat preparation for studies on nociception/antinociception.

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41.3 CHARACTERIZATION OF INHIBITION OF THE TAIL FLICK REFLEX FROM THE LATERAL HYPOTHALAMUS. L.M. Dilts and G.F. Gebhart. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA. 52242.

Stimulation produced antinociception (SPA) can be evoked from a wide variety of sites in the brain, including the diencephalon. The most frequently examined diencephalic site has been the rostral extension of the periventricular gray matter, but SPA can be produced from other sites, including the lateral hypothalmus (LH). The objective of this study was to characterize the descending inhibition of the nociceptive tail-flick (TF) reflex produced by focal electrical stimulation in the LH, including the neurotransmitter(s) mediating the inhibition at the level of the lumbar spinal cord.

Rats were initially deeply anesthetized with pentobarbital (45 mg/kg) for craniotomy and cannulation of the femoral vein and artery. The rats were subsequently maintained in a lightly-anesthetized state (corneal and flexion reflexes present) with an iv infusion of pentobarbital (3-6 mg/kg/hr). Constant current stimulation (100 Hz, 100 µsec, 20-200 µA) with monopolar, cathodal 0.15 mm dia. electrodes was begun 10 sec prior to the application of heat to the tail.

Stimulation in the diencephalon (e.g. VPL) outside of the hypothalmus did not affect the TF reflex. Inhibition of the TF reflex was produced, however, throughout the hypothalamus at intensities of stimulation typically between 75-150 µA. These intensities are considerably greater than required for TF inhibition at sites of stimulation in the brainstem. The area requiring the lowest intensity of stimulation (50-75 µA) to inhibit the TF reflex was a diffuse region of the LH, inferior to the zona incerta and internal capsule, medial to the supraoptic decussation and including the median forebrain bundle. Microinjection of monosodium l-glutamate (100 mM, 0.5 µl) in the LH failed to inhibit the TF reflex, indicating that the descending inhibition was produced by activation of fibers of passage rather than cell bodies.

To further characterize the inhibition from the LH, intrathecal injections of pharmacological antagonists were made at the level of the lumbar enlargement. The stimulation threshold in the LH for inhibition of the TF reflex was determined, after which the effects of methysergide (15 + 30 µg), naloxone (15 + 30 µg) or phentolamine (15 + 30 µg) on the inhibitory stimulation threshold were examined. To date, only phentolamine reliably increased the intensity of stimulation required for inhibition of TF reflex, indicating that spinal adrenoceptors mediate the inhibitory effects of stimulation in the LH.

Supported by DA02879 and NS19912.

41.4 COERULEOSPINAL INHIBITION OF HEAT-EVOKED SPINAL DORSAL HORN NOCICEPTORS IN THE RAT. S.L. Jones and G.F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa City, Iowa 52242.

The involvement of spinopetal efferents from the pontine nucleus locus coeruleus/subcoeruleus (LC/SC) in the centrifugal modulation of spinal nociceptive transmission has been demonstrated functionally in the rat using the nociceptive tail-flick model and electrophysiologically in the cat. However, anatomical differences exist between these species with regard to the organization of the noradrenergic pontine nuclei. The purpose of this study was to characterize, in the rat, the responses of spinal dorsal horn neurons to controlled noxious heating of the footpad in the presence and absence of electrical stimulation of the dorsolateral pons (DLP), in particular the LC/SC. Rats were deeply anesthetized with pentobarbital (45mg/kg) for cannulation of the femoral artery and vein and tracheotomy. They were paralyzed with pancuronium bromide (0.4mg/kg, iv) and ventilated with a gaseous mixture of N_2O:O_2 (2:1) and maintained deeply anesthetized with hourly iv infusions of pentobarbital throughout the duration of the experiment (12-14h). A craniotomy was done to allow for focal electrical stimulation in the LC (monopolar, cathodal constant current stimulation; 100Hz, 100 µs). The spinal cord between segments T12-L1 was exposed by laminectomy. The left hindpaw was fixed in paraffin wax and placed pad upward in a holder; the left tibial nerve was isolated and placed across a pair of stimulating electrodes. All neurons studied to date were driven by stimulation of the nerve at intensities supramaximal for activation of Aα,δ- and C-fibers and also responded to mechanical stimuli and to noxious radiant heating (50°C) of the skin of the foot- or toepads. The heat-evoked responses of all neurons were attenuated by LC stimulation. The mean threshold intensity of stimulation for inhibition of the units examined to date was 26.87 ± 4.73 µA and the mean intensity of LC stimulation attenuating the heat-evoked response at 50°C to 50% of control was 63.31 ± 11.41 µA. Monosodium l-glutamate (100 mM, 0.5 µl) microinjected into the DLP at the same site as electrical stimulation attenuated the neuronal heat-evoked responses (n=7 to date) and decreased blood pressure a mean 20mmHg immediately following the microinjection. Responses of spinal dorsal horn neurons to graded noxious heating of the skin (42-52°C) was a linear monotonic function. Stimulation in the LC/SC resulted in a mean 60.19% decrease in slope of the stimulus-response function without altering the threshold of the response (n=8 to date). Supported by DA02879 and NS19912.

41.5 EFFECT OF DEPLETION OF SPINAL NOREPINEPHRINE (NE) ON DESCENDING INHIBITION FROM THE LATERAL RETICULAR NUCLEUS (LRN). A.J. Janss and G.F. Gebhart. Dept. of Pharmacology, University of Iowa, Iowa City, IA. 52242.

Intrathecal (i.t.) injection of yohimbine or phentolamine significantly increases the threshold of focal electrical stimulation in the LRN (LRNS) required to inhibit the tail flick (TF) reflex, suggesting that LRNS produces an antinociception by releasing NE in the spinal cord (Gebhart and Ossipov, Neurosci. Abs. 10: 98). The effect of depletion of spinal NE on LRNS was evaluated in this study. Intrathecal catheters extending to the lumbar enlargement were surgically implanted in male Sprague-Dawley rats and three days later 20 µg of 6-hydroxydopamine (6OHDA) or vehicle (.1% ascorbate) in 5 µl was administered i.t. Nine days after treatment animals were lightly anesthetized and the threshold of constant current cathodal electrical stimulation (100 Hz, 100 µs) in the LRN for inhibition of the TF reflex was established. 30 µg of yohimbine was injected i.t. and its effects on LRNS and TF latency were determined. The spinal cords of the rats were removed and monoamine content and α_2-adrenoceptor binding estimated:

Pre-Drug	Vehicle-Treated	6OHDA-Treated
TF latency (sec)	2.41 ± .16	2.47 ± .36
LRNS threshold (µA)	19.37 ± 3.20	17.08 ± 7.11
30µg Yohimbine		
TF latency (sec)	1.59 ± .30	1.64 ± .24
% change LRNS	88.12 ± 28.17	165.00 ± 71.42*
Lumbar Monoamines		
NE (µg/g tissue)	.363 ± .042	.040 ± .033*
5-HT (µg/g tissue)	.456 ± .167	.456 ± .137

* Significantly different from vehicle-treated group.

Despite an 89% depletion of spinal NE, there was no change in the threshold of LRNS in the 6OHDA-treated group. Yohimbine produced a significantly greater increase in the LRNS threshold in the 6OHDA- than in the vehicle-treated group. These data suggest the possibility that receptors mediating LRNS-produced antinociception upregulate in NE-depleted animals. Binding studies with [^{3}H]Rauwolscine revealed an increase in α_2-adrenoceptors in lumbar spinal cord depleted of NE. Dose response studies of clonidine-produced inhibition of the TF reflex in lightly anesthetized rats also suggest development of adrenergic supersensitivity following NE depletion; the dose-response curves were shifted leftward 3,7,10 and 14 days following i.t. 6OHDA treatment. Supported by DA02879, NS19912 and T32 GM07337.

41.6 POTENTIATION OF THE ANTINOCICEPTIVE EFFECT OF NOREPINEPHRINE BY THE ADENOSINE ANALOG, 5'-N-ETHYLCARBOXAMIDE ADENOSINE. S. Aran*, N.M. Porter and H.K. Proudfit (SPON: M. Radulovacki). Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, Il 60612

Intrathecal administration of norepinephrine (NE) into the lumbar subarachnoid space of the spinal cord produces a dose-dependent analgesia in the rat. Recently, adenosine and adenosine analogs have been shown to modulate the effects of noradrenergic neurons in the CNS. The present studies were undertaken to determine whether there is an interaction between NE and adenosine in the regulation of pain transmission in the spinal cord.

In the first study, the effects of an adenosine agonist, 5'-N-ethylcarboxamide adenosine (NECA), were assessed on a dose of NE that produced submaximal analgesia. Male Sprague-Dawley rats (400-500 gm) were implanted with intrathecal catheters projecting to the subarachnoid space of the lumbar spinal cord. One week after surgery, animals were tested using the tail flick (TF) and hot plate (HP) tests, divided into three groups and then given the following intrathecal injections 20 minutes apart: NE (3ug) + saline, NE + NECA (1ug) or saline + NECA. Following each drug administration, nociceptive thresholds were determined at fixed intervals for 60-90 minutes. Saline + NECA produced no change in TF latencies. In contrast, the group that received NE + saline showed a significant increase in TF latencies. An even greater increase in TF latencies was observed in animals that received NE + NECA.

In the second study, the capacity of theophylline, an adenosine antagonist, to block the NE + NECA induced antinociception was examined. Rats were pretreated with an intrathecal injection of theophylline (100ug) and then NE + NECA were co-administered. The antinociceptive effect of NE + NECA was attenuated in rats pretreated with theophylline. No changes in HP latencies were observed with any drug treatment in either study.

These data suggest that there is an interaction between the adrenergic and purinergic systems in the spinal cord and provide further support for the involvement of adenosine in the regulation of pain transmission.

(This work was supported by PHS Grant NS18636)

41.7 ANTINOCICEPTION PRODUCED BY MORPHINE INJECTION INTO THE NUCLEUS RETICULARIS PARAGIGANTOCELLULARIS BUT NOT BY INJECTION ONTO SPINAL CORD INVOLVES NOREPINEPHRINE. I.H. Pang and M.R. Vasko. VA Med.Ctr., Univ. Texas Hlth. Sci. Ctr., Dallas, TX 75216

The purpose of this study is to determine if norepinephrine (NE) containing neurons that descend into the spinal cord are important in antinociception produced by morphine (M) microinjected into the nucleus reticularis paragigantocellularis (NRPG) or by intrathecal (i.t.) injection of M onto the spinal cord.

Male Sprague-Dawley rats (250-350g) were anesthetized and implanted with bilateral metal guide cannulae directed at the NRPG and with a polyethylene cannula (PE 10) inserted into the spinal cord subarachoid space with the tip positioned between T10 and L2. After recovery from surgery, spinal cord NE concentrations were depleted by i.t. injection of 200µg of 6-hydroxydopamine HBR (6-OHDA) after pretreatment with trazadone HCl (50mg/kg i.p.). All drug concentrations were calculated as base content. Control rats received trazadone and i.t. injection of vehicle. After 3-5 days, awake, unrestrained rats were administered M sulfate into NRPG (2.5µg/0.2 µl/side) or onto spinal cord (2 or 10 µg/10 µl). Antinociception was determined using the paw-pressure technique. Two hours after M injection, rats were decapitated, the spinal cords removed and homogenized in 0.1N perchloric acid and NE and 5-hydroxytryptamine(5HT) concentrations determined by HPLC with electrochemical detection.

When microinjected into NRPG, M produced profound antinociception (paw-pressure threshold (PPT) before M: 88 ± 4 mmHg; 30min after M: 224 ± 6 mmHg, n=5) which lasted for 120 min. Pretreatment with 6-OHDA significantly attenuated the antinociceptive effects of M (PPT before M: 84 ± 5 mmHg; 30 min after M: 96 ± 9 mmHg, n=5). The neurotoxin decreased the NE concentrations in the spinal cord to less than 15% of control (control: 296.5 ± 32.5 ng/g wet weight tissue; 6-OHDA treated: 30.0 ± 11.8 ng/g; n=5) without affecting the concentrations of 5-HT (control 649.1 ± 48.7 ng/g; 6-OHDA treated: 588.4 ± 82.1 ng/g). Intrathecal injection of 2 or 10 µg of M also produced significant antinociception (2µg PPT before M: 86 ± 2 mmHg; 30 min after M: 145 ± 10 mmHg, n=4; 10µg PPT before M: 86 ± 4 mmHg; 30 min after M: 205 ± 13 mmHg, n=4). Unlike NRPG injections, however, pretreatment with 6-OHDA did not affect the antinociception produced by i.t. injection of either 2 or 10µg of M even though the concentrations of NE in spinal cord were diminished to less than 15% of controls (controls: 264.2 ± 26.1 and 383.4 ± 48.2ng/g; 6-OHDA treated: 39.5 ± 16.3 and 7.1 ± 3.3 ng/g for 2 and 10µg experiments, respectively).

Thus, although antinociception produced by morphine microinjected in NRPG appears to involve NE-containing neurons in the spinal cord antinociception produced by i.t. morphine appears to be independent of NE-containing neurons. (Supported by the Veterans Administration)

41.8 STIMULATION OF THE DORSAL COLUMN NUCLEI (DCN) BY CHRONICALLY PLACED ELECTRODES IN AWAKE RATS INHIBIT THE TAIL FLICK THROUGH SUPRASPINAL MECHANISMS. S.J. Jabbur, N.E. Saadé*§ and S.F. Atweh. Fac. of Med Amer. Univ. of Beirut, Beirut and §Fac. of Sci., Lebanese Univ., Hadath-Beirut, Lebanon.

Dorsal column stimulation (DCst) in humans has been used for the treatment of intractable pain. In order to study the rostral effects of this stimulation in awake rats, we developed an experimental model with chronic stimulating electrodes on DCN.

Fourteen male Sprague-Dawley rats (150-200 g) were anesthetized with chlorpromazine and ketamine and operated under sterile conditions. The interparietal, occipital and atlas bones were exposed and the posterior arch of the atlas was removed; the occipital membrane and the dura were incised to expose the DCN and the rostral part of the cervical spinal cord. Both dorsal columns (DC) were lesioned at the C_1-C_2 level. Two small holes were drilled in the occipital bone on each side. Two insulated copper wires (0.17mm) were introduced through the holes, threaded between the bone and the dura and pulled out through the caudal margin of the occipital bone. The wires were fixed by multiple knots on the inner and outer aspects of the occipital bone and the central ends were bared and placed 0.5 mm apart on either side of the midline on the surface of the two DCN. The peripheral ends of the wires were further fixed to the skull by knotting them through holes in the upper part of the occipital and interparietal bone leaving the distal ends of the wires exposed. The latter were shortened to 1cm and their insulation removed for later connection to the output of a stimulator. The muscles and skin were sutured and the rats were allowed to recover for a period of one week. Five rats underwent a sham operation with no DC cuts or electrode placement.

Baseline tail flick latencies (TFL) were obtained and averaged on all rats prior and after surgery in three (30 min) testing sessions. Conditioning stimulation was then delivered to the DCN while testing the TFL for a period of 10 min at the start of each session in 7 rats. Compared to sham operated rats, DC lesion produced a small but significant increase in TFL. Conditioning stimulation produced a marked increase (more than 100% of control) in TFL. The place of the stimulating electrodes and the extent of the DC lesions were verified at the end of the experiments.

The results are in accordance with our previous electrophysiological demonstration that DC stimulation can modulate pain related activities in the spinal cord through a supraspinal (brainstem) loop (Saadé et al., Brain Res., 310:180, 1984; 335:306, 1985; and in Press, 1985).

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41.9 EFFECT OF MORPHINE ON THE RELEASE OF ENDOGENOUS MONOAMINES INTO SPINAL CORD SUPERFUSATES. H.K. Proudfit, A. Arai,* and M. Monsen.* Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL 60680.

There is evidence which indicates that opiates induce antinociception by activating raphe-spinal serotonergic neurons located in the nucleus raphe magnus, a midline nucleus located in the rostral medulla oblongata. There is additional evidence to suggest that bulbospinal noradrenergic neurons may also be involved in mediating the antinociceptive actions of opiates. The following experiment was designed to directly test whether these monoamines are involved mediating the actions of opiates by measuring the release of endogenous serotonin (5-HT) and norepinephrine (NE) into spinal cord superfusates before and after the systemic injection of morphine.

Male rats (400-500 g) were anesthetized with urethane (1200 mg/kg) and fitted with inflow and outlfow tubes for spinal cord superfusion. Two control samples, three ml each per 30 min period, were collected and then morphine sulfate (5 mg/kg) was injected subcutaneously. Three 30 min samples were collected following the injection. The amount of norepinephrine and serotonin released into the superfusates was determined by isolating the amines on columns containing Bio-Rex 70 exchange resin, lyophilizing the eluate, and quantitating the amount in each sample using HPLC and electrochemical detection.

An additional experiment was done in which animals were superfused and injected with parachloroamphetamine (PCA, 7.5 mg/kg, sc) to induce the release of monoamines in the spinal cord. The purpose of this experiment was to verify that the experimental methods were capable of detecting increases in amine release into the spinal cord superfusates.

The average basal release of NE and 5-HT before the injection of morphine was 0.41 +/- 0.06 and 0.42 +/- 0.06 ng/ml per 30 min, respectively. Following the injection of morphine there were no statistically significant changes in either NE or 5-HT release into the superfusates. However, the injection of PCA induced a significant release of both NE and 5-HT. Thus, the basal release of NE and 5-HT was 0.37 +/- 0.07 and 0.35 +/- 0.06 ng/ml per 30 min, respectively. The efflux of NE in the first three 30 min samples following the injection of PCA was 0.59 +/- 0.09, 0.65 +/- 0.09, and 0.78 +/- 0.14 ng/ml. The 5-HT efflux in these samples was 0.65 +/- 0.09, 0.83 +/- 0.11, and 0.96 +/- 0.17 ng/ml.

These data do not support the proposal that the antinociception induced by systemic administration of opiates is mediated by the activation of bulbospinal monoaminergic neurons. (This work was supported by USPHS Grant NS 18636).

41.10 THE EFFECT OF ETHANOL CONSUMPTION ON THE FORMALIN TEST. D. S. Kirtland*, K. R. Gogas, C. A. Barba*, A. A. Ratzin* and J. T. Cannon. Dept. of Psychology, University of Scranton, Scranton, PA 18510.

In contrast to measures of acute pain, behavior on the formalin test has been found to possess some intriguing properties (e.g., Dubuisson & Dennis, 1977). In part, these differences have led to the suggestion that the formalin test may prove to be a valuable model of some human chronic pain states. A modification of the ethanol training procedure designed by Steward and Grupp (1983) was used. Thirty-nine male Sprague-Dawley rats (300-350 g) were food deprived and maintained at 85% of their free feeding weights. Subjects were divided randomly into three groups (n=13): Group 1 received only water throughout the training period; Group 2 was trained to drink ethanol and then deprived of it three days prior to formalin testing; Group 3 was trained to drink and was maintained on ethanol throughout the study. Subjects were housed individually with water freely available in home cages. One hr per day, 6 days per week, the animals were moved to training cages which contained a fluid source (see below) and their daily portion of food. At first, only water was available during the training sessions. Each animal in the two ethanol groups was subsequently offered increasing concentrations of ethanol (2,4 and 8%) using a 3 day stabilization period as the prerequisite for increasing the ethanol strength. The day after 8% ethanol stabilization, a preference test was run over three days for animals in all three groups. The animals in Group 1 (water) were matched to the ethanol groups based on body weight and were preference tested in a yoked fashion. Subjects in Groups 1 and 3 were formalin tested immediately following preference testing while subjects in Group 2 were offered only water for an additional 3 days before formalin was administered. Formalin testing was done using a modification of Dubuisson & Dennis's (1977) methods. All animals were given a .05 ml injection of formalin (2.5%) into the dorsal surface of the left hind paw. Subjects were then placed in clear Plexiglas tubes. Flinches of the injected paw were recorded during alternate 5 min intervals over a 1 hr period.

A repeated measures ANOVA demonstrated that during preference testing, the ethanol-trained groups consumed a significantly greater percentage of ethanol than did untrained animals ($p<.05$). Data from the two control conditions (Groups 1 and 2) were combined for data analysis. A repeated measures analysis of variance showed a significant main effect for group and time ($p<.05$), with the ethanol group exhibiting fewer flinches than the controls. These results suggest that ethanol consumption can attenuate pain reactivity in the formalin test.

41.11 VARIATIONS IN SACCHARIN INTAKE ARE RELATED TO FORMALIN PAIN REACTIVITY AND THE ANALGESIC EFFECTS OF MORPHINE. K. R. Gogas, D. S. Kirtland* and J. T. Cannon. Dept. of Psychology, University of Scranton, Scranton, PA 18510.

Recent evidence suggests that the intake of a sweet solution may trigger the release of endogenous opioids and that chronic intake may result in tolerance to opioid-mediated phenomena. Lieblich et al. (1983) found that selectively bred rats given chronic access to a 3 mM saccharin solution show tolerance to the analgesic effects of morphine and an opioid form of stress analgesia. Two experiments are presented here which examined the influences of saccharin intake on morphine analgesia and formalin pain reactivity in a common laboratory strain of rats. Forty male Sprague-Dawley rats (300-350 g) were housed individually with food and liquid freely available. All testing was performed during the dark phase of a 12 hr light/dark cycle. In each study, 20 rats were assigned randomly to receive 3 mM solutions of either Na saccharin or NaCl as their only fluid source. Behavioral testing began 7 days after introduction of the preceding solutions. In Experiment 1, all animals were given s.c. injections of morphine sulfate (2.5 mg/kg/ml). Prior to morphine injection, 5 tail-flick trials were conducted at 1 min intervals. Forty min after injection, 5 tail-flick latencies were determined again at 1 min intervals. A repeated measures ANOVA revealed no significant effects for solution, time or solution x time ($p > .05$). However, there was a significant correlation ($r = -.77$, $p < .05$) between total solution intake and post-morphine tail-flick latencies for animals drinking saccharin but not NaCl. In Experiment 2, formalin testing was done using a modification of the Duboisson & Dennis (1977) method. All animals were injected with .05 ml formalin (2.5%) into the dorsal surface of the left hind paw. Subjects were then put into Plexiglas tubes and the number of times that the injected paw flinched was recorded during alternate 5 min intervals over a 1 hr period. A repeated measures ANOVA showed no significant effects for solution or solution x time ($p > .05$). There was a significant main effect for time ($p < .05$). A significant correlation ($r = .88$, $p < .05$) was found between the amount of formalin flinches and total solution intake in saccharin but not NaCl animals.

These results demonstrate that high levels of saccharin intake are associated with decreases in the analgesic effectiveness of morphine and with greater pain reactivity on the formalin test. The lack of significant differences between saccharine and NaCl groups suggests that the preceding correlations may not be due to a causal influence of saccharin, but rather to a third variable that contributes to variations in both saccharin intake and performance on these two pain-related measures.

41.12 MORPHINE ATTENUATION OF CONDITIONED AUTOANALGESIA: IMPLICATIONS FOR ENVIRONMENT-SPECIFIC MORPHINE ANALGESIC TOLERANCE. J. Rochford* and J. Stewart, Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8.

It is known that exposure to cues predictive of noxious stimulation produces analgesia by activating endogenous pain suppression mechanisms, so-called conditioned autoanalgesia (CA). In the present study the effect of morphine administration on the development of CA was examined. We predicted that, by reducing the intensity of noxious stimulation through its analgesic action, morphine would attenuate the development of CA.

Autoanalgesia was induced in rats by administering 1 mA shock for 45 sec for 7 days in a distinctive room. Group RM-M/HC-S received 5 mg/kg morphine in the distinctive room 0.5 hr before shock administration and saline in the home cage; group RM-S/HC-M received the reverse drug treatment while group RM-S/HC-S received saline in both environments. To test for CA all animals were injected with saline (following the first, fourth and seventh shock administration days) or with 5 mg/kg morphine (following the last saline test day) in the distinctive room and then given 50°C hot plate tests at 30, 45 and 60 min post-injection. No shock was administered on the test days. It was found that group RM-M/HC-S displayed shorter response latencies on the hot plate than either of the other two groups on the saline test days. Moreover, the analgesic effectiveness of morphine was reduced in group RM-M/HC-S.

Two further studies investigated whether attenuation of CA would occur with different shock parameters, either 2.5 mA for 180 sec or 1 mA for 15 sec. No significant differences in response latencies on the hot plate were found between groups RM-M/HC-S and RM-S/HC-M at the higher shock intensity. The analgesic potency of morphine was significantly reduced in group RM-M/HC-S at the 1 mA for 15 sec shock intensity, although no significant differences from group RM-S/HC-M were obtained when tests for CA were conducted under saline.

These results provide some support for the possibility that environment-specific morphine analgesic tolerance is attributable, at least in part, to morphine attenuation of CA. That is, the degree of tolerance to morphine may be overestimated when animals are tested in an environment in which they have previously been given noxious stimulation under morphine, provided that the intensity of noxious stimulation is not excessively severe. Furthermore animals exposed to noxious stimulation under morphine will appear hyperalgesic when subsequently given nociceptive tests under saline.

NEUROTRANSMITTERS AND RECEPTORS IN HUMAN DISEASE

42.1 MECHANISMS OF CO_2-INDUCED ANXIETY. S.W.Woods*, D.S.Charney*, G.R.Heninger, W.K.Goodman*, J.Loke*, D.E.Redmond, Jr. (SPON: E. Nestler). Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT., 06508.

Breathing air supplemented with CO_2 stimulates anxiety in humans. In order to elucidate the mechanism of the anxiogenic effect of CO_2, experiments were conducted in 5 chair-adapted male rhesus monkeys, 23 healthy human subjects, and 14 medication-free patients with agoraphobia and panic attacks. METHOD: Five days/week for 6 weeks, the monkeys were chaired and breathed air vented through a transparent hood at 25 l/min for 3.5 hrs. Four test days were scheduled in random order during weeks 3-6 when air, 5%, 7.5%, or 10% CO_2 were given for 3 hrs. after a 30 min. air baseline. All gas mixtures contained 21% O_2. Plasma was obtained for determination of the norepinephrine (NE) metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) 30 and 5 minutes before and 30, 60, 90, 120, and 180 minutes after substitution of test gas mixture. After pulmonary function testing, human subjects underwent testing of ventilatory response to CO_2 as a reflection of central medullary chemoreceptor sensitivity through a hyperoxic hypercapnic rebreathing technique (Read, D.J.C., Aust.Ann.Med., 16:20,1967). Inspired CO_2 concentration rose from 5% to 7-10%, depending on rebreathing duration. A psychiatrist rated all subjects on 31 anxiety symptoms of the Clinician-Rated Anxiety Scale 30 minutes before and immediately after rebreathing. In 8 patients, the rebreathing test was repeated 3 months later following successful treatment with alprazolam in daily dose of 3.1±0.6 mg. (Mean±SEM). RESULTS: In the monkeys on 7.5% and 10% but not on 5% CO_2 days, plasma free MHPG increases over baseline were significant ($p<.05$) at 60, 90, 120, and 180 minutes. Ventilatory response to CO_2 was similar in patients and controls (1.58±0.16 vs. 1.58±0.14 l/min/mmHg, N.S.). Anxiety ratings increased markedly during rebreathing both in patients and controls; anxiety increases were significantly greater in patients ($p<.01$) in comparison to healthy subjects matched for age, sex, and rebreathing duration. Alprazolam treatment robustly attenuated anxiety increases during rebreathing ($p=.0001$). COMMENT: The increases in plasma MHPG in monkeys are consistent with prior preclinical studies which suggest that CO_2 stimulates NE turnover. Differences in anxiogenic sensitivity to CO_2 between patients and controls cannot be explained by variability in chemoreceptor sensitivity but may be due to differences in the regulation of NE or other neuronal systems. Since alprazolam blocks both naturally occurring and CO_2 induced anxiety, CO_2 exposure may provide a useful model to study the pathophysiology of anxiety disorders. Supported by MH25642, MH30924, and MH38007.

42.2 SYNERGISTIC EFFECTS OF ALPHA-2 ADRENERGIC AND OPIATE RECEPTOR BLOCKADE ON ANXIETY AND SEXUAL FUNCTION IN HEALTHY SUBJECTS D.S.Charney*, G.R.Heninger, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT., 06508. (SPON: E.Giller).

Preclinical and clinical studies indicate that brain noradrenergic and opiate neuronal systems have important effects on the regulation of anxiety and sexual function. In addition, there is substantial evidence from neuroanatomical, neurophysiological, biochemical, and behavioral investigations indicating that there are functional interactions between these two neuronal systems. In the present investigation the functional interaction between opiate and noradrenergic neurons was evaluated by determination of the effects of yohimbine and naloxone, alone and in combination, in healthy human subjects. METHODS: The subjects were five males (38±13 years) and four females (37±6 years). They participated in four separate test days: placebo; yohimbine 20 mg p.o.; naloxone 1 mg/kg I.V.; yohimbine and naloxone 15 minutes later. Blood was sampled for plasma cortisol and MHPG and behavioral ratings (visual analogue mood scales, somatic symptom scales) and vital signs were obtained at baseline and at intervals thereafter. Changes in sexual function was assessed by questions from research nurses. RESULTS: Yohimbine and naloxone significantly increased plasma MHPG and cortisol, respectively. These drugs, when given alone, had modest effects on behavior, sexual function, somatic symptoms, and blood pressure, in comparison to placebo. In contrast, the yohimbine-naloxone combination produced large increases in cortisol and patient rated anxiety, nervousness, nausea, palpitations, tremors, and hot and cold flashes, which were significantly greater than the sum of effects produced by the drugs when given separately. All 5 of the male subjects reported full penile erections shortly following the yohimbine-naloxone administration which lasted 60 minutes. There was no associated increase in libido. IMPLICATIONS: The robust synergism of the yohimbine-naloxone combination in producing anxiety, demonstrates an interaction between these two systems, that has important clinical implications for the understanding and treatment of human stress reactions and anxiety disorders. During stressful or anxiety-provoking situations endogenous opioids are released which could have modulatory or inhibitory actions on the increased noradrenergic activity associated with anxiety. New treatments for stress and anxiety disorders could be developed by improving opiate modulation of noradrenergic systems. The ability of the yohimbine-naloxone combination to produce penile erections suggests that a new treatment for impotence could be developed if the negative side effects of the combination can be reduced. Supported by MH36229, MH25642, MH30924 and MH38007.

42.3 PLATELET MONOAMINE OXIDASE ACTIVITY IN DEPRESSION AND ANXIETY. A. Khan, E. Lee, T. Hyde, S. Dager, D. Avery, D. Dunner, Dept. of Psychiatry, Harborview Medical Ctr., University of Washington, Seattle, WA.

Platelet monoamine oxidase (MAO) activity has been reported to be abnormal in patients with major depression and anxiety disorders. It has been suspected that MAO activity may be related to level of anxiety. We report that MAO activity in a large group of patients (n=197) and explore the relationship between MAO activity and degree of anxiety as well as effect of benzodiazepine treatment.

MAO activity was measured in psychotropic free, medically stable adults. The assay was based on benzylamine substrate and has excellent reliability. 56 subjects (48% Fem.) had major depression, 25 (32% Fem.) had generalized anxiety disorder, 53 (66% Fem.) had panic disorder, 29 (65% Fem.) had agoraphobia, and 34 (47% Fem.) had no psychiatric illness. MAO activity was not related to the age of patients. There was no difference with regard to mean MAO activity for the 3 groups of anxious patients. 2-way ANOVA showed that sex (P 0.0001) and diagnosis (P 0.02) contributed to the distribution of MAO activity. In females mean MAO activity was as follows: major depression (10.99) anxiety disorders (12.97) controls (13.41). In males, mean MAO activity was as follows: anxiety disorder (8.68) major depression (10.11) controls (11.85).

In a subset of 40 anxious patients no correlation was found between MAO activity and Hamilton Anxiety Scale ratings prior to treatment. Furthermore, MAO activity was not altered during 2 weeks of treatment with placebo or benodiazepines.

The implications of these findings are discussed.

42.4 PLASMA GAMMA-AMINOBUTYRIC ACID IN PSYCHIATRIC ILLNESS. F. Petty. Psychiatry Service, V.A. Medical Center and University of Texas, Dallas, TX

Abnormalaties in Gamma-aminobutyric Acid (GABA) metabolism have been implicated in several neurological and psychiatric illnesses. Also, psychotropic agents useful in treating psychiatric conditions such as anxiety and depression are thought to exert at least some of their action through GABA mechanisms. Although most GABA is found in CNS, small amounts exist in the periphery and GABA levels can be accurately measured in blood plasma. We have obtained plasma GABA levels in over 300 patients with psychiatric illness and in normal controls. Plasma GABA levels in patients with unipolar depression are significantly lower than control values (35 ± 12 vs. 58 ± 12 ng/ml), this lowering of plasma GABA probably reflects the familial (and therefore genetic) nature of this type of depression. By comparison, patients with secondary depression and with bipolar depression have plasma levels of GABA that are in the control range, although their symptoms in cross-section resemble those of unipolar depressives. Patients with bipolar affective disorder who had recovered had elevated levels of GABA as did bipolar patients in the manic phase of their illness, suggesting that elevated GABA levels may represent a trait marker for bipolar affective disorder. Schizophrenics demonstrated a very large scatter in their GABA levels, perhaps reflecting the heterogenous nature of this disease. Finally, patients with alcoholism had GABA levels that were even lower than those of unipolar depressives, corroborating the well established clinical relation between these syndromes. Effects of diet, activity, and menstrual cycle on plasma GABA will be presented. Plasma GABA may develop into a useful clinical and research tool for further studies on biological mechanisms in affective disorder.

*Supported by the Veterans Administration.

42.5 FURTHER STUDIES OF CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRF-LI) IN CSF OF PATIENTS WITH AFFECTIVE DISORDERS. G.Bissette, F.Spielman, M.Stanley, C.Banki*, M.Fink*, B.Stanley*, L.Traskman-Bendoz*, R.N.Golden*, M.Arato*, and C.B.Nemeroff. Laboratory of Psychoneuroendocrinology, Duke Univ. Med. Center, Durham, N.C. 27710

We have previously reported increased concentrations of CRF-LI in the CSF of patients with diagnoses of major depression (Science, 226:1342-1344, 1984). Here we report CSF concentrations of CRF-LI in several groups of patients with affective disorders from five different institutions. Moreover, patients with other psychiatric diagnoses and controls receiving diagnostic lumbar puncture for neurologic or urologic examination were studied. The radioimmunoassay for CRF-LI uses an antiserum directed toward the 33-41 amino acid region of CRF and iodinated Tyr-CRF as the radioactive trace. Maximum sensitivity is 0.625 pg/tube and the IC_{50} is 30 pg. Duplicate 400 µl aliquots of unextracted CSF samples were lyophilized and equal artificial CSF aliquots were included in the standard curve tubes. Two groups of younger (21-34 yrs., n=21) and older (54-86 yrs., n=12) controls from N. C. Memorial Hospital, Chapel Hill, N. C., had CRF-LI concentrations of 53.0 ± 2.6 pg/ml and 41.3 ± 2.9 pg/ml, respectively. A group of patients (from S.U.N.Y.-Stonybrook) with an RDC diagnosis of primary major depression (n=13, 28-72 yrs.), were also studied as well as two schizophrenic and one bipolar depressed patient before and after ECT. The patients with major depression had CRF-LI concentration of 80.0 ± 6.6 pg/ml before and 61.9 ± 2.4 pg/ml after ECT. The schizophrenic patients had pre- and post-ECT concentrations of CRF-LI that were 55.6 ± 6.8 and 50.7 ± 0.6 pg/ml, respectively. There was no correlation between post-dexamethasone plasma cortisol levels and CRF-LI either before or after ECT for any group. A group of Hungarian psychiatric patients with DSM-III diagnosis of major depression (n=14, 25-69 yrs.) were also studied. Their mean concentration of CRF-LI was 101.0 ± 15.9, and again, no correlation was seen between CRF-LI and post-DST cortisol concentrations. Another group of patients from the Lafayette Clinic, Detroit, Michigan with DSM-III diagnoses of unipolar depression (n=13), bipolar depression (n=19), personality disorder (n=22) or schizophrenia (n=20) were also included. Their mean concentrations of CRF-LI were 47.6 ± 3.7, 55.0 ± 5.1, 51.2 ± 2.9 and 52.0 ± 3.6, respectively. Finally, a group of patients from the NIMH (n=14) with DSM-III diagnoses of major depression were found to have CRF-LI concentrations of 44.7 ± 1.7 pg/ml. These data reveal that certain groups of depressed patients exhibit elevated CSF concentrations of CRF-LI. (Sponsored by NIMH MH-39415)

42.6 BIOGENIC AMINE METABOLITES IN CSF OF SCHIZOPHRENICS BEFORE AND AFTER TREATMENT WITH HALOPERIDOL. C.L. Bowden, K.I. McIntyre*, and M.A. Javors*. Departments of Psychiatry and Pharmacology, The University of Texas Health Science Center, San Antonio, Texas 78284.

Our purpose was to study the effect of the neuroleptic drug haloperidol on the concentrations of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), 5-hydroxyindoleacetic acid (5HIAA), and homovanillic acid (HVA) in cerebrospinal fluid (CSF) of schizophrenic subjects. Thirteen patients were diagnosed with the SADS-RDC criteria for schizophrenic disorder and, in addition, met criteria indicative of greater chronicity of illness and poor response to prior neuroleptic treatment. Once diagnosed, the patients were hospitalized and underwent a drug washout period of at least seven days. At the end of the washout period (day zero), the subjects began a flexible dosage regimen with 20 mg haloperidol per day. During the next 14 days, the dosage was adjusted as clinically indicated, then the dosage was maintained during the next and last 14 days of treatment. Blood and mixed CSF samples were drawn at day zero prior to haloperidol and after 28 days of haloperidol. Mean (± SD) CSF concentrations of MHPG, 5HIAA, and HVA for 13 subjects prior to haloperidol were 7.8 (1.3), 13.1 (4.0), and 27.0 (10.0) ng/ml; after 28 days of haloperidol, they were 6.0 (1.2), 13.5 (4.1), and 34.2 (11.6) ng/ml. The mean MHPG concentration in CSF decreased significantly during treatment ($p < .01$) and mean HVA increased significantly ($p < .001$). The CSF concentration of 5HIAA in these subjects did not change with haloperidol treatment. The increase in HVA concentration from day zero to day 28 correlated significantly with increased age of the subjects ($p < .001$). Furthermore, higher HVA concentrations in CSF at day zero correlated with greater severity of illness by two measures, the Global Assessment Scale ($p < .01$) and the SADS-psychoticism scale ($p < .01$). Lithium was taken concurrently with haloperidol treatment by six subjects. However, there were no differences in the significant relationships mentioned above between the lithium and non-lithium groups. These results indicate that the concentration of HVA in CSF is an index for severity of psychotic illness, and that change in HVA concentration in CSF may be an important indicator of dopaminergic responsiveness. (Supported by a grant from the Kleberg Foundation, San Antonio, Texas).

42.7 REDUCTIONS IN NICOTINIC CHOLINERGIC RECEPTORS MEASURED USING [^{3}H] ACETYLCHOLINE IN ALZHEIMER'S DISEASE. P. J. Whitehouse, A. M. Martino*†, P. G. Antuono*, J. T. Coyle, D. L. Price, and K. J. Kellar†. The Johns Hopkins University School of Medicine, Baltimore, MD 21205; †Georgetown University School of Medicine and Dentistry, Washington, DC 20007

In Alzheimer's disease (AD), presynaptic cholinergic markers, such as choline acetyltransferase (ChAT) activity, are consistently reduced due to dysfunction and death of neurons in the basal forebrain cholinergic system. However, the nature of alterations in cholinergic receptors in this disease is, at present, unclear. Evidence has been presented for increases, decreases, and no changes in receptor densities. In the present investigation, we used [^{3}H] acetylcholine (ACh) to measure both muscarinic cholinergic receptors and nicotinic receptors in left and right frontal, temporal, and occipital cortices from 13 patients with AD (average age 75 years) and 11 intellectually intact matched controls (average age 62 years). Nicotinic receptors were measured using 6 nM [^{3}H] ACh in the presence of 1.5 μM atropine to displace [^{3}H] ACh from the muscarinic cholinergic receptor. Muscarinic cholinergic receptors were assessed by two approaches: incubation with 15 nM [^{3}H] ACh in the presence of 1.5 μM cytisin to displace [^{3}H] ACh from nicotinic receptors and incubation with [^{3}H] quinuclidinyl benzilate (QNB). Nonspecific binding was measured in the presence of 100 μm carbachol. Statistically significant differences in muscarinic cholinergic receptors were not detected as measured by either [^{3}H] ACh or [^{3}H] QNB, although a trend towards increased concentration of muscarinic cholinergic receptors was noted. In AD, a statistically significant (50%) reduction of nicotinic receptors occurred primarily in the left hemisphere which paralleled reductions of ChAT activity in magnitude and distribution. This study demonstrates relationships between changes in neurons of the basal forebrain cholinergic system and alterations in nicotinic cholinergic receptors in forebrain targets.

42.8 AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC AND BETA ADRENERGIC RECEPTORS IN HUMAN SKIN. H.I.Ryer[1]*, B.Nock[2], M.Serby[1]*, S.Katz[3]* and B.S.McEwen[2]. (1)Dept. of Psychiatry, V.A. Medical Center, New York, N.Y. 10010 (2)The Rockefeller University, New York, N.Y. 10021 (3)Dept. of Dermatology, Albert Einstein College of Med., Bronx, N.Y. 10466.

Central cholinergic and adrenergic mechanisms are thought to be involved in a number of neuropsychiatric disorders, e.g. Alzheimer's disease and depression. Peripheral markers for CNS deficits would be of use in diagnosing these disorders. Recent work with skin has shown promise. For example, elderly women with senile dementia of the Alzheimer's type (SDAT) show less sweat gland activity to intradermal injections of choline and carbachol than age matched controls (Eur.J.Clin.Pharm.24:1983). Also, skin fibroblasts from patients with SDAT have been reported to be hyperresponsive to beta adrenergic stimulation (Am.J.Hum.Genet.31:1979). We have used autopsied skin samples from human palm and sole to develop in vitro semi-quantitative autoradiographic techniques for analysis of muscarinic cholinergic and beta adrenergic receptors in human biopsy samples.

Skin was cut into 32-90um thick slices and thaw-mounted onto subbed slides. Tissue was incubated at 25°C for one hr with either (1) 1nM ^{3}H-scopolamine (muscarinic receptors) ± 10uM atropine or (2) 2nM ^{3}H-dihydroalprenolol(DHA; beta receptors) ± 20uM propranolol. The tissue was then exposed to tritium sensitive film for 8-12 weeks. Receptor binding was quantified using a computer-assisted densitometer and tissue was stained with cresyl violet in order to visualize sweat glands.

Specific binding of both scopolamine and DHA was localized to areas of the skin containing eccrine sweat gland tissue. The presence of an unlabeled competitor completely abolished this sweat gland localized binding.

In vitro autoradiography appears to be a useful method for measurement of neurotransmitter receptors in human skin. Biopsy samples from normal and SDAT subjects are presently being assessed.

42.9 PHARMACOLOGICAL TREATMENT OF A FOCUSED BRAIN STEM DISORDER IN MAN. K.A. Bonnet, Millnauser Laboratories, New York University School of Medicine, New York, N.Y. 10016.

A clinical condition consisted of episodic vomiting crisis occurring every 13 days from birth and having a duration of approximately three to five days each time. The individual also exhibited signs of retarded motor development and of facial hemiparesis. The individual had a single seizure at four months of age and was subsequently maintained for three years on phenobarbital and Dilantin. Neurological studies showed relatively little and treatment recommendations were not forthcoming from several major centers. The patient was examined by computerized EEG, plasma catecholamine biochemistry, fundoscope examination, and sinus-atrialarhythmia determination, at points during a well period and at points during a presentation of crisis. Through systematic overlay of the results from each of these investigations it was possible to determine that the primary disorder lay in the hypersensitivity of the afferent sympathetic branch of the vagus and in the brain stem nuclei. Systematic investigation of sphincter regulation confirmed hyperactivity of the pyloric sphincter which was activated at CCK-receptors effecting premature and prolonged closure. The gastric filling that resulted stimulated hypersensitive gastro-esophageal junction receptors triggering the onset of emesis. Systematic investigation of the nuclei in the brain stem medicating this response permitted a selection of a compound for treatment. The receptors for this compound were four-fold more sensitive than the same receptors for this compound in other areas of the brain. Use of the compound at 8 micrograms per day permitted discontinuation of Dilantin, phenobarbital, and released the individual to exhibit a neurological, skeletal and muscular growth spurt that began to approach age-appropriate levels.

Crisis prevention or crisis arrest by this strategy has permitted cognitive growth that was unexpected and has permitted an increase in vocabularly, for example of from 2 to 500 words in a period of only a few months. This type of integrated study and strategy development can be effective in the detection, localization, and systematic focal treatment of brain stem disorders. Supported by Courtney Block Fund for Brain Research.

43.1 ADENYLATE CYCLASE ACTIVITY IN AN ANIMAL MODEL OF DEPRESSION, D.J. Anderson*, J.O. Johnson, and F.A. Henn. Dept. of Psychiatry and Behavioral Science, SUNY/Stony Brook, Stony Brook, NY 11794-8101.

Previous studies have shown that rats exposed to inescapable intermittent foot shock develop behavioral deficits which can be reversed with antidepressant drugs and ECS (Sherman and Petty, 1980). Concurrent with the behavioral deficits is an upregulation of beta-adrenergic receptors in the hippocampus of these rats (Johnson and Henn, 1983). One of the major theories of antidepressant action proposes that all antidepressants cause deamplification of noradrenergic receptor-adenylate cyclase systems (Vetulani and Sulser, 1975). The present study investigates the coupling of upregulated beta-adrenergic receptors with a possible supersensitivity of adenylate cyclase in an animal model of depression.

Male rats (200-250g) were subjected to randomized, uncontrollable foot shock (0.8 mA x 40min). Twenty-four hours later the rats were tested for escape responding in a bar-press paradigm. Approximately one-third of the animals were response deficient (RD, 10-15 failures) and one-third were nondeficient (ND, 0-4 failures). A group of controls was tested without prior shock (C, 0-5 failures). A fourth group of naive controls (N) received no shock.

Two to three hours after testing the hippocampus (HPC), anterior neocortex, and hypothalamus were dissected from individual animals. Each region was sliced 0.26x0.26mm and placed in oxygenated Krebs-Ringer buffer. Adenylate cyclase responsiveness to norepinephrine (NE) was assayed according to Baudry et al. (1976). Basal and stimulated cyclic AMP levels were measured using the protein binding assay of Gilman (1970) in kit form as provided by Amersham, Inc.

Percent maximal response over basal levels were measured in hippocampal slices of the above groups (N's in parentheses):

	RD (5)	ND (5)	C (5)	N (5)
Response %	234±74*	146±36	145±16	119±26

Adenylate cyclase activity in hippocampal slices from response deficient rats was found to be significantly different from the other groups when analysed by a two-tailed Student's t-test and by a random block design ANOVA.

These preliminary data coupled with previous findings of elevated hippocampal beta-adrenergic receptor density in response deficient rats indicate that an amplification of the noradrenergic receptor-adenylate cyclase system may be linked to the behavioral changes seen in this animal model of depression.

43.2 MODULATORY ROLE OF 5-HT ON BETA-RECEPTORS IN AN ANIMAL MODEL OF DEPRESSION, E. Edwards, and F.A. Henn. Dept. of Psychiatry & Behavioral Science, SUNY/Stony Brook, Stony Brook, NY 11794

Rats exposed to forty minutes of intermittent inescapable shock develop a transient learning deficit when later tested on a shock escape paradigm. This behavioral deficit has been shown to be correlated with an up regulation of hippocampal beta-adrenergic receptors (Johnson and Henn, 1983). Serotonergic mechanisms have also been implicated in the medication of the effects of inescapable shock on subsequent behavior (Edwards et al, 1984). The present study examined a possible modulatory role for 5-HT on the regulation of beta-adrenergic receptors after inescapable shock.

Rats were pre-treated with the potent serotonin depletor, p-chlorophenylalanine (PCPA, 300mg/kg i.p). Regardless of inescapable shock exposure, PCPA-treated rats demonstrated significantly less failures than saline controls in a shock escape test (3.8 ± 1.7 failures PCPA-rats vs 11.3 ± 1.5 failures, deficient rats). Concomitant with the blockade of the behavioral deficit, PCPA treatment also resulted in a significant decrease of beta-adrenergic receptors. These results lead to the conclusion that the beta-adrenergic receptor can be controlled by serotonergic mechanisms as suggested by Janowsky et al (1982).

Chronic administration of a specific serotonin uptake inhibitor, Fluvoxamine (12mg/kg i.p x 5 days) resulted in a reversal of the shock induced deficit and a down regulation of hippocampal beta receptors. This is consistent with our finding that most antidepressant treatments down regulate hippocampal beta receptors, even those compounds such as mianserin and fluvoxamine which do not appear to act on beta receptors directly. These serotonergic compounds seem to exert their action by chronic down regulation of 5-HT input to the hippocampus. This may be a multisynaptic pathway and need not involve direct 5-HT action in hippocampus.

All our results suggest that a normal level of 5-HT activity is necessary to maintain beta receptor levels in the hippocampus and a chronic decrease in 5-HT levels will cause down regulation of beta receptors. An understanding of this circuit provides a rational explanation for the mechanism of action of all known classes of antidepressants.

43.3 SELECTIVITY OF THE NOVEL ANXIOLYTICS BUSPIRONE AND TVX Q 7821 FOR 5-HYDROXYTRYPTAMINE$_{1A}$ RECEPTORS. B. C. Hiner*, P. J. Ison* and S. J. Peroutka. (Sponsor: M. Weinrich) Department of Neurology, Stanford University Medical Center, Stanford, California 94305

The exact mechanism of action of the novel anxiolytics buspirone and TVX Q 7821 remains unknown. Unlike diazepam, buspirone has no effect on the benzodiazepine/GABA receptor complex as studied by radioligand techniques. Buspirone does display weak neuroleptic-like activity although its effects on central dopamine systems is not believed to be related to its anxiolytic effects. 5-Hydroxytryptamine (5-HT) receptors have been proposed as the target site for both buspirone and TVX Q 7821 despite the fact that buspirone was initially reported to be inactive at 5-HT$_1$ receptors. Recently, the availability of selective radioligands has allowed 5-HT$_1$ binding sites to be differentiated into 5-HT$_{1A}$ and 5-HT$_{1B}$ subtypes. As a result, the present study analyzed the interactions of buspirone and TVX Q 7821 with 5-HT$_{1A}$ and 5-HT$_{1B}$ sites as well as with a series of eight other receptor binding sites in bovine brain membranes.

Buspirone (IC_{50} = 24 ± 4 nM) and TVX Q 7821 (IC_{50} = 9.5 ± 2 nM) are most potent at 5-HT$_{1A}$ binding sites labeled by ^{3}H-DPAT. Buspirone is 16-fold weaker at dopamine (D_2) sites labeled by ^{3}H-spiperone in striatal membranes (IC_{50} = 380 ± 70 nM) and 58-fold less potent at alpha-adrenergic$_1$ sites labeled by ^{3}H-WB 4101 (IC_{50} = 1,400 ± 500 nM). By contrast, TVX Q 7821 is 6-fold less potent (IC_{50} = 58 ± 10 nM) at alpha-adrenergic$_1$ sites and more than two orders of magnitude less potent at dopamine (D_2) sites (IC_{50} = 3,000 ± 300 nM). Both buspirone and TVX Q 7821 display moderate affinity (i.e., IC_{50} values between 1,000 - 10,000 nM) at 5-HT$_2$ sites labeled by ^{3}H-spiperone in cortical homogenates, histamine (H_1) sites labeled by ^{3}H-pyrilamine and alpha-adrenergic$_2$ sites labeled by ^{3}H-yohimbine. Both drugs are also similar in that they are essentially inactive (i.e., IC_{50} values greater than 10,000 nM) at 5-HT$_{1B}$ sites in the corpus striatum labeled by ^{3}H-5-HT, calcium channel antagonist sites labeled by ^{3}H-nitrendipine, muscarinic cholinergic sites labeled by ^{3}H-QNB and benzodiazepine binding sites labeled by ^{3}H-flunitrazepam.

The major finding of the present study is that buspirone and TVX Q 7821 are potent and selective inhibitors of 5-HT$_{1A}$ binding sites in brain membranes. These two novel anxiolytics share both structural and pharmacological properties. The drugs display moderate affinity for 5-HT$_2$ sites but are essentially inactive at 5-HT$_{1B}$ binding sites. In addition, buspirone and TVX Q 7821 are inactive at benzodiazepine binding sites. These findings suggest that the anxiolytic effects of buspirone and TVX Q 7821 may be mediated by an interaction with a specific subpopulation of 5-HT receptors in the central nervous system.

43.4 MODULATION OF SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS PRODUCED BY NOVEL ANXIOLYTICS (BUSPIRONE, TVX-Q 7821 & DPAT). M.D. Mauk, P.J. Peroutka & J.D. Kocsis. Deptartment of Neurology, Stanford Medical Center and VA Medical Center, Palo Alto, CA 94304.

Unlike diazepam, the novel anxiolytics buspirone and TVX-Q 7821 possess very little affinity for benzodiazapine/GABA binding sites. However, these compounds have recently been shown to display potent and selective affinity for 5-HT$_{1A}$ binding sites (Hiner, et al, this volume). Beyond this, little is known about the CNS actions of these compounds. Two observations; 1)that serotonin (5-HT) affects the amplitude of population spikes in hippocampal slice and 2)that the hippocampus is a rich source of 5-HT$_{1A}$ binding sites, suggested to us that further characterization of the CNS actions of buspirone and TVX-Q 7821 could be accomplished using in vitro hippocampal slice.

Hippocampal slices (400u) were prepared with a vibratome using tissue derived from female Wistar rats. Slices were placed in a submersion chamber and were superfused with a continuous flow of modified Krebs and maintained at 33-34°. Field potentials elicited by monopolar stimulation of Schaffer collaterals were recorded in the pyramidal layer of CA-1. When the field potentials had been stable for at least 20 min., the slices were superfused with Krebs containing various concentrations of the compounds under study. Dose-response curves were obtained by applying increasing concentrations of the compounds every 30 min. until complete abolition of the population spike was observed. Reversal of the effect was then assessed by washing the slices in normal Krebs solution.

Application of buspirone, TVX-Q 7821 and DPAT as well as diazepam produced a dose dependent and reversible reduction of the population spike. Effective concentrations were 25-100 uM. In one experiment, K^+-sensitive electrodes were used to monitor the field potential and extracellular K^+ concentration. Application of buspirone (100uM) produced a complete abolition of the population spike and was accompanied by an increase in extracellular K^+.

Previous experiments have shown that an increase in K^+ conductance and a decrease in membrane resistance are responsible for the effects of 5-HT on population spike amplitude. The present data indicate that the action of buspirone is similar to that of 5-HT suggesting that buspirone, TVX-Q 7821 and DPAT are 5-HT$_{1A}$ agonists in the hippocampus. Diazepam produces a similar effect on the amplitude of the population spike. However, on the basis of previous binding data and physiological characterization it seems likely that diazepam and buspirone affect the hippocampus via different ionic mechanisms. The similarity of action suggests, but our data do not demand, that modulation of hippocampal synaptic transmission may mediate the anxiolytic actions of diazepam, busbirone and TVX-Q.

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43.5 ANGIOTENSIN RECEPTOR BINDING IN DEVELOPING SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. B. Salaymeh* and J.A. Weyhenmeyer, College of Medicine, University of Illinois, Rockford, IL 61107, and Department of Anatomical Sciences, University of Illinois, Urbana, IL 61801

Although the peripheral renin-angiotensin system is considered to play an important role in blood pressure regulation and extracellular fluid volume homostasis, recent physiological and pharmacological data have indicated that the biologically active end product of the RAS, ANG II, can exert a profound neuromodulatory influence in the CNS. These central effects include an increase in arterial blood pressure, an increase in fluid intake, and a release of pituitary hormones. Although the role of ANG II in the development of hypertension remains as yet unresolved, Okuno et al. (Hypertens. 5:653, 1983) have demonstrated that chronic blockade of the angiotensin system prevents the development of hypertension in the young spontaneously hypertensive (SH) rat. The purpose of this study was to investigate the binding characteristics of ANG II receptors in the developing SH rat and its normotensive control, the Wistar Kyoto (WKY) rat.

Age-matched 4-, 8-, 12- and 16-week SH and WKY rats were sacrificed by decapitation while under CO_2 anesthesia. Brains were rapidly removed at 4°C and the hypothalamus-thalamus-septum-midbrain region was microdissected. Tissues were homogenized in 50 mM Tris containing 150 mM NaCl and 5 mM EDTA and centrifuged at 50,000 g for 30 min. The resulting pellets were suspended in Tris containing 5 mM dithiothreitol and 0.2% BSA. The homogenates were subsequently incubated in the presence of 0.003 to 1.0 nM ^{125}I ANG II in the presence (blocked) or absence (unblocked) of 4 μM ANG II for 30 min at 25°C and filtered under vacuum. The filters were washed with cold Tris and counted in a gamma counter with 75% effiency.

Our results demonstrate that although ANG II receptor levels decrease in both SH and WKY rats during development, there are significant differences in the ANG II binding capacities between the two strains. At 4 weeks of age, the ANG II receptor levels in the SH rat are significantly higher than in its normotensive control (WKY). At 8, 12 and 16 weeks of age, no significant differences were observed in the receptor levels of SH and WKY rat. These data indicate that ANG II receptor down regulation is greater in the developing SH rat than its normotensive (WKY) control, and suggest that the ANG II receptor may play a role in the pathogenesis of hypertension.

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43.6 ANGIOTENSIN II LEVELS IN MICRODISSECTED BRAIN AND BRAINSTEM NUCLEI FROM SPONTANEOUSLY HYPERTENSIVE AND WISTAR KYOTO RATS. J. M. Meyer, D. L. Felten, and J. A. Weyhenmeyer, Neural and Behavioral Biology Program and College of Medicine, University of Illinois, Urbana, IL, 61801; Department of Anatomy, University of Rochester School of Medicine, Rochester, NY, 14642.

Angiotensin II (ANG II), a naturally occuring brain peptide, has been implicated in the CNS regulation of blood pressure, fluid intake, sodium intake and hormone release. Hoffman et al. (Am. J. Physiol., 232:H4426, 1977) demonstrated that the intraventricular administration of ANG II results in a more significant pressor response in the spontaneously hypertensive (SH) rat than its normotensive control, the Wistar Kyoto (WKY) rat, suggesting an increased ANG II sensitivity in the SH rat. The purpose of this study was to quantitate ANG II levels in microdissected nuclei of SH and WKY rat brain and brainstem, including the paraventricular nucleus of the hypothalamus (PVH), A1 region, nucleus of the solitary tract (NTS), and locus coeruleus (LC). Each of these nuclei has previously been shown to contain ANG II-like immunoreactivity (Weyhenmeyer and Phillips, Hypertens. 4:514, 1982) and has been implicated in cardiovascular regulation.

Twelve-week old male SH and WKY rats, recorded for blood pressure and weight, were anesthetized and perfused transcardially with ice cold phosphate buffered saline. Nuclei were microdissected over dry ice, rapidly frozen and stored at -90°C. Tissues were homogenized in 0.1N HCl, boiled for 5 min and centrifuged at 30,000 g for 30 min at 4°C. The supernatant was collected and the pellet re-extracted with 0.1N HCl. Supernatants were pooled and purified on Sep Pak cartridges according to the method of Raizada et al. (Am. J. Physiol., 247:C115,1984). Samples were analyzed by high performance liquid chromatography using a reverse phase C18 column and by a competitive inhibition radioimmunoassay using a specific ANG II antibody.

Preliminary evidence indicates that both SH and WKY rats contain picomolar levels of ANG II in the microdissected nuclei under study. However, significantly higher levels of ANG II were found in the PVH, NTS, and LC of the SH rat as compared to its normotensive control. These data suggest neurochemical differences between the genetically predisposed hypertensive rat and its normotensive counterpart and support previous findings that suggest a down regulation of ANG II receptor levels in adult SH rats.

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43.7 EVIDENCE OF A ROLE FOR CATECHOLAMINES IN THE EVOLUTION OF INFARCTION IN ISCHEMIC HIPPOCAMPUS J. Weinberger, J. Nieves - Rosa. Dept Neurology, Mt. Sinai Sch of Med, N.Y., N.Y. 10029

The striatum and hippocampus are brain regions with a selective vulnerability to ischemic infarction. Previous studies in our laboratory have demonstrated an increased sensitivity of catecholamine nerve terminals to ischemic damage and have implicated catecholamines in the evolution of infarction in ischemic striatum. In the present study, the hippocampi of Mongolian gerbils with stroke due to unilateral carotid artery ligation were examined by the Falck-Hillarp technique to determine if there was a relationship between vacuolization of the neuropil and the presence of catecholamine derived fluorescence as seen in striatum. Brains were removed from stroke animals at 2, 4, 7, 12 and 16 hours after carotid artery ligation, freeze dried at -35°C for 48 hours and incubated with paraformaldehyde to form fluorescent isoquinolines. Specimens were examined with a Leitz transmission fluorescence microscope employing BG12 excitation filters and a K510 barrier filter. Morphologic damage to the neuropil was assessed by the Nissl technique, employing a cresyl violet stain.

At 2 (n=3) and 4 (n=3) hours after stroke, no vacuolization was seen in the ischemic hippocampi. Fluorescence of the nerve terminals was similar in the ischemic and control hippocampi. At 7 hours (n=3) and 12 hours (n=3), severe vacuolization of the neuropil was present adjacent to the granular cell layer of the CA_1 region and in the pyramidal cell layer of the dentate gyrus. Large concretions of fluorescence were seen in these vacuoles, probably representing swollen catecholamine nerve terminals. This fluorescence could be removed by washing the sections with a 0.1% solution of sodium borohydrate in 95% isopropanol and could be restored by reincubation with paraformaldehyde, confirming that the fluorescent material was derived from catecholamine. By 16 hours (n=3), fluorescence had faded, and there was no progression of vacuolization of the neuropil compared to 7 hours after stroke.

To further explore whether there was relationship of catecholamines to ischemic vacuolization, catecholamines were depleted by pretreating gerbils with a-methyl-p-tyrosine (AMPT) 400mg/kg i.p. 6 hours prior to carotid ligation. In these treated animals, ischemic vacuolization and associated fluorescence were not seen 7 hours after stroke (n=7), but appeared 16 hours after stroke (n=7).

There is a temporal and spatial coincidence of catecholamine derived fluorescence with vacuolization of the neuropil in the ischemic hippocampus. These changes can be delayed by depleting catecholamines with AMPT. The results suggest that catecholamines may play a role in the evolution of ischemic infarction in the hippocampus similar to that seen in the striatum.

43.8 EFFECTS OF LOW CONCENTRATIONS OF Cd^{++} ON THE BASAL RELEASE OF CATECHOLAMINES. A. Obeso*, S. Fidone and C. Gonzalez. Dept. of Physiology, School of Medicine, U. of Utah, Salt Lake City, Utah.

It is well documented in animal models that acute administration of Cd^{++} produces hypertension and that chronic exposure to this cation results in hypertension comparable to human essential hypertension. The pathogenic effects of Cd^{++} in producing hypertension in animal models is dose dependent; only moderate levels of Cd^{++} produce hypertension while high levels are ineffective. These findings in laboratory animals agree with epidemiological studies in humans. In these latter studies, a positive correlation was found amongst the Cd^{++} levels (moderate) in the tissues, increased blood catecholamine levels and the presence of hypertension. On the other hand, people living in Cd^{++} polluted areas have a normal incidence of hypertension despite high tissue levels of Cd^{++}. These well documented facts, the mechanism(s) involved in the genesis of hypertension induced by Cd^{++} is unknown.

In the present experiments, we found that low Cd^{++} concentrations (2-7 μM) induced a moderate but significant (≈ 20%) increase in the basal release of ^{3}H-DA (synthesized in vitro from ^{3}H-tyrosine) from the rabbit carotid body. In two vascular preparations (rabbit common carotid artery and rat aorta) preloaded with ^{3}H-NE (0.25 μM; 30 min), we found that 5 μM Cd^{++} in the superfusion fluid (air-equilibrated Tyrode's) produced a significant increase in the basal release of ^{3}H-NE, with minor effects on the release evoked by high K^+_o. The same effect was also observed with 2 μM and 7.5 μM Cd^{++} in the superfusion media. At higher concentrations (>20 μM), Cd^{++} produced inhibition of both the basal and the high K^+_o evoked release.

Since the concentrations of Cd^{++} that resulted in increased release of ^{3}H-catecholamines are within the range of those found in association with hypertension in animal models, our data, although preliminary, provide a possible explanation for the association between moderate levels of Cd^{++}, high blood catecholamine levels and hypertension.

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43.9 PROLACTIN-A CLUE TO THE MECHANISM OF HALOPERIDOL-INDUCED ACUTE DYSTONIA? D.D. Miller*, L.A. Hershey, B.M. Arafah*, J.P. Duffy* and D.J. Greenblatt*. Depts. of Psychiatry, Neurology and Medicine, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106 & Div. of Clin. Pharmacol., Tufts U. Sch. of Med., Boston, MA. 02111.

Although acute dystonia (AD) is a common adverse effect of antipsychotic agents, its pathophysiologic mechanism is unknown. Some investigators have suggested that a central imbalance of dopaminergic and cholinergic activity is involved. To better understand this reaction, we examined 21 acutely psychotic inpatients before and during their treatment with haloperidol (HDL) without prophylactic anticholinergics. We analysed various factors, looking for a difference between patients who developed AD (n=12) and those who did not (n=9). All patients whose AD was severe enough to have a rating of 2 or more on the scale of Chien et al (AJP,1974) had blood drawn within 30 minutes of developing this reaction (prior to administration of benztropine). Those patients who did not develop AD had blood drawn at similar times for comparison. Serum samples were analysed for HDL concentrations using the GLC method of Abernethy et al (J Chromatogr, 1985). Anticholinergic activity in serum was measured utilizing the QNB binding assay of Tune & Coyle (Arch Gen Psych, 1980). Prolactin (PRL) concentrations were measured by radioimmunologic assay. The technicians running these assays were unaware of whether or not the patients had developed AD. When pretreatment PRL concentrations were subtracted from those measured at the time of the drug reaction, the AD group showed a significantly larger increase in PRL than those who never experienced AD ($p<0.05$). Since serum PRL is a peripheral measure of central dopaminergic blockade, our findings suggest central dopamine antagonism as the basis for drug-induced AD. Since QNB is only a peripheral measure of anticholinergic activity, we cannot rule out the possibility of a central imbalance of dopaminergic and cholinergic activity. It will be important to examine whether stress might explain the increase in PRL concentrations in AD patients. We plan to measure serum cortisol and growth hormone levels in these serum samples in order to provide some insight into this question.

43.10 **A COMPARISON OF SUBSTANCE P AND SEROTONIN-IMMUNOREACTIVE NERVE TERMINALS IN SPINAL CORDS OF RATS AND GUINEA PIGS WITH ACUTE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE).** **D. Vyas*, D. Bieger, and S.R. White. Fac. of Med., Memorial Univ. of Newfoundland, St. John's, NFLD., Canada, A1B3V6 and Dept. of VCAPP, Col. Vet. Med., Washington State Univ., Pullman, WA 99164**

Adult male Lewis rats and juvenile female Hartley guinea pigs were inoculated for EAE with hindfoot injections of homozoic spinal cord emulsified in complete Freund's adjuvant (CFA). Control animals of each species were injected with CFA only. EAE animals developed paraplegia 11-17 days post inoculation, at which time they were anesthetized and perfused with 4% paraformaldehyde in PBS. CFA animals, matched with paraplegic EAE animals for time since inoculation, were treated in an identical manner. Spinal cords were removed and stored in fixative for at least 48 hours. Sections from cervical, thoracic and lumbar spinal cord (40 µm thick) were stained for serotonin (5HT)-and substance P (SP)-like immunoreactivity using the peroxidase-antiperoxidase method. 5HT and SP antisera were purchased from ImmunoNuclear.

Grossly distorted 5HT-positive axons were observed in the ventral and lateral funiculi at cervical, thoracic and lumbar spinal cord levels in both rats and guinea pigs with acute EAE. However, EAE-induced changes in the morphology of 5HT-positive terminals in the spinal cord gray matter differed for the two species. Depletion of 5HT-immunoreactive fibers and terminals in gray matter was striking in EAE guinea pigs; lumbar gray matter was almost completely denuded of 5HT-positive staining. In EAE rats, 5HT-positive staining was still evident in spinal cord gray matter, but marked alterations in the morphology of stained fibers and terminals compared to those of CFA controls were observed. 5HT-immunoreactive varicosities were enlarged in EAE rats compared to controls and intervaricose segments which were clearly visible in CFA rats were absent in the EAE rats in lumbar and thoracic gray matter.

Although some distorted SP-positive axons could be observed in the dorsolateral funiculus of the spinal cords of EAE rats and guinea pigs, there was not a marked or consistent change in morphology or density of SP-immunoreactive terminals in spinal cord gray matter of either species. The extensive damage to bulbospinal 5HT-immunoreactive axons and terminals occurring during acute EAE appeared to be associated with relative sparing of SP-immunoreactive neurons. The effects of the disease on those axons and terminals which contain co-localized 5HT and SP are currently being examined.

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43.11 GLYCINE RECEPTOR IN SPINAL CORD OF THE MUTANT MOUSE SPASTIC: EVIDENCE FOR NORMAL RECEPTOR STRUCTURE Cord-Michael Becker*, Irmgard Hermans*, Bertram Schmitt*, Heinrich Betz. Institute for Neurobiology, ZMBH, University of Heidelberg, D-6900 Heidelberg, FRG.

The glycine receptor from spinal cord is an M_r = 246K glycoprotein composed of three polypeptides of 48K, 58K, and 93K (Pfeiffer et al.; J. Biol. Chem. 257:9389; 1982). Homozygotes of the mutant mouse "spastic" are characterized by a specific reduction of ^{3}H-strychnine binding to homogenates from various regions of the CNS as compared with unaffected controls (White, W.F. and Heller, A.H.; Nature 298:655; 1982).

Scatchard analysis of ^{3}H-strychnine binding to crude synaptic membranes revealed a single binding site with a B_{max} of 267 ± 62 fmol/mg protein and 864 ± 220 fmol/mg protein for spastic homozygotes and control animals, respectively. K_D values were not significantly different with 7.68 ± 0.95 nM for spastic and 7.46 ± 0.85 nM for control mice. Competitive binding assays with glycine revealed K_i values of 22.2 ± 2.4 µM for spastic mice and 19.0 ± 1.7 µM for controls. Also, inhibition of ^{3}H-strychnine binding by glycinergic ligands was the same with mutant mice and control animals.

SDS-PAGE revealed the same polypeptide pattern for glycine receptor purified from spastic mice and controls, each consisting of three polypeptides of M_r = 48K, 58K, and 93K. Also, monoclonal antibodies directed against different subunits of the receptor protein produced an identical ELISA reaction pattern with glycine receptor purified from spinal cord of rat, normal, and spastic mice.

Immunofluorescent staining of sections from mouse spinal cord with monoclonal antibodies showed the same patchy distribution of fluorescence on the cell membrane of anterior horn neurons for both spastic and control mice.

These results strongly suggest that the pharmacology and the structure as well as the synaptic location of glycine receptors is unaltered in spastic homozygote mice. The reduction of Bmax for ^{3}H-strychnine binding observed in the mutants appears to result from a regulatory rather than a structural effect of the mutation on glycine receptors.

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43.12 DECREASED PLATELET [^{3}H]IMIPRAMINE BINDING SITES IN DOWN'S SYNDROME. S.W. Tang, J. Bruni*, J. Berg*, and A. Davis. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8.

Defective accumulation and storage of serotonin (5-HT) in blood platelets is one of the many metabolic abnormalities discovered in Down's syndrome (DS). Platelets are known to accumulate, store, and transport 5-HT in a similar fashion to that of serotonergic neurons in the central nervous system (CNS). A defect in platelet 5-HT storage, as seen in DS, may therefore reflect abnormal 5-HT metabolism in the CNS. This may in turn contribute to the mental deficiency observed in DS. In order to investigate the nature of the 5-HT problem in DS, radioactively labelled imipramine ([^{3}H]IMI) was used. Imipramine, a tricyclic antidepressant, appears to label the 5-HT uptake mechanism on both platelets and serotonin nerve terminals.

Subjects with a previously established diagnosis of DS (n=12, age 27 ± 5, mean ± S.E.) were recruited from the Metro Toronto Association for the Mentally Retarded (MTAMR). Two control groups also took part in the study. The parents of the Down's individuals comprised one group (n=16, age 63 ± 9). One or both parents were assayed on the same day as their child in order to control for possible assay and seasonal variation. The other control group consisted of individuals assayed independently and of comparable age (n=33, age 32 ± 7) to the Down's group in order to control for the possible influence of age. Platelet [^{3}H]IMI binding was performed according to the method of Raisman et al. (Europ. J. Pharmacol.,61:373, 1980) with slight modification. Scatchard analysis was performed to calculate the receptor density (B_{max}) and affinity constant (K_d) for [^{3}H]IMI binding.

Results show no significant difference in K_d values between Down's subjects and controls. However, Down's syndrome subjects had significantly lower B_{max} values for [^{3}H]IMI binding than controls. The observed B_{max} values were 630 ± 132 (mean ± S.D.) fmol/mg protein for the Down's platelets, 942 ± 190 fmol/mg protein for the parental controls, and 1009 ± 190 fmol/mg protein for the younger control group. The difference in B_{max} values between the Down's group and both control groups is significant ($p < .001$) as analyzed by either the two-tailed Student's t-test or by the paired t-test.

In conclusion, our results indicate a significant decrease in platelet [^{3}H]IMI binding in Down's syndrome. This finding may help us understand the 5-HT deficiency observed in DS platelets. Further, this demonstrates the usefulness of [^{3}H]IMI binding as a probe to further investigate the molecular mechanism of 5-HT transport across cellular membranes in both normal and pathological states.

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43.13 EVIDENCE THAT THE BENZODIAZEPINE ANTAGONIST CGS-8216 COUNTERACTS THE SYMPTOMS OF HEPATIC ENCEPHALOPATHY. M. Baraldi. Inst. of Pharmacology, Modena University, Via Campi 287, 41100 Modena, Italy.

The CNS derangement which occurs during the developement of hepatic encephalopathy (HE) due to fulminant hepatic failure induced in rats by Galactosamine has been associated with a denervation supersensitivity phenomenon of GABA receptors (Science, 216:427, 1982) and of Benzodiazepine recognition sites (Clin. Sci.,67:167, 1984). This finding prompted us to test the potential usefulness of the Benzodiazepine antagonist CGS-8216 to counteract the CNS generalized depression which characterizes HE. The acute injection of CGS-8216 temporally wakes up the rats from the comatose state, normalizes the pattern of visual evoked potential recordings and down regulates the Benzodiazepine binding sites labelled by ^{3}H-Diazepam. CGS-8216 has been classified as a Benzodiazepine antagonist with inversive-agonist properties which may explain the above mentioned results. On the other hand, this agent seems to interact also with the purinergic system in the brain. In this context it is likely to mention that Adenosine receptor agonists are potent CNS-depressants whereas Adenosine receptor antagonists are potent CNS-stimulants. To test whether CGS-8216 counteracts the symptoms of HE not only by antagonizing the Benzodiazepine receptor supersensitivity but also by blocking the Adenosine receptors, we performed binding experiments in vitro and behavioral studies in vivo. CGS-8216 displaces Adenosine agonist (|-|N^{6}-phenylisopropyl-^{3}H-adenosine and 5'-N-ethylcarboxamido-^{3}H-adenosine) and antagonist (1-3-diethyl-8-^{3}H-phenylxantine) binding to brain membranes with an IC_{50} of 5-10 uM wich is very similar to that of theophylline but higher than that of |-|N^{6}-phenylisopropyl-adenosine (0.08 uM). From these data and from behavioral experiments (CGS-8216 counteracts in vivo the sedative effects of adenosine derivatives) one can surmise that CGS-8216, like methylxantines, is an adenosine receptor antagonist. Finally, by studying the characteristics of Adenosine receptors in HE, we found that during the developement of the coma there is no change of ^{3}H-PIA binding while there is an increased affinity of the antagonist ligand ^{3}H-DPX. All these data taken together seem to indicate that CGS-8216 has antagonistic properties on Adenosine receptors and that its usefulness in counteracting the CNS depression of HE could be due to the antagonistic effect on both Benzodiazepine and Adenosine receptors.

43.14 TYROSINE HYDROXYLASE ACTIVITY IN EXPERIMENTAL DIABETES MELLITUS. R.W. Hamill, F.K. Northington*, S. Banerjee, Dept of Neurology, CBR & Pharmacology, Univ Roch Med Ctr/Monroe Community Hospital, Rochester, NY 14624

Autonomic dysfunction in man is a common manifestation of diabetes mellitus (DM); frequently experienced disabilities include orthostatic hypotension, sweating abnormalities, bladder dysfunction, impotence, and altered gastrointestional motility. Experimental DM in rats is also associated with autonomic abnormalities: streptozotocin (STZ), a B-cell cytotoxic agent, treatment results in altered cardiovascular and gastrointestional function and is associated with ultrastructural and biochemical changes in sympathetic innervation. In order to examine the neurochemical aspect of experimentally induced DM, tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis, was examined in peripheral sympathetic ganglia following induction of the diabetic state.

In initial studies STZ (65mg/kg) or vehicle was injected intravenously and 8 weeks later animal weight, blood sugar and stellate ganglion TH activity were determined in 8 control and 8 experimental animals. Diabetic animals exhibited blood sugars of 415+/-130mg/dl compared to control values of 200+/40mg/dl and a 23% weight loss. Stellate ganglion (SG) TH activity was reduced by 35% (control-4483+/-362pmoles/gang/hr; diabetic-2990+/-283 pmoles/gang/hr.p<0.01). In order to determine whether these effects might be a direct toxic effect of STZ, TH activity was examined within 72 hrs. of induction of glycosuria. Enzyme activity was unchanged. The effect of experimental DM on the sympathetic axis was evaluated by examining TH activity in the superior cervical (SCG), stellate(SG) and hypogastric (HG) ganglia for 12 weeks following induction of DM:

Tyrosine Hydroxylase Activity (pmoles/gang.hr)

	Control	Diabetic	Insulin Rx'd
SCG	2522±293	2145±332	2968±450
SG	3659±289	1702±234	2742±258
HG	1685±200	1417±243	2054±253

(Insulin Rx'd was initiated one month prior to sacrifice)

These studies indicate that STZ induced DM effects neurotransmitter synthesizing enzymes in specific peripheral ganglia and short term insulin treatment appears to partially reverse these effects. (Supported in part by Univ of Rochester/MCH Research Fund and NIA grant AG03234).

43.15 EFFECTS OF PARASYMPATHETIC DECENTRALIZATION ON AUTONOMIC RECEPTOR FUNCTIONS AND ON THE NON-CHOLINERGIC CONTRACTILE RESPONSE TO ELECTRICAL FIELD STIMULATION IN CAT URINARY BLADDER. K.-E. Andersson*, A. Mattiasson*, C. Sjögren*, M. Atta* and A. ElBadawi. Dept. Obst. Gyn., Univ. of Toronto, Canada, Dept. Urol., Univ. of Lund, Sweden and Dept. Pathol., SUNY-Upstate Med. Ctr. Syracuse, New York 13210.

Previous investigations have shown that parasympathetic decentralization of the lower urinary tract in cats is associated with morphological changes in the autonomic innervation and receptor functions. The aim of the present study was to investigate in vitro whether the contractile response to electrical field stimulation remaining after muscarinic receptor blockade, the responses to α-adrenoceptor agonists and antagonists, and the muscarinic receptor functions on the adrenergic nerves were changed after parasympathetic decentralization.

Male cats were parasympathetically decentralized by means of ventral sacral root division. The animals were killed for investigation after three weeks (n=6) and ten weeks (n=6) after decentralization and the results obtained were compared to those of normal controls (n=6). Strips of detrusor muscle (mucosa removed) were dissected and mounted in organ baths maintained at 37° C, and isometric tension was recorded. Pieces of tissue from the bladder muscle were pre-incubated with ^{3}H-noradrenaline, and the release of ^{3}H in response to electrical stimulation was investigated.

The results showed that the non-cholinergic part of the electrically induced contraction decreased in amplitude after parasympathetic decentralization, and maximum was reached at lower frequencies of stimulation than in controls. The non-cholinergic part of the contraction was not decreased by α-adrenoceptor blockade. Both before and after decentralization there was a weak and inconsistent response to α-adrenoceptor stimulation with phenylephrine and noradrenaline (>10^{-5} M), but never to clondine. In the release studies muscarinic receptor stimulation with carbachol concentration-dependently decreased the electrically induced release of ^{3}H by 19±4% (10^{-7} M) and 51±5% (10^{-6} M). Corresponding figures after decentralization were 41±5% and 73±3%. No differences were found between cats investigated three and ten weeks after decentralization.

The results suggest that no changes in post-junctional α-adrenoceptor functions occur after parasympathetic decentralization in cats. However, there seems to be a pre-junctional supersensitivity of the muscarinic receptors controlling noradrenaline release, similar to the post-junctional supersensitivity previously demonstrated to occur on bladder smooth muscle.

43.16 *EXPERIMENTAL AUTO-IMMUNE MYASTHENIA GRAVIS AND MYASTHENIA GRAVIS : COMPARISON OF SOME IMMUNOLOGICAL RESULTS.* *M. Geffard, M.L. Souan*, and C. Gouyou-Beauchamps*. IBCN-CNRS, 1 rue Camille Saint-Saëns, 33077 Bordeaux cedex, France.*

Myasthenia gravis (MG) is an auto-immune disease with a breakdown in the self-recognition of the acetylcholine receptor (ACh-R) due to the production of anti-ACh-R antibodies. The consequent loss in the ACh-R activity leads to a characteristic muscular weakness. Though the humoral and cellular mechanisms of this disease have extensively been studied, the triggering mechanism of the auto-immune process has not been elicited yet. Animal models have therefore been studied by using two main procedures : (i) injection into animals of purified ACh-R to raise antibodies and study the structure and function of the receptor complex; (ii) immunization of matched allotypic animals with antibodies directed against an ACh-agonist in order to raise anti-idiotypic antibodies. From Jerne's network theory, we developed a more direct route : we induced an experimental auto-immune myasthenia gravis (EAMG) by injecting into rabbits an acetylcholine-conjugate which both mimicked the molecular structure and the biological activity of acetylcholine. After six months of immunization, the rabbits developed severed symptoms of MG. The EAMG mechanism was analyzed at the immunological and clinical levels : (a) Injections of an ACh-conjugate into rabbits induced the development of both idiotypic and anti-idiotypic antibodies. Using a modified ELISA method, competition experiments allowed to determine for anti-ACh antibodies the self-displacement which occurred between 10^{-8} and 10^{-6} M, indicating a quite high antibody affinity. After purification, the auto-anti-idiotypic antibodies recognized (i) the antigen combining site of the idiotypic antibodies and (ii) the ACh-R, as demonstrated by competition with |^{125}I|-α-bungarotoxin vis a vis the purified receptor.

(b) The rabbits were examined throughout the immunization period. Signs of muscular weakness were noticed within the first few weeks. At the end of the immunization period, the rabbits showed marked signs of respiratory distress and electromyographic disturbances : increased jitters, neuromuscular blockings ....

This type of model allowed to further understand the mechanisms involved into the human myasthenia gravis. Using a modified ELISA method, serum from MG patients was found to contain an antibody that recognized the ACh determinants of our ACh-conjugate. The presence of anti-ACh antibodies in MG sera suggests a possible pathophysiologic mechanism involving the idiotypic/anti-idiotypic regulation.

43.17 ANTIIDIOTOPIC MODIFICATION OF THE IMMUNE RESPONSE IN EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. M.A. Agius, C.G. Geannopoulos and D.P. Richman, (SPON: J.W. Crayton). Department of Neurology, University of Chicago, Chicago, Illinois 60637

The maintenance of tolerance and immune regulation in autoimmune diseases involves idiotypic-antiidiotypic network interactions. To modify the anti-acetylcholine receptor response in chronic experimental autoimmune myasthenia gravis, we have injected female Lewis rats with two purified syngeneic anti-idiotopic monoclonal antibodies (HC-4A and HC-29), that are directed against an anti-acetylcholine receptor antibody capable of inducing experimental myasthenia gravis. One of the anti-idiotopic antibodies (HC-4A) recognizes a crossreactive (public) idiotope on six other anti-acetylcholine receptor monoclonal antibodies. Injections of each antiidiotopic monoclonal antibody, or a control monoclonal antibody, were made intraperitoneally; for each antibody a one-half milligram per kilogram and a one-half microgram per kilogram dose was used. Animals were actively immunized two weeks later with acetylcholine receptor and assessed four months later. All animals had elevated total serum anti-acetylcholine receptor antibody titers despite absence of weakness or decremental electromyographic findings. The anti-acetylcholine receptor titers were significantly lower in the animals treated with high doses of antiidiotopic antibody (0.6 ± 0.10 micromoles per liter, mean ± SEM), compared with animals treated with low doses or controls (1.33 ± 0.26, $P < 0.05$). In addition acetylcholine receptor content analysis by immunoprecipitation revealed normal levels of free receptor (total receptor content minus receptor complexed to antibody) for the high dose anti-idiotope group (13.5 ± 2.0 picomoles of alpha-bungarotoxin binding sites per gram of extracted membrane protein) while the other groups had amounts reduced to thirty percent of normal (4.1 ± 1.0, $P < 0.005$). These results show that pretreatment with high doses of antiidiotopic antibody suppresses the myasthenic immune response in Lewis rats.

43.18 HUMAN x HUMAN HYBRIDOMAS FROM LYMPHOCYTES OF A PATIENT WITH MYASTHENIA GRAVIS SECRETE MONOCLONAL ANTIBODIES TO ACETYLCHOLINE RECEPTOR. D.A. Blair* and D.P. Richman* (SPON: R. Rosenberg). Dept. of Neurology, University of Chicago, Chicago, IL 60637

Peripheral blood lymphocytes from a patient with myasthenia gravis (MG) were fused with polyethylene glycol to a hypoxanthine phosphoribosyl transferase-deficient non-immunoglobulin-secreting variant of the human lymphoblastoid line, GM4672. Fused cells were cultured in selective media. Four weeks after fusion, resultant human x human hybridoma cells were screened for anti-acetylcholine receptor (AChR) activity with an ELISA assay (Mihovilovic and Richman, Journal of Biological Chemistry 259:15051, 1985). The antibodies produced were studied in this assay for their ability to bind to membrane-bound AChR from Torpedo californica as well as purified solubilized AChR from the same source. Several cell lines have been cloned by limiting dilution; these lines have been carried for six months and are stable in their monoclonal antibody production. For the five lines in which we have determined immunoglobulin class, all are IgM.

Thirteen cell lines have been studied for their ability to bind AChR. Results of a typical ELISA assay are as follows: OD_{450}

Cell line	Solubilized AChR	Membrane-bound AChR
MIL 11	.141	.597
MIL 12	.127	.598
MIL 13	.131	.556
MIL 16	.310	.470
MIL 19	.146	.368
MIL 20	.107	.431
11A-3	.079	.323
11A-4	.199	.529
8A-2	.214	.391
8B-2	.138	.153
16B-5	.047	.208

(For 238 unreactive supernatants, including GM4672 culture supernatant, x ± S.D. = .004 ± .01.)

In addition, two of our cloned lines, 11A-4 and 8A-2 have exhibited saturation kinetics with membrane-bound Torpedo AChR when tested with this assay.

To our knowledge, this is the first production of human x human hybridomas secreting anti-AChR monoclonal antibodies. These data suggest that the concept of restricted species cross-reactivity of antibodies present in MG deserves re-evaluation.

43.19 RADIONUCLIDE IMAGING OF DENERVATED SKELETAL MUSCLES IN LIVE ANIMALS AND MEASUREMENT OF THE DEGRADATION RATE OF THEIR EXTRA-JUNCTIONAL ACETYLCHOLINE RECEPTORS. A. L. Politoff, F. Silva* and C. G. Nascimento*. Neurophysiology Lab., Rush Medical College, Chicago, ILL 60612 and Univ. of P. R. Medical School, San Juan, PR 00936.

Visualization of denervated skeletal muscles in live animals has been achieved by gamma camera radionuclide imaging after an intravenous bolus injection of a non-paralyzing dose (20 μg/Kg) of radioiodinated α-Bungarotoxin (131-I-α-BuTX), with a specific activity of approximately 10^4 Ci/Mol (Politoff et al., J. Cell Biol., 97:240a, 1983). The rationale of the procedure is that α-BuTX binds with high specificity and in an essentially irreversible manner to skeletal muscle acetylcholine receptors in vitro and in vivo, after systemic injection. In the present experiments, rabbits with a 6-7 day old unilateral muscle denervation and contralateral sham operation were injected 131-I-α-BuTX as before and imaged 24 hours later through a gamma camera. General anesthesia (Na penthobarbital) was required to eliminate movement artifacts. Sequential images were obtained at 5 min intervals throughout at least one hour. At the end of each sequence the surfaces containing the orthogonal projections of the denervated and sham operated regions of the legs were enclosed by identical rectangles and counts in each rectangle were integrated over time and area (C_{TA}). The time course of C_{TA} at each side was plotted: C_{TA} was always larger and decreased faster over the denervated than over the sham operated region. Slight movements of the animal caused large shifts in C_{TA}: Complete, bilateral C_{TA} curves were obtained only in 3 anesthetized animals. The difference in C_{TA}'s (denervated minus sham operated region) was not abolished by pretreatment with cold iodide nor mimicked by injection of a radioactively equivalent dose of 131-INa. Furthermore, monoiodotyrosine is not incorporated into proteins. Thus, the difference is consistent with and can be attributed to denervation-induced increase in extrajunctional acetylcholine receptors. The difference C_{TA} curve was best fitted to an exponential function: The time constant ranged between -2.28 and -2.59 x 10^{-3} per min ($p < 0.006$), equivalent to half lifes between 4.5 and 5.1 hours. These values are slightly shorter than those found in the literature, all of which were obtained using invasive and destructive methods for the quantification of the receptors, in contrast with our in vivo conditions. It is suggested that the addition of iso-rate constant contour maps to the images will enhance current radionuclide detection of denervated muscles in vivo.

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43.PO EVIDENCE FOR INVOLVEMENT OF CENTRAL GABA MECHANISMS IN AMYGDALOID KINDLED SEIZURES IN THE RAT. W.S. Schwark and W. Löscher*. New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 and School of Veterinary Medicine, Free University of Berlin, West Berlin, West Germany.

The role of central GABAergic systems in the amygdaloid kindling model of epilepsy in the rat was studied using drugs which modulate GABAergic activity and by examination of neurochemical aspects of GABA function in specific brain regions. Two inhibitors of GABA uptake from the synaptic cleft, N-(4,4-diphenyl-3-butenyl) guvacine (SKF 100330-A) and N-(4,4-diphenyl-3-butenyl) nipecotic acid (SKF 89976-A), produced profound inhibitory effects on all examined parameters of kindled seizure activity, including seizure severity, seizure latency, seizure duration and after discharge duration. In contrast to diazepam, which produced marked sedation and ataxia at ED50 dosages (0.0067 mmol/kg ip), ED50's of SKF 100330-A and SKF 89976-A (0.014 and 0.045 mmol/kg ip, respectively) resulted in no CNS depressant or other side effects. Other GABAmimetic drugs, including the specific GABA receptor agonists muscimol, progabide and THIP (gaboxadol), either had no inhibitory effect on kindled seizures or exhibited anticonvulsant action only in dosages which resulted in sedative effects.

Parameters relating to central GABA dynamics, including activity of the key GABA synthetic enzyme glutamic decarboxylase (GAD, EC4.1.1.15), GABA levels and postsynaptic [^{3}H]GABA binding, were examined in brain regions of sham-operated controls and fully kindled rats in which at least 1 week elapsed since the last kindled seizure. Of the 12 brain areas studied, i.e., olfactory bulb, frontal cortex, corpus striatum, hippocampus, right (kindled) amygdala, thalamus, hypothalamus, tectum, substantia nigra, pons, medulla oblongata and cerebellum, significant changes were localized to the amygdala, corpus striatum and substantia nigra. Evidence for reduced GABAergic function in the substantia nigra of kindled rats (control GAD activity 286±23.2 nmol/hr/mg protein vs. 161±8.9 in kindled rats; control GABA level 13.1±2.8 nmol/mg protein vs. 8.0±1.5 in kindled rats; control [^{3}H]GABA binding 385±67.5 fmol/mg protein vs 213±47.8 in kindled rats) is in agreement with several recent reports which indicate that the substantia nigra is an important regulatory site of seizure initiation and propagation. The present data suggest that alterations in central GABAergic systems may play an important role in the pathogenesis of seizures in the kindling model of eiplepsy.

Supported by a grant from the Fogarty Senior International Fellowship program (F06 TW00920-01).

43.PO Neuropeptide Y Levels in a Gene Mutation Causing Central Noradrenergic Hyperinnervation. Jeffrey L. Noebels, M. Flint Beal, Joseph B. Martin, V.R. Holets Whittemore, and Tomas Hokfelt. Departments of Neurology, Massachusetts General Hospital, Boston, MA., and Histology, Karolinska Institutet, Stockholm, Sweden.

Neuropeptide-Y (NPY) interneurons and fibers are present throughout the CNS and the peptide coexists in particular with tyrosine hydroxylase-containing cell bodies in the locus coeruleus (LC). NPY interacts at noradrenergic synapses and intraventricular exposure increases cortical synchronization. A single gene mouse mutation, tottering (tg), causes a striking and selective proliferation of LC (A6) axon terminals with a 100-200% increase in forebrain and cerebellar noradrenaline (NE) levels. The mutant expresses a neurologic syndrome of ataxia and absence seizures with spike-wave synchronization in cortex and hippocampus which can be corrected by denervation of the LC projection. To evaluate whether the tg gene co-regulates NPY levels in the LC fibers, and if altered, whether NPY might share a role with NE in disease expression, 4 tg/tg homozygotes were examined by immunohistochemistry for regional NPY innervation patterns, and 6 tg/tg were analyzed by RIA to quantify regional NPY levels. Each group was compared with age-matched +/+ control mice.

Neuroanatomical examination revealed no certain difference in NPY immunofluorescence innervation patterns between mutant and wild type mice. RIA assays of NPY content in selected brain areas showed a 20% decrease in frontal cortex (mean 41.9 pg/mg wet weight vs 52.2 pg/mg, $p<.04$) and a 60% decrease in hippocampus (9.5 pg/mg vs 24.5 pg/mg $p<.001$) in the mutants. The striatum (36.6 pg/mg vs 35.3 pg/mg) and brainstem (6 pg/mg vs 5.2 pg/mg) were unchanged. Cerebella in both genotypes contained unmeasurably low levels of NPY.

These studies identify lowered cortical and hippocampal NPY levels as a second neurochemical abnormality in the tottering mutation, and suggest that NPY probably co-localizes with only a small subgroup of LC neurons which do not hyperinnervate the tottering CNS. Whether the highly selective decrease in regional NPY levels is a cause or effect of noradrenergic hyperinnervation and inherited absence seizures, or an unrelated pleiomorphism of the tottering gene requires further study.

44 SYMPOSIUM. BEHAVIORAL FUNCTIONS OF CHOLECYSTOKININ IN THE CENTRAL NERVOUS SYSTEM. J.N. Crawley, NIMH (Chairperson); J.-J. Vanderhaeghen*, Free University of Brussels; B.G. Hoebel, Princeton University; Ph. De Witte*, Universete de Louvain; C.A. Baile, Washington University; E. Stellar, University of Pennsylvania.

Cholecystokinin (CCK) is an eight amino acid peptide which was discovered in mammalian brain by Jean-Jacques Vanderhaeghen in 1975. While the functions of gastrointestinal CCK in digestive processes and feeding behaviors have been well documented, the functional significance of central CCK opens a new field of investigation. In the brain, CCK satisfies many of the criteria for a neurotransmitter, i.e. neuronal localization, synthesis and metabolism within central neurons, neuronally stimulated release, postsynaptic receptors, and neurophysiological actions. This symposium will explore the behavioral functions of central CCK, by presenting the most recent studies on the actions of intracerebrally administered CCK on motor behaviors, feeding, self-administration, and self-stimulation.

Dr. Vanderhaeghen will introduce the session with an overview of the anatomical localization of central CCK in mammalian brain, including coexistences of CCK with other transmitters and peptides. Dr. Crawley will present evidence that picogram concentrations of CCK potentiate dopamine-induced hyperlocomotion and apomorphine-induced stereotypy in the mesolimbic pathway, where CCK and dopamine co-exist, but not in the nigrostriatal pathway, where CCK and dopamine are found in separate neurons. Dr. Hoebel will describe new studies on self-administration of CCK into the nucleus accumbens, and evidence for CCK-induced satiety in the paraventricular nucleus of the hypothalamus. Dr. De Witte will discuss findings suggesting that CCK attenuates brain stimulation reward in the hypothalamus after both peripheral and central administration, an effect not blocked by vagotomy, and a comparison of CCK actions with those of other neuropeptides and neurotransmitters in a drug discrimination paradigm. Dr. Baile will present information on changes in hypothalamic CCK concentrations after a meal, after a fast, or after chemically modulated feeding in normal rats, Zucker obese rats, and in sheep. Dr. Stellar will present new data showing that nanogram doses of CCK, administered into the third ventricle, inhibit runway performance and consumption of a food reward.

These studies suggest that CCK localized in central nuclei has discrete behavioral actions, which are functionally distinct from the actions of peripherally localized CCK. Time will be allocated for discussion of additional new research from other laboratories. Questions from the audience will be encouraged.

45 WORKSHOP. COMPUTER-ASSISTED ANALYSIS OF AUTORADIOGRAPHS: APPLICATIONS TO THE 2-DEOXUGLUCOSE METHOD. D. L. McEachron, Drexel Univ. (Chairperson); C. R. Gallistel, Univ,. of Penn.; N. R. Adler, Univ. of Penn.; C. Chu*, Drexel Univ.; O. J. Tretiak*, Drexel Univ.

The ^{14}C-2-deoxy-D-glucose (2-DG) method has been used extensively in recent years to visualize and locate changes in neurophysiology resulting from specific stimuli. Concurrently, computer-assisted analysis of the resultant autoradiographs is enjoying an increasing popularity. A number of questions remain concerning both the 2-DG method itself and the power and limitations of computer analysis. Most prominent of these are: What are the potential sources of error in the various applications of the 2-DG technique? Are normalized data valid or should local glucose utilization always be used? What are the assumptions inherent to the analysis of autoradiographs and what influence do these assumptions have on the choice of image processing hardware? What statistical methods should be used and in what way does the 2-DG technique influence the choice?

Dr. Gallistel will review the history and development of the 2-DG method with special emphasis on the assumptions involved in its application including questions of normalized data. Dr. Adler will discuss the utility of the 2-DG method as an interface between behavior and neurophysiology, commenting on what 2-DG uptake really measures neurophysiologically and how that effects data interpretation. Dr. Tretiak will examine the problems of data acquisition and interpretation from an engineering point of view once an autoradiograph has been generated. Dr. Tretiak will focus upon some of the intrinsic physical limitations involved in image analysis using the 2-DG method as an example and how this is applicable to other imaging problems. Dr. Chu will then present a particular solution to problems of computer imaging of autoradiographs in the form of a microcomputer - based image analysis system developed at Drexel University. Dr. Chu will discuss both the hardware and the software development in detail. Dr. McEachron will examine problems of standardization and statistical evaluation both as a question in the development of a computer imaging system and how these problems influence experimental designs. Dr. McEachron will discuss both practical and theoretical issues created by using the 2-DG method, including issues such as pattern recognition, which have yet to be resolved. The Drexel University microcomputer-based system (DUMAS) will be available for examination and demonstration at the workshop.

46.1 EVALUATING SPECIFICITY IN IN SITU HYBRIDIZATION HISTOCHEMISTRY. M.E. Lewis, S. Burke, T.G. Sherman, and S.J. Watson. Mental Health Res. Inst., University of Michigan, Ann Arbor, MI 48109.

In situ hybridization histochemistry is a valuable technique for anatomically localizing and quantifying changes in specific mRNA. In immunocytochemistry, controls have been devised to avoid "false-positive" staining; similarly, we have explored several procedures for evaluating the specificity of hybrids detected in situ. In these studies, we have used synthetic oligonucleotide or cloned cDNA probes complementary to different regions of proopiomelanocortin (POMC) mRNA. (1) Peptide-mRNA colocalization: Using 4% formaldehyde-perfused tissue, it has been possible to demonstrate cellular colocalization of POMC peptide (e.g. ACTH) immunoreactivity and POMC probe hybrids. (2) Competition studies: Prehybridization of pituitary mRNA with unlabeled alpha-MSH oligonucleotide appropriately attentuates the intermediate lobe (IL) oligonucleotide hybridization signal. However, when the alpha-MSH probe is prehybridized to an equimolar amount of message sense oligonucleotide before tissue incubation, both specific IL and nonspecific (posterior lobe; PL) signals are completely eliminated. Thus, the "preadsorption control," commonly used in immunocytochemistry, is logically inappropriate for in situ hybridization histochemistry. (3) Colocalization of multiple probes: Using adjacent thin sections, probes complementary to different regions of the same mRNA should hybridize to the same cells. We are presently exploring this procedure with multiple POMC as well as dynorphin oligonucleotide probes. (4) Thermal stability of hybrids: Since base-pair mismatches reduce the thermal stability of DNA-RNA hybrids, determination of the Tm (temperature at which 50% of hybrids dissociate), compared to the calculated theoretical Tm value, provides an index of specificity. We have confirmed the usefulness of this measure by showing excellent agreement between obtained and theoretical Tm values (69^{o}C) for hybridization of the alpha-MSH probe to IL. The nonspecificity of low level "hybridization" to PL was confirmed by the much lower apparent Tm (50^{o}C). (5) Northern analysis: The identity of an apparent mRNA target of a probe in situ should be confirmed by demonstrating that the probe hybridizes to an extracted RNA of appropriate size. Using this procedure, we have confirmed that the alpha-MSH oligonucleotide and a much longer POMC cDNA both hybridize to a single pituitary RNA species of a size previously determined for POMC mRNA (1230 nucleotides). These multiple controls provide important, complementary information for evaluating the specificity of hybrids found in situ. Furthermore, for several of these controls, synthetic oligonucleotides and other constant length probes have clear advantages over nick-translated cDNA probes.

46.2 BRATTLEBORO RAT TRANSCRIBES VASOPRESSIN mRNA IN HYPOTHALAMUS: EVIDENCE FROM IN SITU HYBRIDIZATION. J.T. McCabe, J.I. Morrell, H. Schmale*, R. Ivell*, D. Richter*, and D.W. Pfaff. The Rockefeller University, New York, N.Y. 10021 and The University of Hamburg, Hamburg, Federal Republic of Germany

Hereditary diabetes insipidus exhibited by the Brattleboro rat is caused by an inability to synthesize detectable amounts of vasopressin (VP) hormone in the neurohypophyseal system. Gene sequence analysis of genomic and cDNA libraries from Brattleboro rats indicate that the nucleotide sequence encoding VP hormone is normal, but that there is a single base-pair deletion in the region encoding the neurophysin moiety attached to the carboxyl end of VP (Schmale & Richter, 1984, Nature, 308: 705). This deletion base-pair in the Brattleboro's neurophysin segment of the VP gene, downstream to a normal VP sequence, appears to block synthesis of a normal VP-neurophysin-glycoprotein molecule.

Tritiated- and 35-sulphur-labelled (35-S) probes, generated from genomic and cDNA libraries of normal rats, were hybridized to fresh-frozen, cryostat-cut 6 μ sections, that were subsequently fixed (3:1 ETOH:Acetic acid), deproteinated (1 μg/ml pepsin in 2xSSC:Acetic acid, pH 3.5) and air-dried before prehybridization and probe application (method: McCabe et al., 1985, J Histochem Cytochem, submitted). An 18-hr hybridization period was followed by ammonium acetate:ETOH dehydration steps and standard autoradiographic procedures.

Tritiated probes encoding the 3' sequence of the neurophysin region of the VP gene, labelled magnocellular neurons in the supraoptic (SON) and paraventricular (PVN) nuclei of normal and Brattleboro rats. Comparable with immunocytochemical descriptions in normal rats, VP probes labelled magnocellular neurons in the lateral subnucleus of the PVN and ventral portions of the SON. Cell grain counts over SON neurons indicated the mean number of grains per cell for Brattleboro rats were approximately 25% the number seen in the normal rat. In situ hybridization with 35-S labelled probes also labelled magnocellular neurons in the Brattleboro hypothalamus, but with higher background levels. Prolonged (sixty-day) autoradiographic exposure periods with 35-S probes decisively demonstrated VP-gene transcription in the Brattleboro rat. The present results are congruent with recent Northern Blot analyses and transfection studies that show the defective VP gene is transcribed in Brattleboro rats (Schmale, et al., 1984, EMBO J, 3: 3289). This in situ hybridization analysis provides neuroanatomical confirmation that the defective VP gene is transcribed in cells which ordinarily synthesize VP hormone.

Supported by grants PHS NS07214 (JTM) and HD16327 (JIM).

46.3 STIMULATION OF DYNORPHIN AND VASOPRESSIN mRNA EXPRESSION IN THE BRATTLEBORO RAT WITH CHRONIC INTERMITTENT OSMOTIC CHALLENGE. T.G. Sherman, O. Civelli*, J. Douglass*, E. Herbert* and S.J. Watson. M.H.R.I., University of Michigan, Ann Arbor, MI 48109, and *Dept. of Chemistry, University of Oregon, Eugene, OR 97403.

The coexistence of prodynorphin peptides within vasopressin magnocellular neurons of the rat hypothalamus has prompted several studies in our laboratory into the regulation of the expression of each of these peptide hormone mRNAs. Previously we have shown that with chronic osmotic challenge (salt-loading), these mRNAs are coordinately stimulated to levels 8-10 fold higher for vasopressin and 3-4 fold higher for dynorphin. Despite the inability of homozygous diabetes insipidus rats of the Brattleboro strain to synthesize AVP under most conditions, these animals continue to synthesize, process and secrete prodynorphin. Examination of their hypothalamic content of prodynorphin mRNA, in fact, revealed that they contained nearly twice as much prodynorphin mRNA as control Long Evans rats, with heterozygotes containing an intermediate amount. Furthermore, a 24 hour dehydration of these animals failed to elicit a further increase in this RNA as it did in controls. Longer dehydration or salt-loading experiments were difficult, for these animals would fail to survive even moderate salt-administration for more than 24 hours. To investigate whether homozygous animals expressed a dynorphin mRNA which was regulated differently than normals, we developed a means by which these rats could be chronically dehydrated without severe body weight loss or death. Animals were provided with 2% saline for 18 hours per day (from 4pm to 10pm), and with water for the remaining 6 hours. Body weights of homozygotes weighing 280-300 grams would lose up to 50-60 grams in an 18 hour dehydration, only to regain that and more in the hydration period. Control and heterozygote animals under this regiment fluctuated less widely. After a six day course of this treatment, individual PVN, SON and SCN areas were punched, and their isolated poly(A)RNAs examined by Northern analysis. Very little or no change in dynorphin mRNA content was recorded in the PVN and SCN areas, whereas, each group of normals, homozygotes, and heterozygotes had 2-3 fold increases in SON dynorphin mRNA levels. This finding is substantiated by in situ hybridization analysis for both dynorphin and AVP mRNAs with oligonucleotides. Work is continuing on estimating the total change in vasopressin mRNA in these nuclei, and whether or not changes can be detected between the expressionn of the normal versus the aberrant vasopressin mRNAs in heterozygotes.

46.4 QUANTITATIVE IN SITU HYBRIDIZATION STUDIES OF VASOPRESSIN AND SOMATOSTATIN mRNA DISTRIBUTIONS AND DYNAMICS. G.R. Uhl. Department of Neurology and Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Study of neuropeptide gene expression may provide a means for monitoring functional activities of peptidergic neuronal groups. In situ hybridization can allow quantitative detection of peptide-specific mRNAs with good anatomic resolution and can complement studies of mRNA populations in tissue extracts.

We have used a chemical/enzymatic "synprobe" technique to synthesize homogeneous single-stranded ^{3}H- and ^{35}S-radiolabeled cDNAs of 1500 and 12,000 Ci/mMole respectively. These probes can specifically recognize vasopressin and somatostatin mRNAs in brain sections.

Several physiologic conditions altering region-specific peptidergic activity alter mRNA hybridization in parallel fashion. Dehydration of rats doubles vasopressin hybridization in the supraoptic and paraventricular nuclei. Vasopressin releases related to hypoxia and to diurual cycles are also associated with regional mRNA level changes in preliminary studies. Conversely, vasopressin mRNA levels appear normal in hypothalamic nuclei of genetically vasopressin-deficient Brattleboro rats given free access to water.

Cells in different peptidergic neuronal groups may express substantial differences in peptide mRNA populations. Studies of somatostatin-hybridizing cells suggest that positive cells in the periventricular hypothalamus may express more than four times the hybridizable preprosomatostatin mRNA levels found in positive cells in the cerebral cortex or striatum. Levels of vasopressin hybridization in hypothalamic nuclei are substantially greater than levels found in extrahypothalamic zones thought to contain vasopressin-immunoreactive neurons.

Could these studies be applied to human brain tissue obtained postmortem? Using the rat (Spokes-Koch) model of the human postmortem process, we have found substantial stability of hypothalamic vasopressin mRNA up to twelve hours postmortem. Hybridization techniques can thus conceivably provide information about peptide mRNA variations related to involvement of peptidergic neurons in different brain circuits or different physiologic, genetic, and pathologic processes.

46.5 QUANTITATION OF VASOPRESSIN AND OXYTOCIN mRNA : AN IN SITU HYBRIDIZATION HISTOCHEMICAL STUDY OF NORMAL, ADRENALECTOMIZED AND BRATTLEBORO RATS. W. Scott Young, III*, Eva Mezey, and Ruth Siegel, Lab. of Cell Biol., NIMH, Bethesda, MD 20205

The levels of vasopressin (VP) and oxytocin (OT) in the supraoptic (SON) and paraventricular (PVN) nuclei change under various physiological stimuli. In contrast, VP is not detected in the brain of the Brattleboro rat. We have used in situ hybridization histochemistry to measure the mRNA levels in normal, adrenalectomized and Brattleboro rats to determine how they relate to peptide levels.

Sprague-Dawley rats were adrenalectomized (Adx) and perfused 2 weeks later with 4% paraformaldehyde and 10% sucrose. Some received colchicine 2 days prior to perfusion. Brattleboro rats were perfused similarly. Twelve micron thick frozen sections were hybridized with 48-base synthetic oligonucleotides directed toward the glycopeptide region of VP and N-terminus of OT. The probes were kinased with γ-^{35}S ATP. Sections and ^{35}S-impregnated brain-paste standards then were apposed to emulsion-coated coverslips for 4 days.

VP and OT mRNA-containing cells were found in the expected subdivisions of the SON and PVN. No labelling was seen with a VP probe of mRNA sense. In magnocellular neurons, we detected 7000 and 5200 VP mRNA copies in the SON and PVN, respectively. Adx raised the levels to 12,000 and 7000. Oxytocin mRNA levels were 8400 and 7300 with only a slight reduction, if any, after Adx. The parvocellular PVN contained about 10% of VP mRNA of the magnocellular PVN (by regional volume). Colchicine reduced levels of VP mRNA in all 3 areas before and after Adx with the exception of the Adx parvocellular PVN where levels increased, indicating a confounding effect of colchicine. Heterozygous Brattleboro rats had 8700 and 7300 copies of VP per SON and PVN magnocellular neuron, respectively. Homozygous rats had 30 to 50% fewer copies.

This study emphasizes the sensitivity of in situ hybridization histochemistry which will permit more detailed examinations of mRNA levels at steady state and after various stimuli. For example, our results show that Adx produces larger increases in the magnocellular than the parvocellular VP mRNA in the PVN.

Although magnocellular VP and OT mRNA levels are abundant, other mRNA species in lower amounts should be amenable to similar study. We do not know at present what percentage of the message we are detecting and this will likely be the limiting factor in absolute quantitation.

W.S.Y. is a Pharmacology Research Associate of NIGMS.

46.6 GLUCOCORTICOID SENSITIVE STIMULATION OF VASOPRESSIN mRNA SYNTHESIS IN THE PARAVENTRICULAR NUCLEUS. Frank Baldino,Jr. Betty Wolfson*, Rene Arentzen*, Robert W.Manning*, Jan Reid* and Leonard G. Davis. Central Research and Development, E. I. du Pont de Nemours and Co., Wilmington, DE 19898

Immunocytochemical studies have shown that adrenalectomy produces changes in the content and distribution of arginine-vasopressin (AVP) immunoreactivity in the paraventricular nucleus (PVN) of the hypothalamus. The purpose of this study was to determine whether glucocorticoids mediate the adrenalectomy-induced transcription of AVP mRNA within this region. In situ hybridization methodology with synthetic oligodeoxynucleotide probes and immunocytochemistry was used to detect the distribution of AVP-immunoreactive perikarya, AVP mRNA and alpha-tubulin mRNA. Probe specificity was determined by Northern blot hybridization against a rat AVP cDNA clone. The hybridization signal was detected with autoradiography on X-ray film or with photographic emulsion and quantitated with scanning densitometry. The results show that AVP mRNA is co-distributed with AVP immunoreactivity in the posterior magnocellular subdivision of the PVN and its accessory nuclei, the supraoptic nucleus (SON) and the suprachiasmatic nucleus (SCN). In adrenalectomized rats (7 days) the density of the hybridization signal is increased in the PVN and SON. Moreover, the distribution of AVP mRNA is altered in the PVN; a 3 fold increase in the area comprising the signal was observed. At the cellular level autoradiographic grains were detected in CRF-immunoreactive neurons throughout the medial parvocellular subdivision of the PVN. No changes were noted in the distribution of AVP mRNA in the SON or SCN. Although without effect in control animals, treatment with dexamethasone (240 µg/day for 7 days) prevented the increase in AVP mRNA produced by adrenalectomy. On the other hand, adrenalectomy did not influence the density or distribution of the hybridization signal produced by a probe for alpha-tubulin mRNA indicating that not all mRNA species are affected by this treatment.

These results demonstrate, at the cellular level, that adrenalectomy induces a glucocorticoid sensitive stimulation of AVP mRNA synthesis in the PVN. Thus considerable plasticity in gene expression is retained in the hypothalamus of the adult rat.

46.7 ANALYSIS OF PROOPIOMELANOCORTIN GENE EXPRESSION DURING RAT PITUITARY DEVELOPMENT BY IN SITU HYBRIDIZATION. D.I. Lugo and J.E. Pintar*. Dept. Anatomy and Cell Biology, Columbia Univ., P&S. New York, NY 10032.

The proopiomelanocortin (POMC) gene codes for a variety of peptide hormones which may have important functions during embryogenesis as well as post-natally. POMC gene expression has been detected as early as embryonic day 13 (e13) by hybridization of isolated nucleic acid to radiolabelled POMC probe. Moreover, POMC is synthesized at this stage and the pattern of POMC proteolytic cleavage resembles that of the adult intermediate lobe; it is not until e18 that peptides characteristic of the adult anterior lobe first appear. Although these data suggest that the intermediate lobe begins synthesis of POMC before the anterior lobe, immunocytochemical studies have shown that POMC peptide accumulation in the anterior lobe precedes the intermediate lobe by at least one day. Therefore, it is of interest to determine when and where in the pituitary POMC mRNA is first synthesized and whether changes in the relative amounts of POMC mRNA in the anterior versus the intermediate lobe coincide with specific developmental events. Since in situ hybridization can be used to determine both the cellular sites of specific mRNA and relative levels of a particular mRNA, we have used this approach to investigate POMC gene expression in the fetal and neonate rat pituitary gland.

Pregnant Sprague-Dawley rats were decapitated and the fetuses from day 12 (e12) to e20 were dissected. Young fetuses (less than e17) were fixed by immersion in 4% paraformaldehyde pH7 while older fetuses as well as neonate rats (post-natal day 1 (p1) to p17) were intracardially perfused; the pituitaries were then dissected, embedded and sectioned using a cryostat. The sections were hybridized with either P^{32}-labelled POMC cDNA and subsequently exposed to X-ray film or with H^3-labelled POMC cDNA and subjected to autoradiography. Experiments with P^{32}-labelled probe demonstrated higher film exposure over the intermediate lobe compared to other pituitary regions as early as e17. After 3.5 week exposure of sections hybridized with ^{3}H-labelled probes, most cells of the intermediate lobe and a few cells in the anterior lobe of e20 fetuses appeared labelled. Thus, by e20 the pattern of silver grains over the pituitary gland following in situ hybridization with POMC cDNA resembles that of the adult pituitary gland. Longer exposure times of sections from yonger embryos should prove effective in identifying sites of POMC gene expression at earlier ages. In addition, semi-quantitative comparison of grain densities over different pituitary cells from embryos of varied ages will provide an indication of the relative POMC mRNA levels and their variations during development.

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46.8 QUANTITATION OF POMC GENE EXPRESSION IN THE RAT PITUITARY AT THE LEVEL OF THE INDIVIDUAL CELL BY IN SITU HYBRIDIZATION. JL Roberts and JN Wilcox*, Center for Reproductive Sciences, Columbia Univ., College of Physicians and Surgeons, New York, NY 10032

The great advantage of in situ hybridization is its ability to resolve cDNA:mRNA hybrids at the level of the individual cell. This has many uses in localizing cells synthesizing an mRNA of interest. Perhaps the greatest potential of the technique is the possibility of quantifying specific mRNAs in individual cells. If the amount of cDNA in the hybridization reaction is saturating, then the amount of cDNA probe hybridized will be related to the amount of complementary mRNA in that cell. The number of silver grains over an individual cell should be related to the amount of radioactive cDNA probe hybridized, thus providing an estimate of mRNA levels in that cell. To demonstrate the potential of using this technique to study gene regulation at the cellular level we have used as a model the effect of glucocorticoids and dopamergic compounds on proopiomelanocortin (POMC) mRNA levels in individual cells of the rat pituitary.

In the present series of experiments we have been able to document changes in POMC gene expression in pituitary sections and in cell cultures by quantifying silver grains after in situ cDNA:mRNA hybridization. Female rats were injected ip with 1mg Bromocryptine (BROMO) per day for 1 or 3 days, the animals sacrificed and the pituitaries processed for in situ hybridization to a [3H] labelled POMC cDNA probe. After developing the slides the number of silver grains per cell were determined and paired with the cell area using a computer based grain counting system (Bioquant II, R&M Biometrics). A comparison of the grain densities (grains/pixels per sq mm) over individual cells of the intermediate pituitary indicated that there was a significant reduction in silver grain density to 50% and 26% of control after 1 or 3 days of BROMO treatment respectively.

In a separate experiment the effect of dexamethasone (DEX) was analyzed on cells in culture in which POMC mRNA levels are down-regulated by glucocorticoids. AtT20 cells were grown for 2 days in media containing 10^{-9}, 10^{-8}, 10^{-7}, or 10^{-6}M DEX. The slides were then fixed in ETOH:HAc and hybridized to a [3H] labelled POMC cDNA probe. Comparison of silver grain densities over individual cells treated with various concentrations of DEX indicated that there was a significant decrease in POMC mRNA to 65% of controls in cells treated with 10^{-6}M DEX.

Results from these experiments parallel known changes in POMC mRNA levels as previously determined by dot blot analysis and suggest that we can see significant changes in grain densities after in situ hybridization when mRNA levels differ by more than 2 fold.

46.9 LOCALIZATION OF POMC mRNA IN RELATED NEURONS FUNCTIONALLY DEFINED BY THEIR AXONAL PROJECTIONS: COMBINING IN SITU HYBRIDIZATION WITH FLUORESCENT AXONAL TRACING. J.N. Wilcox*,B.M. Chronwall, T.L. O'Donohue and J.L. Roberts. Center for Reproductive Sciences, Columbia Univ. P&S, New York, NY 10032 and NINCDS, NIH, Bethesda, MD 20205.

In situ cDNA:mRNA hybridization is a technique that has been developed for the visualization of cDNA:mRNA hybrids in individual cells. Recently this technique has received considerable attention as a method by which regulation of gene expression might be measured at the level of the individual neuron. However, to use this technique to study regulation of gene expression in the brain when the cell population under study does not respond uniformly, it will be necessary to divide hybridizing cells into functional subgroups that can be located in experimental and control tissues. Secondary techniques must be employed to define related cell types if there are no clear anatomical distinctions. With this problem in mind we have developed a procedure by which hybridizing cells may be classed according to their axonal projections by combining the techniques of in situ hybridization with retrograde axonal tracing of neurons using the fluorescent tracer Fast Blue (FB) on the same tissue section.

Cell bodies synthesizing proopiomelanocortin (POMC) mRNA are located in the periarcuate region of the rat hypothalamus. FB is injected by stereotaxic implant into the preoptic nucleus, a terminal field of the POMC system where it is taken up by axon terminals and fibers of passage. Animals are sacrificed 3 days after injection, 10 micron sections taken on a cryostat, and the tissue processed for in situ hybridization. Arcuate cells remain strongly labelled with the FB dye after in situ hybridization to a [3H] labelled POMC cDNA probe. By alternately viewing the slide under fluorescence or polarized light epilumenescence illumination it is possible to identify cells containing both FB and POMC mRNA. We estimate that about 10% of the POMC neurons detected by in situ hybridization in the medial arcuate region also project to the preoptic nucleus. Thus, this procedure sharply deliniates a small population of cells that project to the preoptic nucleus. Using these techniques it now becomes possible to measure mRNA regulation in functional subsets of neurons defined by their axonal projections.

46.10 LOCALIZATION OF CELLS CONTAINING PREPROENKEPHALIN mRNA IN THE RAT FOREBRAIN BY IN SITU HYBRIDIZATION. R.E. Harlan, B.D. Shivers*, G.J. Romano*, [1]R.D. Howells* and D.W. Pfaff. The Rockefeller University, New York, NY 10021 and [1]The Roche Institute for Molecular Biology, Nutley, NJ 07110.

Methionine enkephalin is cleaved from preproenkephalin, whose mRNA has recently been cloned and sequenced. Using a 435 bp cDNA complementary to mRNA coding for amino acids 56 through 200 of the rat preprohormone (Howells et al. PNAS 81:7651, 1934), we have localized cells in the rat brain containing this mRNA. The cDNA was nick translated to a specific activity of about 10^8 cpm/μg for ^{32}P or 10^7 cpm/μg for ^{3}H.

Cryostat sections of either paraformaldehyde-perfused brains soaked in sucrose containing 0.02% diethyl pyrocarbonate (DEP), or fresh-frozen, sectioned brains postfixed in paraformaldehyde containing 0.02% DEP were incubated in prehybridization buffer, followed by hybridization buffer (including 0.6 M NaCl and 50% formamide) containing labeled cDNA (50,000-125,000 cpm/20 μl for ^{32}P; 10,000-30,000 cpm/20 μl for ^{3}H) for 3 days at room temperature or 37 C. Sections were rinsed twice, ten minutes each in 2X SSC (1X SSC = 150 mM NaCl, 15 mM Na citrate) including 0.05% inorganic pyrophosphate, followed by 36-48 h of 0.5X SSC, 0.05% inorganic pyrophosphate, all at room temperature. Following dehydration, the sections were dipped in emulsion, and exposed at 4 C for 10-30 days for ^{32}P and 14-51 days for ^{3}H. Autoradiograms were developed and the sections stained with fast green and cresyl violet.

Controls: Sections incubated in hybridization buffer:formamide without probe, or pretreated with RNase before hybridization contained no labeled cells.

In the forebrain, well-labeled cells were located in: anterior olfactory nucleus (especially medial); frontal cortex; piriform cortex; caudate/putamen (especially in lateral aspects); nucleus accumbens; olfactory tubercle; diagonal bands of Broca; lateral septum; preoptic area (medial and lateral); bed nucleus of the stria terminalis; amygdala (especially the central nucleus); paraventricular nucleus; anterior hypothalamus; perifornical region; ventromedial hypothalamic nucleus (especially ventrolateral); dorsomedial hypothalamic nucleus (especially caudal part of ventral division); lateral hypothalamus; arcuate nucleus; premamillary nuclei (dorsal and ventral); medial mamillary nucleus and lateral geniculate. Analysis of midbrain, hindbrain and spinal cord is in progress.

In most cases, these results correlate well with those based on immunocytochemistry, although in situ hybridization revealed many more labeled cells. In situ hybridization has not demonstrated, to date, neurons that can synthesize preproenkephalin in the hippocampus or some cortical areas, as suggested by immunocytochemistry.

46.11 LOCALIZATION OF CELLS CONTAINING LHRH-LIKE mRNA IN RAT BRAIN. B.D. Shivers*, R.E. Harlan, [1]J.F. Hejtmancik*, [2]P.M. Conn*, and D.W. Pfaff. The Rockefeller Univ., NY, NY 10021, [1]Baylor College of Medicine, Houston, TX 77030, [2]Univ. of Iowa, Iowa City, IA 52242.

The purpose of this study was to localize neurons in the rat brain which synthesize the reproductive peptide, luteinizing hormone-releasing hormone (LHRH) by in situ hybridization. Rat brain sections were probed with two synthetic, radiolabeled oligomers complementary to different portions of the human LHRH mRNA sequence (Seeburg and Adelman, Nature 311: 666, 1984), and the formation of hybrids was detected autoradiographically.

Probe 1 was a 59-mer complementary to the sequence coding for amino acids -5-20 of the proposed human LHRH preprohormone which includes the region coding for the decapeptide. Probe 2 was a 60-mer complementary to a sequence in the open reading frame of human LHRH mRNA coding for amino acids 33-52. These oligomers were end-labeled with either (γ-^{32}P)ATP and T4 polynucleotide kinase, or (^{3}H)CTP and terminal transferase. Sections received 50,000-150,000 cpm/20μl for ^{32}P oligomers, or 12,000 cpm/20μl for ^{3}H oligomers.

Following incubation in prehybridization buffer:50% formamide to reduce nonspecific binding, 10μm sections of frozen, paraformaldehyde-fixed, diethyl pyrocarbonate-treated female rat brains (n=8) were hybridized for 3 days in a buffer which included 0.6M NaCl and 50% formamide and then rinsed two times, ten minutes each in 2xSSC (1xSSC= 0.15M NaCl; 15mM Na citrate) including 0.05% inorganic pyrophosphate followed by two rinses, in 48 hours, of 0.5xSSC, 0.05% inorganic pyrophosphate, all at room temperature. Sections were then dehydrated, dipped in emulsion, exposed at 4 C, the autoradiograms developed, and the sections stained with fast green and cresyl violet.

Autoradiogram exposures of 3-4 weeks for ^{32}P-labeled probe 1, and 60 days for ^{3}H-labeled probe 1 yielded labeled cells distributed in forebrain regions shown previously to contain immunoreactive LHRH cell bodies (Shivers et al. Neuroendocrinology 36: 1, 1983). The majority of probe 1-labeled cells were in the diagonal bands of Broca and the medial preoptic area. Some labeled cells were observed in the anterior hypothalamic area and were often found immediately dorsal to the supraoptic nuclei. Occasionally a labeled cell was observed in the medial basal hypothalamus. Labeled cells often appeared fusiform in shape.

Sections incubated in hybridization buffer:formamide without probe, or pretreated with RNase before hybridization, or hybridized with probe 2 contained no labeled cells.

Additional studies will be required to characterize the LHRH-like mRNA contained in these cells, and to determine whether its message level is regulated by steroids.

46.12 LOCALIZATION OF GLUTAMATE DECARBOXYLASE (GAD) MRNA IN MOUSE BRAIN BY IN SITU HYBRIDIZATION. C.W.Wuenschell*[1], R.S.Fisher[2], and A.J.Tobin[1] (SPON: N.Wexler). [1]Dept. of Biology and Molecular Biology Institute, and [2]Mental Retardation Research Center, Univ. of California, Los Angeles, CA 90024.

We are using in situ hybridization to locate specific mRNAs in individual cells in mouse brain in order to assess gene expression during development. In this initial report, adult mouse brain revealed GAD mRNA-containing cells in all the major brain regions known to contain cells positive for the GAD enzyme.

We have subcloned our GAD cDNA into the transcription vector pSP65 in both orientations with respect to the viral-specific promoter. Transcription from these two templates by the sp6 viral polymerase produces either "antisense" RNA molecules complementary to the mRNA, which can be used as a hybridization probe for mRNA, or RNA identical in sequence to the mRNA which serves as a negative control.

In situ hybridization was performed on 10 um frozen sections fixed with 4% paraformaldehyde. RNA probes were labeled with ^{35}S to specific activities of 3-5 X 10^8cpm per microgram. Hybridizations were performed overnight at temperatures ranging from 42° to 50° in 50% formamide, 750 mM NaCl. Following hybridization, non-specifically bound probe was removed by treatment with ribonuclease A, followed by washing at 43° in 0.1 X SSC and defatting with xylene. Autoradiographic detection was performed with Kodak NTB2 emulsion.

The antisense hybridization probe labeled many scattered cells throughout the globus pallidus, caudate-putamen, and the inferior and superior colliculi. In the cerebral cortex, stellate neurons appeared to be labeled, as did a sparse population of basket cells in the hippocampus. The negative control probe gave randomly distributed silver grains in all of these areas. Purkinje cells of the cerebellum and, to a lesser extent, granule and periglomerular cells of the olfactory bulb appeared to be labeled more heavily by the antisense than by the negative control probe.

This work was supported by grants to AJT from NIH (#NS20356 and NS22256) and from the Dystonia Medical Research Foundation. CWW has been supported by a USPHS Training Grant in Genetic Mechanisms (#GM7104).

47.1 3-D ANALYSES REVEAL SUBPOPULATIONS OF LHRH NEURONAL CELL BODIES FOLLOWING GONADECTOMY. King, J.C., G. Kugel*, D. Zahniser*, K. Wooledge* and D.A. Damassa. Department of Anatomy and Cellular Biology and the Image Analysis Laboratory, Tufts-New England Medical Center. Boston, MA 02111. Neuronal cell bodies capable of synthesizing LHRH are widely dispersed over several nuclear groups of the basal hypothalamus. If only some LHRH neurons, and not the entire population, increase secretion following gonadectomy, this change in activity might not be detected by RIA. It was the purpose of this study to specify subpopulations of LHRH cells identified by immunocytochemistry in male and female rats, one day, six days and three weeks after gonadectomy. The immunoreactive species of LHRH detected within neuronal cell bodies did not change in any endocrine condition. Only antisera capable of binding extended forms of decapeptide detected LHRH within neuronal perikarya and not antisera requiring either the amidated Gly C terminal or both amidated Gly C terminal and cyclicized Glu N terminal. Populations of LHRH cells were reconstructed in three dimensions across the basal forebrain using computer assisted techniques. Comparisons between populations were made by simultaneous viewing two or more color-coded reconstructed populations. We have previously reported sex differences in changes in the size of LHRH populations following gonadectomy. Further analyses indicate that variations in populations occurred largely in subgroups of neurons that surrounded a central core of neuronal cell bodies in which LHRH was detected in both males and females regardless of the endocrine condition. The core subgroup consisted of cells in several preoptic nuclei, the rostral preoptic area, periventricular preoptic and ventral preoptic areas. Peripheral subgroups layered in an onion-skin fashion about this central core and extended into the diagonal band of Broca, lateral preoptic and anterior hypothalamic regions and medial septal nucleus. We suggest that subgroups receive different types of afferent input and that changes in afferent stimulation to peripheral, but not core, subgroups are induced by gonadectomy. This work was supported by NSF PCM8402540.

47.2 IMMUNOLOCALIZATION OF THE TRH PROHORMONE IN THE RAT CENTRAL NERVOUS SYSTEM. R.M. Lechan, Ping Wu*, Serene Forte* and Ivor M.D. Jackson. Divisions of Endocrinology, Tufts-New England Medical Center, Boston, MA 02111 and Brown University, Rhode Island Hospital, Providence, R.I. 02902.

To study the distribution of the TRH prohormone in the rat CNS, we raised an antiserum (342) against a synthetic, cyclic decapeptide, cys-lys-arg-gln-his-pro-gly-lys-arg-cys (ProTRH-SH), based on the deduced amino acid sequence of the frog skin TRH precursor (Richter et al, EMBO, J. 3, 617, 1984). Brain tissue was fixed with 4% paraformaldehyde by intracardiac perfusion and sectioned on a vibratome. All immunocytochemical studies were performed by the indirect peroxidase-antiperoxidase technique using antiserum #342 at a final dilution of 1:750 containing 0.2% Triton X-100.

Immunoperoxidase was present predominantly within the cytoplasm of neuronal perikarya, closely juxtaposed to the nucleus, and conspicuously absent from axon terminals. Similar to the distribution of the tripeptide, TRH, in rat brain (Lechan and Jackson, Endocrinology, 111, 55, 1982), neurons immunoreactive for proTRH were present in the hypothalamus, preoptic area and medullary raphe. In addition, immunoreactive proTRH was present in neurons in the glomerular and external plexiform layers of the olfactory bulbs, caudate-putamen complex, hilus of the dentate, amygdala, central gray, pontine nuclei, dorsal raphe, cochlear nuclei, nucleus solitarius and flocculus of the cerebellum. Unique to the cerebral cortex, the soma of some neurons in layer II were observed to contain immunoreactive material within a large, cytoplasmic inclusion, often extending into first order dendrites or the axon hillock. All immunoreaction product was abolished by preincubation with Pro-TRH-SH at 10^{-6}M but not TRH at 10^{-5}M.

These studies support the belief that TRH biosynthesis in the mammalian CNS occurs by posttranslational cleavage of a larger precursor protein and indicates that processing of the prohormone occurs primarily within the neuronal cell body. In addition, immunoreactive proTRH appears more widely distributed than TRH. This raises the possibility that the prohormone could be processed to intermediate forms that would not be recognized by an antiserum specific for p-glu-his-pro-NH_2, TRH.

47.3 ELECTRON MICROSCOPIC LOCALIZATION OF ANGIOTENSIN AND ANGIOTENSIN CONVERTING ENZYMES IN THE RAT SUBFORNICAL ORGAN. V.M. Pickel, J. Chan*, D. Ganten[1] and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and [1] German Institute of High Blood Pressure Res., Univ. of Heidelberg, Heidelberg, Germany.

The octapeptide, angiotensin II (AgII) is a circulating hormone and a putative transmitter in neural circuits involved in drinking and blood pressure regulation. The humoral and neural pathways containing AgII overlap in regions of the central nervous system (CNS) which are outside the blood-brain barrier including the subfornical and certain other circumventricular organs. Immunocytochemical labeling with the peroxidase-antiperoxidase method alone or combined with immunocolloidal gold was used to examine the ultrastructural localization of angiotensin and angiotensin converting enzyme (EC 3.4.15.1), generously supplied by Dr. R.J. Soffer, Cornell Univ. Med. College, in the subfornical organ of adult male rats. The antisera (CE and DE) to angiotensin cross-reacted most extensively with Ag II and III. Angiotensin-like immunoreactivity (AGLI) was diffusely localized in the extracellular spaces near blood vessels, in a few perikarya and dendrites and in numerous axon terminals. The axon terminals contained small clear and large dense core vesicles and made synaptic contacts principally with dendrites. In addition to the diffuse cytoplasmic labeling, pinocytotic and multivesicular bodies in dendrites, perikarya and terminals also contained AGLI. The immunoreactivity for angiotensin converting enzyme was found on the luminal surface of blood vessels, the apical and basal surfaces of certain ependymal cells, the plasmamembranes of intrinsic neurons, and througout a few small axon terminals. In dual localization studies employing peroxidase and colloidal gold markers, AGLI and the converting enzyme were associated with a few neuronal perikarya and processes. We conclude that the humoral and neuronal afferents containing AGLI coverage on neurons in the subfornical organ and that the converting enzyme has a localization compatible with a role in generation of angiotensin II in the cerebrospinal fluid, in the extracellular spaces from plasma constituents and within neurons in the subfornical organ. (Supported by NIH grants HL18974 and MH00078.)

47.4 **LOCALIZATION OF IMMUNOREACTIVE MET-ENKEPHALIN IN WIDELY DISPERSED INDIVIDUAL VESICLES IN THE GLOBUS PALLIDUS.** H. D. Coulter. Dept of Anatomy, Univ of Minn, Minneapolis, MN 55455.

The purpose of this study was to determine the subcellular location of immunoreactive met-enkephalin in tissue not treated with aqueous fixatives and dehydration media. Globus pallidus from rats was frozen at liquid helium temperature, dried in a freeze-drying apparatus, fixed with osmium tetroxide vapor, and embedded in epoxy resin. 25-100 nm thick serial sections were placed on glass slides for light microscopy and on grids for electron microscopy. Sections on slides were treated with alcoholic NaOH to remove resin, 3% hydrogen peroxide, anti-met-enkephalin (R451 from Immunonuclear at 1:100), and FITC-labeled sheep anti-rabbit IGG. Grids were stained with anti-met-enkephalin at a dilution of 1:30,000 followed by goat anti-rabbit IGG labeled with colloidal gold. For control experiments, serial sections were treated with antisera pre-absorbed with 0.1 mM met-enkephalin. Fluorescence images were obtained with a silicon-intensified-tube television camera. Photographs were taken directly from the television monitor, and printed at a final magnification of 7500 X. Electron microscopic negatives were printed at the same magnification for direct comparison.

Staining was represented in the light microscope by scattered 200-400 nm areas of fluorescence. The positive staining represented flaring of much smaller structures, because fluorescence images in serial experimental sections could not be correlated with one another except in sections thinner than 50 nm. In the electron microscope, colloidal gold was found associated with 80-100 nm vesicles, of average electron density, widely dispersed in the neuropil. Usually one and no more than four vesicles were found in profiles of individual neuronal processes. Adjacent absorption control sections were negative both in the light and electron microscope.

In immunocytochemical studies carried out with pre-embedding staining, immunoreactivity for enkephalin is widely dispersed throughout the cytoplasm of positive cells, with staining found on mitochondria, neurotubules, vesicles of various types, nuclei, and background cytoplasm. In contrast, this approach utilizes post-embedding staining of ultrathin sections of frozen-dried tissue. It reveals precise subcellular localization of immunostaining in a peculiar class of vesicles, overlooked until now, that are found primarily in axonal processes. Supported by NSF grant BNS 80-21004 and NIH grant RO1 NS 18428.

47.5 DIFFERENTIAL PROJECTIONS OF LOCUS COERULEUS NEURONS CONTAINING TYROSINE HYDROXYLASE AND NEUROPEPTIDE Y AND/OR GALANIN. V.R. Holets[1], T. Hökfelt[1]*, L. Terenius[2]* and M. Goldstein[3]. Dept. of Histology 1, Karolinska Institute, Stockholm, Sweden, Dept. of Pharmacology[2], Uppsala Univ., Uppsala, Sweden and Neurochem. Res. Lab.[3], New York Univ. Medical Center, New York, NY.

Populations of locus coeruleus (LC) neurons contain the peptides neuropeptide Y (NPY) and/or galanin (GAL) together with noradrenaline. In the present study, we investigated the projections of these populations of LC neurons to the cerebral cortex (CTX), hypothalamus (HYP) and spinal cord (SC) using retrograde tracing combined with immunohistochemistry.

Rats (n=4 for each group) were injected with Fast Blue (1% in distilled water) 3 days before receiving an intraventricular injection of colchicine. The following injections were made: simultaneous injections into 5 sites in the right CTX, a unilateral injection into the paraventricular and dorsomedial nuclei of the HYP and bilateral injections into the T6 segment. The animals were perfusion fixed 24-36 hr after the colchicine injection. The LC and the injection sites were sectioned (10 µm serial sections) and photographed for Fast Blue labeling. Adjacent sections were then processed for immunohistochemistry using antisera raised against NPY, tyrosine hydroxylase (TH) and GAL. Photographs of the sections were compared to the photographs of the Fast Blue labeling. The number of Fast Blue neurons labeled, the number of TH-, NPY- and GAL-immunoreactive neurons and the number of neurons which contained TH-, NPY- or GAL- immunoreactivity and Fast Blue were counted.

99.17% ± 0.37 (S.E.M.) of the LC neurons contained TH immunoreactivity. Of the total population of TH neurons in the LC, 25.45% ± 2.04 contained NPY and 83.55% ± 0.74 also contain GAL. Following injections into the HYP: 16.07% ± 2.69 of the NPY immunoreactive neurons, 14.21% ± 2.97 of the TH immunoreactive neurons and 15.93% ± 2.47% of the GAL immunoreactive neurons also contained Fast Blue. Following injections into the CTX: 3.20% ± 0.16 of the NPY neurons, 13.82% ± 0.66 of the TH neurons and 4.37% ± 0.32 of the GAL neurons were also retrogradely labeled. Following injections into the SC: 7.97% ± 0.70 of the NPY neurons, 14.51% ± 2.26 of the TH neurons and 5.07% ± 0.55 of the GAL neurons were also retrogradely labeled.

A differential projection to the CTX, HYP and SC of those LC neurons which contain NPY versus those which contain GAL was observed. This may point out functional divisions in the LC based on the peptide contained within the neurons and their afferent projections.

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47.6 COLOCALIZATION OF NEUROPEPTIDES IN AFFERENT PATHWAYS TO THE URINARY BLADDER AND COLON: DEMONSTRATION WITH DOUBLE COLOR IMMUNOHISTOCHEMISTRY IN COMBINATION WITH AXONAL TRACING TECHNIQUES. M. Kawatani, M.B. Houston*, M. Rutigliano*, S. Erdman*, and W.C. de Groat, Dept. Pharmacology, Univ. of Pittsburgh, Pittsburgh, PA, 15261.

A major part of the afferent innervation to cat urinary bladder and colon originates in the sacral dorsal root ganglia (DRG) and is carried in the pelvic nerve (PN). Previously we have shown that a large percentage of visceral afferent neurons projecting to the PN contain neuropeptides (eg., VIP 42%, leucine enkephalin (LENK) 30%, substance P (SP) 27%, and CCK 23%). The total percentage of cells containing individual peptides exceeded 100% indicating that some of these visceral afferent neurons must contain more than one peptide.

This possibility was examined using axonal tracing methods (fluorescent dyes; fast blue and diamidino yellow) in combination with immunohistochemical techniques to identify two peptides on the same section. Different dyes were injected into the bladder or colon wall 7-20 days prior to sacrifice. Colchicine (5 µg) was injected into sacral DRG 31-48 hrs before sacrifice to increase peptide levels. Peptide immunoreactivity was examined using two techniques: (1) a double staining technique in which two antisera prepared in different species were applied to the same sections, then processed with bridge antibodies linked to different fluorochromes (FITC and TRITC) or (2) application of one antiserum to a series of individual thin (6 µm) cryostat sections, where DRG cells could be identified on multiple sections.

Bladder afferent neurons (BA) and colon afferent neurons (CA) which were small to medium size (<40 µ m) were present in the S1-S3 DRG with the largest numbers in S2. BA exceeded the number of CA by 50-100%. Dye injected into the colon also labeled 1-2% of BA. Approximately equal proportions of BA and CA contained SP (32% vs 27%) and LENK (10% vs 12%), however, VIP was present in a considerably higher percentage (50% vs 12%) of BA. Somatostatin was present in a low percentage (<1%) of BA and CA.

Double color immunohistochemistry revealed that 20% of BA contained both VIP and SP. Other combinations of peptides, eg., VIP-LENK, SP-LENK were present in less than 10% of BA and CA. This is in contrast to the more prominent colocalization noted in the general population of sacral DRG cells eg., VIP-SP 60-80%, LENK-SP 40-65%, VIP-LENK 20-40%.

In summary the present results indicate that several types of neuropeptides are present in sacral afferent pathways to the colon and bladder. VIP was most prominent in bladder afferents whereas other peptides were present in both afferent pathways. Certain peptides (VIP-SP, VIP-LENK) were colocalized in these pathways.

47.7 GABAERGIC, SUBSTANCE P-IMMUNOREACTIVE NEURONS IN MONKEY CEREBRAL CORTEX E.G. Jones and S.H.C. Hendry Dept. of Anatomy, University of Calif., Irvine, Irvine, CA 92717

Using immunocytochemical methods, we have discovered a large population of substance P immunoreactive neurons in the monkey cerebral cortex. Sections from monkeys (Macaca fascicularis) perfused with 2% paraformaldehyde and 0.1% glutaraldehyde were incubated in a rat monoclonal antibody to substance P. The anti-substance P antibody was localized with either the avidin-biotin method, using 3,3'-diaminobenzidine HCl as a chromogen, or with an FITC-conjugated goat anti-rat IgG. Control experiments were done using anti-substance P antibody adsorbed with an excess of substance P, bombesin, caerulein, somatostatin, neuropeptide Y and cholecystokinin. The staining was blocked only after substance P adsorption. Substance P positive neurons are exclusively non-pyramidal cells and are present in all areas of the monkey cerebral cortex. The stained cell bodies and processes form two populations: 1) a minority of cell bodies are intensely stained and give rise to long prominently beaded processes. In each area of the cortex, these cells are widely scattered throughout the layers but are most numerous in the subcortical white matter. 2) The majority of substance P positive cell bodies are lightly stained and seldom give off stained processes. They are, instead, embedded in a neuropil that contains numerous, small punctate profiles that are also lightly stained. A marked difference exists among areas of the cerebral cortex in the density and laminar distribution of the lightly stained cell bodies and profiles. They are most numerous in areas of the prefrontal and temporal cortex, in areas of the superior parietal cortex and in area 18 of the occipital cortex. In these areas the cells are clustered in layers IV and V. Relatively few of the cells are found in the granular sensory areas (area 17 and areas 3b,1 and 2) or in the precentral motor area (area 4); there they occur mainly in layers II-III and VI. Using methods for simultaneous detection of two antigens by immunofluorescence, we have found the cell bodies and punctate profiles lightly stained for substance P to be also immunoreactive for GABA. The intensely stained substance P cell bodies and processes display no GABA immunoreactivity but they are also stained for neuropeptide Y. Numerous GABA and NPY immunoreactive are not stained for substance P. We conclude that two populations of substance P neurons exist in the monkey cerebral cortex; one is also GABA positive, gives rise to numerous punctate profiles and varies markedly in density and distribution among areas. The other is also neuropeptide Y positive, gives rise to long beaded processes and in each area consists of neurons present mainly in the subcortical white matter. Supported by NIH Grant NS 21377.

47.8 **INNERVATION OF FOREBRAIN STRUCTURES BY VENTRAL MESENCEPHALIC NEURONS CONTAINING BOTH CHOLECYSTOKININ-AND TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITIES: A FLUORESCENT TRIPLE-LABELING STUDY.** K.B. Seroogy, K. Dangaran, S. Lim, and J.H. Fallon. Department of Anatomy, University of California, Irvine, CA 92717.

The distribution and forebrain projections of dopamine-containing cells in the midbrain substantia nigra-ventral tegmental area (SN-VTA) have been thoroughly described. Recent work from our laboratory has demonstrated the forebrain projection patterns of cholecystokinin (CCK)-containing neurons in this same region. CCK and tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, have been co-localized within subpopulations of these mesencephalic neurons. The purpose of this study was to describe the forebrain projections of those neurons of the SN-VTA containing both CCK and TH and to determine if these projections are distinct from those of neurons containing TH or CCK alone.

The technique of indirect immunofluorescence for two antigens combined with fluorescence retrograde tracing was used. Adult female albino rats received .04-.15 ul injections of the fluorescent dyes Fast Blue or Stilbene Gold into the prefrontal cortex (PFC), caudate-putamen (CP), nucleus accumbens (NAC), or amygdala (AMY). Four to six days later, colchicine was injected intracerebroventricularly and locally near the SN-VTA. After one or two days, the animals were perfused and cryostat tissue sections immunocytochemically processed for the simultaneous localization of TH and CCK using sheep anti-TH (supplied by J. Haycock, Rockefeller Univ.) and rabbit anti-CCK (Immunonuclear Corp.).

The distribution of cells in the SN-VTA containing only TH, only CCK, or both TH and CCK immunoreactivities was similar to previous reports although a higher incidence of co-localization was observed in this study. In all cases following tracer injection into each of the forebrain areas, neurons in the SN-VTA were found to be triple-labeled with both CCK and TH immunoreactivities plus the retrogradely transported dye. With the injection of tracer into the PFC or NAC, triple-labeled neurons were primarily located in the middle VTA. After dye injection into various loci of the CP, triple-labeled neurons were mainly found in the medial SN, with relatively fewer observed in the lateral VTA and middle SN. Following tracer injection into the AMY, the majority of triple-labeled cells were located in the SN pars lateralis and lateral SN. Generally, cells double-labeled for CCK immunoreactivity and dye were located more dorsally in the SN-VTA, whereas cells double-labeled with TH immunoreactivity and dye were found more ventrally throughout this region. These results indicate that CCK projections of the SN-VTA parallel the ascending dopaminergic projections of this area. Particularly noteworthy is the presence of dye-labeled, CCK immunoreactive neurons with or without TH immunoreactivity following injection placement in the CP which suggests that CCK is more significantly involved in the nigrostriatal pathway than previously thought. (Supported by NSRA 5F31MH09090 from NIMH to K.B.S. and NIH grant NS 15321 to J.H.F.)

47.9 LIGHT AND ELECTRON MICROSCOPIC CHARACTERIZATION OF SOMATOSTATIN IMMUNOREACTIVE NEURONS IN RAT VISUAL CORTEX. D. L. Meinecke* and A. Peters. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Results of studies using antisera to somatostatin (SOM), a potential neuromodulator, to examine the types and distribution of SOM immunoreactive neurons in the rat visual cortex have been conflicting. Some authors report these neurons belong to certain non-pyramidal classes, while others include non-pyramidal neurons and some pyramidal neurons. To better identify the types of SOM immunoreactive neurons in the rat visual cortex, and to resolve whether or not they include pyramidal neurons, we used antisera to SOM (Dako) to examine immunoreactive neurons in the light and electron microscope. Adult Sprague-Dawley rats were anesthesized and perfused with buffered solutions of 4% paraformaldehyde for light microscopy, or 5% acrolein for electron microscopy. Blocks of visual cortical areas were cut into 20-40 um slices on a vibratome, reacted with SOM antisera, and immunostained with the Avidin/Biotin system (Vector). SOM immunoreactive neurons were present in all layers of the visual cortex, but were concentrated in layers II/III and V/VI. We could identify bipolar, multipolar, bitufted, horizontal, and subcortical white matter cells. SOM immunoreactive bipolar neurons were present in equal numbers in layers II/III and V/VI and measurements of their sizes revealed they included both the small and large varieties described by Peters and Kara (J. Comp. Neurol. 234:242-263, 1985). Ultrastructurally both large and small bipolar neurons had clefted nuclei, and the large bipolars had prominent stacks of ER at both poles of their cell bodies. Immunoreactive multipolar neurons were also present in layers II/III and V/VI and had rounded cell bodies with several randomly oriented dendrites. Some of these neurons had thin dendrites while others had two or more stout dendrites which tapered as they left the cell body. In the EM, multipolar neurons had folded nuclei and made asymmetric and symmetric axosomatic synapses. Some immunoreactive neurons resembled pyramidal cells because they had a thick, tapering ascending dendrite which soon branched and other prominent dendrites extending from the base of the cell body. These proved to be non-pyramidal neurons, for when examined in the EM their somas possessed asymmetric synapses, and their dendrites lacked spines. Consequently, we suggest that they belong to the population of multipolar neurons with stout dendrites. These results demonstrate SOM immunoreactive neurons all belong to non-pyramidal neuronal classes, some of which use GABA, and others acetylcholine as their putative neurotransmitter.

Supported by NIH grants NS 07016 and T32 NS 07152.

47.10 **PROOPIOMELANOCORTIN-DERIVED NEUROPEPTIDE IMMUNOREACTIVE CELLS OF THE VENTROMEDIAL ARCUATE NUCLEUS ESTABLISH DIRECT SYNAPTIC CONNECTIONS WITH LH-RH NEURONS OF THE MEDIAL PREOPTIC AREA IN THE RAT** Leranth, Cs., MacLusky, N.J. and Naftolin, F. (SPON: W. Collins) Section of Neuroanatomy and Dept. of Obstetrics and Gynecology, Yale University Medical School, New Haven, CT 06510

Pharmacologic evidence suggests that hypothalamic opioid peptide-containing neurons are involved in the control of LHRH release. The present studies were performed to determine whether direct synaptic connections exist between cells in the arcuate nucleus which contain proopiomelanocortin (POMC)-derived peptides (ACTH, β-endorphin, α-MSH) and LHRH-containing neurons in the preoptic area. To establish whether axons from POMC peptide-containing cells form synapses in the preoptic area, rats were pretreated with colchicine (Sigma; 80μg into the lateral ventricle) 24h prior to sacrifice then perfusion fixed (Somogyi and Takagi, Neuroscience 7:1779, 1982). The brains were sectioned on a Vibratome and immunostained for POMC-derived peptides using the peroxidase ABC technique (Hsu et al, J. Histochem. Cytochem. 29:577, 1981). Immunoreactive axo-dendritic synapses were observed most frequently in the medial and ventrolateral parts of the preoptic area. In the medial preoptic area, the synaptic contacts were primarily on dendritic shafts, while in the ventrolateral region they were on dendritic spines. To determine whether some of these synapses might terminate directly on LHRH-immunoreactive cells, we used a recently developed pre-embedding double-label electron microscopic immunostaining procedure in which peroxidase and avidin-ferritin serve as contrasting electron-dense markers (Leranth et al, Histochemistry 82:165, 1985). Direct synaptic connections were observed in the medial preoptic area between axons immunoreactive for the POMC-derived peptides and the dendritic shafts of LHRH neurons. To study the origin of these axons, a combination of immunocytochemical and retrograde horse radish peroxidase (HRP) tracing techniques was employed. Animals were injected with HRP in the medial preoptic area. Two days later, Vibratome sections through the arcuate nucleus were immunostained for ACTH and reacted for HRP using the diaminobenzidine-glucose oxidase method (Zaborszky et al Neuroscience Letters 52:219,1984). The majority of ACTH-immunoreactive perikarya were HRP-negative. HRP-labeled ACTH-immunoreactive neurons were observed primarily in the ventromedial arcuate nucleus, close to the third ventricle. HRP-labeled but ACTH immunonegative neurons were observed distributed throughout the arcuate nucleus. These studies suggest that a subpopulation of POMC neurons in the ventromedial arcuate nucleus may modulate the activity of LHRH cells in the preoptic area through a direct synaptic connection. (Supported by grant HD13587 from NIH, NICHHD and the A. Mellon Foundation.)

47.11 SUBSTANCE P-, CALCITONIN GENE-RELATED PEPTIDE-AND CHOLECYSTOKININ/GASTRIN-LIKE IMMUNOREACTIVE TRIGEMINAL GANGLION CELLS SUPPLY THE EYE: QUANTITATIVE EVALUATION USING IMMUNOHISTOCHEMISTRY AND RETROGRADE TRACING. Y.Kuwayama*, R.A.Stone, G. Terenghi*[1], and J.M.Polak[1]. Department of Ophthalmology, University of Pennsylvania School of Medicine, Scheie Eye Institute, Philadelphia, PA. 19104; and [1]Department of Histochemistry, Hammersmith Hospital, London W12 OHS, U.K.

Using immunohistochemical techniques, substance P (SP), calcitonin gene-related peptide (CGRP) and cholecystokinin/gastrin (CCK) have been localized to peripheral ocular nerve fibers; these nerves are believed to originate in the trigeminal ganglion. In the present study, the combination of immunohistochemistry and retrograde axoplasmic tracing not only confirmed the trigeminal origin of these nerves but also established their quantitative relationship.

Under general anaesthesia, guinea pigs received intraocular injections of the cholera toxin B subunit conjugated to fluorescein isothiocyanate (B-FITC). Two days later, the animals were perfused under pentobarbital anaesthesia with Zamboni's fixative. Sequential cryostat sections of trigeminal ganglia were stained by an indirect immunofluorescence technique with primary antisera to SP, CGRP, and cholecystokinin-octapeptide and with a rhodamine-conjugated secondary antiserum.

Within the entire trigeminal ganglion, the comparative ratio of immunoreactive nerve cells was SP:CGRP:CCK=1:3:0.2. Retrograde labelling of neurons by B-FITC was seen only in the anteromedial region of the ipsilateral trigeminal ganglion. Approximately 5-10% of all B-FITC labelled cells were immunoreactive for SP; 20%, for CGRP; and less than 1%, for CCK.

47.12 THE ORIGIN OF CALCITONIN GENE-RELATED PEPTIDE (CGRP)-IMMUNOREACTIVE NERVES IN THE URINARY TRACT. J. Wharton*, H.C. Su*, T. Katagiri*, M.A. Ghatei*[1], P.K. Mulderry*[1], J. Ballesta*, S.R. Bloom[1] and J.M. Polak. Department of Histochemistry and [1]Medicine, Royal Postgraduate Medical School, London W12, U.K.

We report here the distribution and origin of CGRP- immunoreactive (CGRP-IR) afferents in the urinary tract by combined immunocytochemistry and retrograde labelling with True blue. Adult rats and guinea pig were used (200-300 gm) and anaesthetised with Diazepam (10 mg/kg^{-1} i.p.) and Hypnorm (5 mg/kg^{-1} i.p.). True blue (5% w/v) was injected at 2 - 3 sites (1-6μl total volume) in the rat kidney (hilum), ureter, bladder (dome and trigone), and upper urethra. All injections were on the left side and 4 rats were used per area. Seven days after injection they were perfused transcardially with phosphate buffered saline (PBS) and 4% paraformaldehyde. Animals were also treated with capsaicin, either as neonates (rats; 50 mg kg^{-1} s.c. on the 2nd and 3rd day after birth) or adults (guinea pigs; stepwise over 5 days with 25, 50, 100, 200 and 400 mg kg^{-1} s.c. or i.p.). Controls received vehicle alone (10% alcohol, 10% Tween 80 and 80% saline). Tissues were collected up to 3 months later and fixed in 4% paraformaldehyde or 0.4 benzoquinone in PBS (w/v). After washing in PBS containing 15% sucrose the tissues were processed either as cryostat sections or whole mounts and immunostained with fluorescent or PAP techniques. A specific antiserum was raised in rabbits against synthetic rat CGRP (1-37) conjugated to bovine serum albumin by glutaraldehyde and used at a dilution of 1/200-1/2000.

Numerous CGRP-IR nerves were found distributed throughout the urinary tract, from the renal pelvis to urethra, running in the adventitia, muscle and sub-epithelial layers and in association with blood vessels. No immunoreactive cell bodies were identified in these tissues. The CGRP immunostaining of nerve fibres was almost totally abolished in capsaicin treated rats and guinea pigs, as was substance P immunostaining. The injection of True blue resulted in the retrograde labelling of neuronal cell bodies in specific dorsal root ganglia, the level of which was dependent on the site of injection. Although labelling was also noted in the pelvic, inferior mesenteric and coeliac ganglia, CGRP-IR perikarya were only demonstrated in dorsal root ganglia. These occurred in all the levels examined (T_5-S_2) and were mainly of the smaller type. In those ganglia possessing True blue labelled cells a sub-population of CGRP-IR cells was identified which contained both substances. The results demonstrate that the urinary tract of the rat and guinea pig receives CGRP-IR afferent nerve fibres from perikarya in dorsal root ganglia, the distribution of which is organ-specific.

48.1 AXONAL CONTRIBUTION TO SUBTHRESHOLD CURRENTS IN APLYSIA BURSTING PACEMAKER NEURONS. Richard H. Kramer (Spon. I. Levitan) Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA 94720.

Voltage-clamp studies of molluscan somata have revealed an assortment of ionic conductances which generate the bursting pacemaker cycle. The cell bodies of bursting neurons have been used because of technical advantages (size and identifiabilty) and because they can retain bursting activity following isolation. However, recent evidence suggests that bursts can continue in the absence of voltage changes in the soma, and that spikes during spontaneous bursts are initiated in the axo-dendritic tree (Treistman, Brain Res. (1980) 187:201-205). In this study, the role of the axon in the generation of bursting activity was investigated by using two electrode voltage clamp in Aplysia bursting cell somata in conjunction with intra-axonal recordings. The results demonstrate directly that the axon contributes significantly to the subthreshold currents which underlie bursting pacemaker activity.

Experiments were done on bursting cells L2-L6 of the Aplysia abdominal ganglion. Two electrodes in the soma were used for voltage-clamp; a third electrode containing 6-carboxyfluorescein was used to iontophoretically pass dye into the cell so that the axon could be visualized with a fluorescence microscope. Dye-filled axons were impaled 300-500 uM from the cell body. Depolarizing voltage-clamp pulses in the soma resulted in uncontrolled regenerative responses in the axon. A component of the axonal response was resistent to block by TTX, but was blocked by Ca^{2+} channel blockers (Co^{2+} or Mn^{2+}). The axonal Ca^{2+} spike did not produce noticeable current "notches" in the cell body, although they did distort the time course and magnitude of inward currents generated in response to the somatic voltage-clamp pulse.

Spikes were generated selectively in the axo-dendritic tree by voltage clamping the soma to a constant value (-40 mV) while passing depolarizing current into the axon. Axon spikes were followed by a depolarizing afterpotential (DAP), and bursts of spikes were followed by a long (>10 sec) post-burst hyperpolarization (PBH). As in the soma (Kramer & Zucker, J. Physiol., June, 1985) the PBH was blocked by Ca^{2+} channel blockers, was unaffected by external tetraethylammonium, and did not reverse at E_K; hence the axonal PBH is not due to Ca-activated K^+ current.

The axonal afterpotentials contribute to slow currents resulting from somatic voltage clamp pulses. In some L2-L6 cells, elimination of axonal activity (by axonal hyperpolarization, or by axotomy) blocks the slow currents. In other L2-L6 cells, elimination of axonal activity reduces the slow currents by less than 50%. Hence, the axonal contribution to subthreshold bursting currents is quite variable in different cells. Supported by NSF grant BNS 82-02416 and NIH grant NS 15114.

48.2 PHYSIOLOGIC IMPLICATIONS OF INITIAL SEGMENT STRUCTURE IN FROG SPINAL GANGLION CELLS. J. Rosenbluth, E. Matsumoto* and A. Blight. Depts. Physiology and Rehab. Medicine, New York University School of Medicine, New York, NY 10016

Intracellular recordings from frog spinal ganglion cells have demonstrated an "NM" spike, thought to originate in the non-myelinated initial segment (IS), preceding the "S" spike, thought to originate in the soma (Ito, 1957, Jap. J. Physiol. 7: 297). The threshold for the NM spike is significantly lower than that for excitation of the soma (Ito, 1959, Jap. J. Physiol. 9: 20), suggesting electrophysiological similarities between the initial segment and the node of Ranvier. Morphological examination of the initial segment and axon hillock (AH) regions of frog spinal ganglion cells shows both similarities to and differences from nodes of Ranvier. In freeze-fracture replicas both display unusually high concentrations of E-face intramembranous particles, but at the node these are restricted to a ~ 1 μm band between collars of paranodal axoglial junctions. Additional particles are found under the myelin sheath in the paranodal axolemma and in the juxtaparanodal portion of the internode, but these are isolated by the overlying myelin lamellae and by the extensive paranodal axoglial junctions at both ends of each internode. In the AH and IS, in contrast, E-face intramembranous particles are distributed over a much broader area without obvious boundaries. Moreover, in thin sections the sheath overlying the IS displays multiple interruptions with no evidence of paranodal-type axoglial junctions that might restrict current flow to portions of the axolemma. As a result, much larger numbers of axolemmal particles are accessible to current fluxes during the action potential than is the case at the node of Ranvier. If the E-face particles in the AH and IS plasma membrane represent voltage-sensitive sodium channels, as has been proposed for nodal E-face particles, the morphological findings suggest the presence of much larger overall numbers of sodium channels in the AH and IS than at a typical node, inasmuch as the total area is at least an order of magnitude greater and the particle density comparable. The morphological observations are therefore compatible with very large sodium currents in the AH and IS regions. Our in vitro studies confirm that S and NM spikes are both eliminated by low concentrations of tetrodotoxin before the nodal (M) spike, deeper in the ganglion, is noticeably affected. None of these components is significantly reduced by replacement of calcium with manganese or cadmium. Local amplification of the action potential current in the AH and IS regions would serve to discharge the large somatic membrane capacitance to threshold rapidly. A delayed action potential in the soma could reactivate the axon, as is in fact observed when depolarization of the soma is slowed by shunting of its membrane resistance. Supported by grants from the NIH (NS 07495) and Muscular Dystrophy Association.

48.3 **CONDUCTION IN DEMYELINATED NERVE MEASURED BY CHANGES IN LIGHT ABSORPTION OF POTENTIAL-SENSITIVE DYES.** P. Shrager, S.Y. Chiu*, J.M. Ritchie*, D. Zecevic, and L.B. Cohen. Dept. of Physiology, University of Rochester Medical Center, Rochester, NY 14642 and Depts. of Physiology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Frog sciatic nerves were focally demyelinated by injection of 1% lysolecithin 5-15 days prior to the experiment. Desheathed nerves were soaked in Ringer's solution containing a pyrazoxonol dye (RH155), kindly supplied by Amiram Grinvald and Rina Hildesheim, 1 mg/ml, for 1-2 hours. The optical path was placed between pairs of Pt wires for external stimulation and recording. A Leitz Ortholux II microscope formed a real image of the nerve on a photodiode array oriented with rows of elements parallel to the axons. The incident light was filtered at 705 nm. Absorption changes were recorded from control regions of the nerve following moderate stimuli. Conduction velocities were measured by calculating the time to peak change in light intensity. In control segments these velocities were in excess of 5 m/sec, values typical of A and B myelinated fibers in this preparation. In the demyelinated zone optical signals decreased in amplitude, generally reaching minimum levels of detection 0.5-1 mm beyond the transition region. These signals 're-emerged', however, at the distal end of the 2-3 mm demyelinated zone indicating that the decrease in amplitude was not due to conduction block in increasing numbers of fibers. Conduction velocities in the demyelinated area (measured near the boundary) were 0.4-0.8 m/sec, significantly lower than in control segments.

4-Aminopyridine (4-AP), a K^+ channel blocker, has been shown to widen the frog myelinated nerve action potential about 2-fold. Optical signals, which represent the summed changes in many fibers with a spectrum of conduction velocities, doubled in amplitude after addition of 2.5 mM 4-AP. Optical records also showed that after K^+ channel block action potentials were markedly enhanced in the demyelinated zone and conduction could be followed throughout this region.

Thus, optical techniques can be used to monitor accurately electrical excitability in demyelinated nerve. This method may ultimately be of value in studies of the central nervous system, in regions in which conduction is not readily followed electrophysiologically.

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48.4 VOLTAGE-DEPENDENT ION CHANNELS IN GLIAL CELLS. B.A.Barres, L.L.Y. Chun*, and D.P.Corey. Program in Neuroscience, Harvard Medical School, Neuroscience Group, Howard Hughes Medical Institute and Department of Neurology, Massachusetts General Hospital, Boston, Ma. 02115.

The traditional notion that glial cells have passive membranes permeable only to potassium has been revised by recent patch-clamp studies of cultured mammalian glial cells that reveal voltage- and ion-dependent ion currents (Chiu et al., Nature 1984; Quandt and MacVicar, Soc. Neurosci. Abstr. 1984). We have used the postnatal rat optic nerve preparation developed by Raff and coworkers to prepare neuron-free cultures of each of three glial cell types (type 1 and type 2 astrocytes and oligodendrocytes). Each type can be positively identified by a unique surface antigenic phenotype, which correlates with a specific morphology in culture. For studies with freshly dissociated cells in suspension, cells were labelled with antibodies and observed with a fluorescence microscope during electrophysiological study. Because pretreatment with antibodies was time-consuming and subject to the possibility that it might change physiologic properties of the cell studied, we further developed a Percoll density gradient separation technique with which we can rapidly separate out greater-than-90% pure populations of each glial subtype.

Ionic currents were studied in cultured glial cells using the whole cell and cell-attached patch-clamp recording configurations. These reveal a voltage-sensitive sodium current in type 2 astrocytes which has a voltage-dependent inactivation and is blocked by TTX. This conductance differs quantitatively from mammalian neuronal sodium conductance in its timecourse and voltage-dependence. We have also identified a voltage-dependent calcium current in these cells which is blocked by cobalt and is characterized by a strong voltage-dependent inactivation. There is also a TEA-sensitive potassium current.

In contrast, oligodendroglial cells were not observed to have sodium or calcium conductances but exhibit a large voltage-sensitive potassium permeability that is different in its characteristics from that of the type 2 astrocyte. Whole cell and single channel recording reveal it to be inactive only at large negative potentials, and to pass inward current better than outward current (Corey, Barres and Chun, this meeting).

Two important issues are unresolved: First, at what point do the electrophysiological characteristics of the oligodendrocyte and the type 2 astocyte diverge in development, since these arise from a common progenitor cell, the O2A? Second, are these ion channels normally present in glial cells in vivo, or are they artifacts of the tissue culture environment? These questions are now being studied by recording from cells in suspension immediately after optic nerve dissociation.

48.5 AN INWARDLY RECTIFYING POTASSIUM CHANNEL IN OLIGODENDROGLIA. D.P.Corey, B.A.Barres, and L.L.Y.Chun*. Neuroscience Group, Howard Hughes Medical Institute and Department of Neurology, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Boston, Ma. 02114

Oligodendroglial cell membranes have been shown to be exclusively permeable to potassium (Kettenman et al., J. Neurosci., 1983) and this permeability has been thought to be passive, that is, not voltage-sensitive. To determine whether there is a voltage dependence to the potassium permeability in oligodendroglia, we have used the patch-clamp recording technique to study oligodendroglia cultured from postnatal rat optic nerve with the procedure developed by Raff and coworkers.

Oligodendroglia were first studied with the whole cell patch-clamp recording configuration, using the following solutions (in mM). Outside: 20 KCl, 100 TMA-Cl, 2 Ca; inside: 120 K aspartate, 10 EGTA, and no added Ca. In all cells studied under these conditions we observed a large current at potentials more positive than about -110 mV, which reversed near the potassium equilibrium potential. Inward current was increased when external potassium was raised. The current-voltage relationship showed marked inward rectification: outward current was much less than inward current even with these solutions. This current was completely blocked by barium but was not blocked by 20 mM TEA or 1 mM 4-AP.

Preliminary experiments were done with single channel recording in the cell-attached configuration, using a 130-mM KCl solution in the micropipette. In one case a channel was observed which activated at 50 mV below resting potential, and was almost always open at resting potential or above. The single channel conductance at negative potentials was 140 pS at 21°C in this solution. The single channel current-voltage relation demonstrated marked inward rectification, with little current flow in an outward direction.

This channel differs from many inward rectifiers that have been studied in that it is positively activating, but it is very similar to an inwardly rectifying potassium channel recently observed in cultured hippocampal neurons (Sullivan and Cohen, Biophys. J., 1985). It differs from channels observed in cultured mouse spinal cord oligodendroglia (Kettenman et al., Pflugers Arch., 1984) primarily in its large conductance and in its rectifying current-voltage relation. The difference could be explained by a heterogeneity of oligodendroglia, but more likely reflects the low external potassium concentrations used in the spinal cord studies.

This channel may play an important role in the ability of oligodendroglia to accumulate and spatially buffer potassium.

48.6 MODULATION OF REPETITIVE FIRING IN BULLFROG SYMPATHETIC GANGLION CELLS BY TWO DISTINCT K CURRENTS, I_{AHP} AND I_M. P.Pennefather†, S.W. Jones and P.R.Adams. Dept. Neurobiology, SUNY Stony Brook, NY 11794 Fac. Pharmacy, Univ. of Toronto, Toronto, Ont, M5S 1A1†.

I_{AHP} is a voltage insensitive K current that is evoked by Ca influx during an action potential (Pennefather et al. P.N.A.S. 1985 in press). I_M is a voltage sensitive K current that is half maximally activated at -35 mV. At potentials near threshold for action potential generation (-40 to -30 mV) both I_M and I_{AHP} show exponential kinetics with time constants in the hundreds of milliseconds. Both currents are small (1-3 nA at -40 mV) compared to K currents responsible for spike repolarization but, because of their slow kinetics, modulate repetitive firing.

Maximal activation of receptors for muscarine, LHRH or ATP cause a 90% inhibition of I_M and a 30% inhibition of I_{AHP}. Apamin and the neuromuscular blocking agents d-tubocurarine (dTC) and pancuronium inhibit maximally 80% of I_{AHP} without affecting I_M. We have used 10 μM muscarine and 200 μM dTC (10X their half maximal blocking concentrations) to investigate the influence of I_M and I_{AHP} on repetitive firing.

Membrane currents and membrane potential were measured by repeated switches between single electrode voltage and current clamp (hybrid clamp). Cells, with resting potential $<$-45 mV and input resistance $>$70 MΩ, were manually clamped to -60 or -55 mV. Depolarizing current steps of 1-2 nA typically evoked 1-3 spikes after which the membrane potential settled to a new level of around -45 mV, i.e. a potential where sufficient I_M is activated to balance the injected current. In the presence of dTC this step evoked a burst of spikes for about 100 ms after which the potential settled to a level similar to that in the absence of dTC. The duration of the discharge reflects the the time needed to develop sufficient I_M. When I_M is inhibited by muscarine the current step causes a greater steady depolarization. There are a few more spikes early in the step and spikes occur irregularly thereafter. Muscarine and dTC together have a synergistic effect on repetitive firing. Yet, even when both I_M and I_{AHP} are inhibited, repetitive firing is not maintained.

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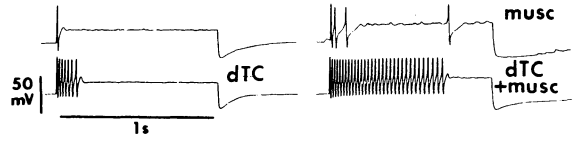

2nA step, holding potential -60 mV, AP's clipped by chart recorder

48.7 COMPUTER SIMULATION OF BULLFROG SYMPATHETIC GANGLION CELL EXCITABILITY. C. Koch and P. R. Adams. The Center for Biological Information Processing, Massachusetts Institute of Technology, Cambridge, Mass., 02139 and Department of Neurobiology & Behavior, State University of New York, Stony Brook, N.Y., 11794.

Using conventional microelectrodes and patch clamp techniques, seven distinct voltage-dependent currents have been characterized in type B bullfrog sympathetic ganglion cells. We have now modeled the electrical behavior of these cells under current-clamp conditions in terms of a fast sodium current (I_{Na}), a calcium current (I_{Ca}), a transient K^+ current (I_A), a non-interacting muscarine-sensitive K^+ current (I_M), a large sustained delayed rectifying K^+ current (I_K), a fast Ca^{++}-dependent K^+ current (I_C), a slow Ca^{++}-dependent K^+ current (I_{AHP}), intracellular calcium buffering and diffusion, extracellular potassium accumulation, membrane pumps and nicotinic, muscarinic and peptidergic -induced synaptic conductance changes. The underlying equations for these processes were developed using either experimental data or literature values. This interactive and graphic computer program accounts for the electrical behavior of the cell under a variety of conditions (e.g. voltage-clamp). In particular, the model satisfactorily reproduces the waveform of individual action potentials, both before and after inhibiting Ca^{++}-dependent potassium currents, and for a wide variation of input impedances. The model reveals that under normal conditions, an action potential leads to a large transient increase in I_C, which is replaced by a similar increase in I_K if calcium influx is blocked. In agreement with experimental data (see Pennefather, Jones and Adams, this volume), the model predicts that maintained repetitive firing during depolarizing current steps will only be seen if both I_M and I_{AHP} are inhibited at the same time. These two currents both subtract from the stimulus current, and hence act cooperatively, albeit in response to different stimuli (i.e. voltage or calcium).

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48.8 DIFFERENTIAL EFFECTS OF Co^{++} AND TEA ON SPIKES OF FROG DORSAL ROOT GANGLION NEURONS. S. D. Stoney, Jr., Dept. of Physiology, Medical College of Georgia, Augusta, GA 30912.

Four types of neurons are distinguishable in frog dorsal root ganglia (DRG) based on shape of the somatic spike, orthodromic conduction velocity (CV) and least conduction interval (LCI) for pairs of orthrodromic impulses at their intraganglionic axon branch point (Hershberger and Stoney, Neurosci. Abstr. 10:109, 1984). I have attempted to determine the causes for different spike shape by superfusing DRG neurons with solutions containing Co^{++}, Co^{++} & TEA, TEA, TEA & Co^{++}, or excess Ca^{++}. 10mM Ca^{++} caused the appearance or accentuation of a hump on the spike falling phase for all neurons tested, suggesting that a Ca^{++}-sensitive outward current contributes to repolarization. Co^{++} (10mM, 3-5 mins) followed by Co^{++} & TEA (10mM each, 3-5 mins) had significantly different effects on the repolarization phases of different types of neurons. Spikes of F neurons (CV≈20 m/sec; LCI≈1.7 msec; brief & smooth) were slightly affected by Co^{++} alone and greatly affected by the addition of TEA. The maximum rate of repolarization (-dv/dt) was decreased by 39% by Co^{++} alone and an additional 243% by TEA. Spike duration at 50 mv (D50) was increased by 70% and 174% by the two treatments. In contrast, H1 neuron spikes (CV, etc., the same as F neurons) were strongly affected by Co^{++} and by TEA. D50 was increased by 130% and 120% while -dv/dt was decreased by 59% and 57% by Co^{++} and Co^{++} & TEA, respectively. Spikes of H2 neurons (CV ≈ 7 m/sec; LCI≈2.2 msec; broad with slight hump on falling phase) were more strongly influenced by Co^{++} than by TEA. D50 was increased by 73% and 28% while -dv/dt was decreased by 60% and 28% by the two treatments. Spikes of H3 neurons (CV≈7 m/sec; LCI≈4.8 msec; very broad with marked hump on falling phase) were reversibly blocked by Co^{++}. Other neurons were treated with 10mM TEA followed by TEA & Co^{++} (10mM each). Co^{++} caused a lengthening of F, no change in H1 and a shortening of H2 neuron TEA spikes. These results suggest that differences in spike shape among different types of frog DRG neurons are due to quantitative differences in the extent to which g_{Ca}, $g_{K(Ca)}$ and $g_{K(V)}$ are activated during the spike. The importance of g_{Ca} and $g_{K(Ca)}$ in spike electrogenesis appears to be graded across neuron types H3>H2>H1>F. This is nearly identical to the way CV (H3<H2<H1 = F) and LCI grade across neuron types (H3>H2> H1 = F). Supported by BRSG # 2S-07-RR-05365-23.

48.9 EXPRESSION OF IONIC AND SYNAPTIC MECHANISMS UNDERLYING ELECTRICAL EXCITABILITY IN DEVELOPING PURKINJE NEURONS. D.L. Gruol and C.L. Franklin*, Div. of Preclin. Neurosci. and Endocrin., Scripps Clinic and Research Foundation, La Jolla, CA 92037

The Purkinje neuron (PN) of the cerebellum exhibits characteristic patterns of electrical activity generated by synaptic input and endogenous, voltage-sensitive mechanisms. Similar patterns of activity are express by mature PNs derived from fetal rat tissue and differentiated in culture (Gruol, Brain Res., 263, 1983). In culture, PN activity typically consists of regular patterns of simple spikes, generated by pacemaker potentials, with intermittent bursts events similar to complex spikes (CS). We have investigated the expression of these mature electrophysiological characteristics during the course of PN development using the cerebellar culture system as a model. Initially, immunohistological techniques were used to identify the immature PNs in culture and extracellular recordings were used to characterize the activity patterns (Franklin and Gruol, Neurosci. Abst, 1984). We have now used intracellular recordings to examine the underlying membrane and ionic mechanisms, as a first step in establishing the timing and sequence in expression of the specific ion channels mediating the activity. The cultures are prepared from 20 day rat embryos (1 day before birth) and earliest culture age at which PNs could be identified was 4 DIV (DIV= days in vitro). At this age, the majority of PNs were silent. However, the excitatory transmitter glutamate could evoke action potentials (APs) in some of the silent PNs, indicating that this age represented a transition period in development of electrical excitability. By 6 DIV, which is well before the main period of synaptic and dendritic development, the majority of PNs displayed slow, spontaneous activity consisting of synaptic events and APs. In some of these PNs, the APs were soley generated by the synaptic events, which tended to occur in intermittent patterns. In other PNs, the endogenous mechanisms were the predominant triggering factor and patterns were more regular. Both the synaptic and endogenous mechansism evoked doublet spikes of full amplitude, as opposed to the single and complex type spikes typical of later stages. Na+, Ca++, and K+ channel blockers significantly altered the activity patterns and current evoked APs of these young PNs, indicating that a variety of ion channels had been expressed by this early developmental stage. These data indicate that both synaptic and endogenous mechanisms are expressed early in PN development, that chemical excitability and synaptic input mediate the first patterns of spontaneous activity and that expression of the ionic mechanisms underlying AP generation precede expression of the endogenous mechanisms responsible for maintaining the activity. (supported by NIH grant NS21777)

48.10 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF IDENTIFIED, INTRINSIC CORTICAL NEURONS IN DISSOCIATED CELL CULTURE. J.M. Nerbonne[1], A. Burkhalter[2] and J.E. Huettner[3], Depts. of Pharmacology[1] and Neurosurgery[2], Washington Univ. Sch. of Med., St. Louis, MO 63110 and Neurobiology[3], Harvard Med. Sch., Boston, Mass. 02115.

It is likely that the membrane properties of individual cortical neurons are functionally as important as the attributes of their interconnections. Characterizing these properties should, therefore, contribute to the understanding of the mechanisms underlying cortical information processing. Using the whole-cell patch-clamp recording technique, we have determined the properties of voltage-activated currents in callosal projecting neurons in dissociated cell culture.

Neonatal Long-Evans rats were injected at postnatal day 3-5 with rhodamine labelled beads, a retrograde tracer suitable for in vitro visualization of neurons (Katz et al., Nature, 310: 498 (1984)). Injections were made into primary visual cortex (area 17) of the left hemisphere. Histological examination after 2-5 days, revealed bead labelled cells distributed throughout the contralateral area 17, in layers 3-5. For the preparation of cultures, tissue pieces from this area were dissociated (Huettner and Baughman, Soc. Neurosci., 165 (1984)). A large number of labelled cells are obtained; these cells retain beads and survive for several weeks in culture. Lucifer Yellow injections of labelled cells revealed extensive process outgrowth and functional synaptic contacts are formed.

In the whole-cell recording configuration, with 140 mM Na^+ and 5mM Ca^{++} in the bath and 140 mM K^+ in the pipette, depolarizations from holding potentials -30 to-80 mV, revealed inward and outward currents. In all cells studied under these conditions, a fast TTX-sensitive inward Na^+ (I_{Na}) current and an outward K^+ (I_K) current, resembling the delayed rectifier, were seen. With 140 mM Cs^+ in the recording pipette, K^+ currents were blocked and depolarizations revealed a second (slow) inward current, carried by Ca^{++} and blocked by Co^{++} (I_{Ca}). When I_{Na} and I_{Ca} were blocked (1uM TTX and 5 mM Co^{++}), two components of outward current were distinguished: a fast transient K^+ current (I_A) and the slower, non-inactivating I_K. With Ca^{++} in the bath, it is evident that at least one component of outward current is Ca^{++}-dependent.

Using this methodology, we have identified voltage-activated currents suggested previously by intracellular recordings in cortical slices. These currents are recorded from cells within hours after isolation and most likely are present in vivo. In addition, their properties are not altered in vitro, as similar results are obtained 6 hr. to 2 wk in culture. Employing this approach, we anticipate that we shall similarly be able to study transmitter modulation of ionic currents in identified cells.

48.11 VOLTAGE-DEPENDENT CURRENTS IN EMBRYONIC CULTURES OF DROSOPHILA NEURONS. Lou Byerly, Section of Neurobiology, Department of Biological Sciences, University of Southern California, Los Angeles, California 90089.

Patch clamp studies are being performed on Drosophila neurons in cultures prepared by disaggregating whole gastrulating embryos as described by Seecof et al. (Exp. Cell Res 69:161, 1971). Neuroblasts differentiate in vitro during the first 24 hr into neurons that can form functional neuromuscular junctions with the myocytes in the culture (Seecof et al., Proc. Natl. Acad. Sci. USA 69:566, 1972). The nerve cell bodies (3-8μm in diameter) are usually found in clusters from which radiate bundles of nerve processes, but isolated nerve cell bodies are also present. Tight seals of 5-20GΩ readily form between the membrane of these nerve cell bodies and patch clamp electrodes. Frequently spontaneous biphasic currents (5-15pA in amplitude) are recorded from these on-cell patches, indicating the presence of action potentials in the neuronal somas. When the patch electrode contains an extracellular solution (125mM Na^+, 6mM K^+, 5mM Ca^{2+}, 5mM Mg^{2+} and 10mM HEPES, pH 7.4), outward single-channel currents are observed during depolarization of the patch. These single-channel currents are probably carried by K^+ and indicate single-channel conductances of several sizes, the largest being about 40 pS. Before characterizing the single-channel currents, a study of the macroscopic currents of the Drosophila nerve cell body is being undertaken, using whole-cell voltage clamp studies. Although seals to the membrane are formed equally well when the patch electrode contains an intracellular solution (144mM K^+, 10mM HEPES, 5mM EGTA, pH 7.3), pulses of negative pressure tend to break the seal instead of the patch membrane. When a whole-cell clamp is successfully established, the total resistance is 5-10GΩ. With 144mM K^+ in the patch electrode only net outward currents are evoked by depolarizing voltage pulses, when the cell body does not have processes. These currents activate rapidly (fully activating in 5ms at 0mV) and then slowly inactivate (decaying about 30% in 100ms at 0mV). This conductance is first activated about -30mV and reaches 5nS at 0mV (50 times larger than the conductance at -60mV). Repetitive transient inward currents are frequently recorded during depolarizing pulses in neurons with processes, probably indicating Na currents from regions of the membrane where the voltage is not controlled.

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48.12 COMPLEMENTATION ANALYSIS OF SHAKER MUTANTS IN DROSOPHILA. L. C. Tinpe*(SPON: L. Y. Jan). Howard Hughes Medical Institute and the Dept. of Physiol., Univ. of Calif., San Francisco, San Francisco, Calif. 94143

Previously described mutations of the Shaker locus either eliminate a transient potassium current, the A current, or alter its inactivation kinetics (Salkoff and Wyman, Nature 293: 228-230, 1981). Using intracellular recording and voltage clamp techniques I have examined the phenotypes of sixteen additional Shaker mutants, and have used complementation tests to study the genetic complexity of the locus. Ten of the sixteen mutants have no detectable A current in pupal indirect flight muscle. Measurement of A current peak amplitudes in Sh^{null}/Sh^+ heterzygotes shows that while some of the null mutations are almost completely recessive, others are partially dominant. Crosses between different members of the null class give progeny which likewise have no A current. These ten null mutations therefore identify a single locus required for the presence of normal A current.

In four of the remaining mutants A current is present, but the peak amplitude is reduced compared to its level in wild type. One of the four, B55, is an X:Y chromosomal translocation with a breakpoint in or near Shaker. Male pupae of the genotype Sh^{null}/proximal B55 have A current, demonstrating that Shaker is proximal to the B55 breakpoint.

The two mutants of a final class have close to normal A current in pupal muscle, but have the same severely mutant phenotype of synaptic transmission at the larval neuromuscular junction as do the null mutations. These mutations map to the Shaker region by recombination. Tests against null alleles show non-complementation of the abnormal phenotype at the neuromuscular junction. Since altered synaptic transmission at the larval neuromuscular junction is thought to be due to a defect in a neuronal potassium channel (Jan, Jan and Dennis, Proc. R. Soc. Lond. B. 198: 87-108, 1977), these mutations seem to identify a region of the Shaker locus necessary for the expression of the A channel in nerve but not in muscle.

49.1 NEUROCHEMICAL PROFILE OF FENGABINE, A NEW ANTIDEPRESSANT DRUG. B. Scatton, T. Dennis*, B. Zivkovic*, K.G. Lloyd, S. Arbilla, S.Z. Langer and G. Bartholini*.(SPON : B. Onteniente). Biology Department, LERS, Paris 75013, FRANCE.

FENGABINE (SL 79.229) [2-(butylimino)(2-chlorophenyl) methyl -4-chlorophenol] is a new GABA-mimetic agent which possesses marked therapeutic properties in patients with major depression. However, its neurochemical profile in animals differs from that of tricyclic antidepressants. Thus, fengabine does not inhibit the re-uptake of cerebral norepinephrine or serotonin ; whilst, after acute and chronic treatment, this agent increases the turnover of cerebral norepinephrine in the rat as indicated by the increased utilization rate of norepinephrine and the rise in 3,4-dihydroxyphenylethyleneglycol and normetanephrine levels in the hypothalamus, septum and nucleus accumbens (ED_{50}=50-75 mg/kg i.p.). Fengabine (100 or 200 mg/kg i.p., b.i.d.) given repeatedly for two, but not one, weeks, caused a strong desensitization of isoprenaline-stimulated adenylate cyclase in slices of the septum and cerebral cortex. This desensitization seems to be due to an uncoupling of the enzyme from the β-receptor as repeated treatment with fengabine neither decreases basal activity of the enzyme nor down-regulates the β-adrenergic recognition site. No desensitization of the adenylate cyclase stimulated by dopamine (striatum) or by sodium fluoride (septum) occurred under these conditions. Furthermore, chronic treatment with fengabine failed to change the density of cortical α_1 and α_2-adrenoceptors. However, the compound given repeatedly for 6-18 days enhanced $GABA_B$ binding in the rat frontal cortex as do classical antidepressants. The effects of fengabine on noradrenergic transmission are relatively selective as neither acute nor repeated administration of this drug influences serotonin-related parameters and diminishes slightly striatal acetylcholine and dopamine turnover. Moreover, chronic treatment with fengabine did not affect the density of ^{3}H-spiperone binding to 5-HT_2 receptors in the frontal cortex. Finally, in agreement with its GABA mimetic activity, fengabine decreases cerebellar cyclic guanosine 3',5'-monophosphate levels upon acute treatment in the rat. In conclusion, the acceleration of cerebral norepinephrine turnover by fengabine suggests that this compound increases the amine concentrations in the synaptic cleft as do the classical antidepressants, although by a different mechanism (probably via an increase in the firing rate of noradrenergic neurons). Activation of central noradrenergic transmission may be involved in the antidepressant action of the compound.

49.2 PUMILIOTOXIN B: A POTENTIAL BIOCHEMICAL TOOL FOR THE STUDY OF PHOSPHATIDYLINOSITOL METABOLISM. F. Gusovsky*, E.B. Hollingsworth* and J.W. Daly. Laboratory of Bioorganic Chemistry, NIADDK, NIH, Bethesda, MD 20205.

Pumiliotoxin B (PTX-B), an alkaloid from the skin of the neotropical frog Dendrobates pumilio, possesses marked myotonic and cardiotonic activity (Mensah-Dwumah and Daly, Toxicon, 16:189, 1978; Albuquerque et al., Mol. Pharmacol., 19:411, 1981; Daly et al., J. Med. Chem., 28:482, 1985). The molecular mechanism for these actions of PTX-B are poorly defined, but may involve effects on the mobilization of calcium that is required for excitation-secretion coupling at synaptic terminals and excitation-contraction coupling at postsynaptic sites.

Biochemical actions of PTX-B now have been investigated with respect to phosphatidylinositol (PI) turnover in guinea pig cerebral cortical synaptoneurosomes and mouse atrial preparations. PI turnover was measured as accumulations of [^{3}H]inositol phosphates in the presence of 10 mM LiCl in preparations prelabeled with [^{3}H]-inositol. Effects on PI turnover, in particular the formation of inositol triphosphate, which is known to release calcium from internal storage sites, was considered as one possible basis for the pharmacological actions of PTX-B.

PTX-B was found to significantly increase PI turnover in brain preparations as do a variety of biogenic amines and muscarinic agonists. In synaptoneurosomes, PTX-B elicits significant PI turnover at concentrations as low as 0.5 μM with maximal accumulation of [^{3}H]inositol phosphates (140 to 170 percent of control) occurring at concentrations of 5 to 10 μM. The effects of PTX-B are not blocked by muscarinic or α-adrenergic agonists and are, therefore, not mediated via such receptors. Phorbol-12-myristate-13-acetate prevents both PTX-B-elicited PI turnover and PI turnover elicited by muscarinic and α-adrenergic agonists in synaptoneurosomes. PTX-B also elicits accumulations of [^{3}H]inositol phosphate in mouse atria and, therefore, may represent a biochemical tool for investigation of PI turnover and its role in physiological functions in a variety of cells and tissues.

49.3 GABA-MEDIATED SYNAPTIC INHIBITION IN RAT SUPRAOPTIC NUCLEUS (SON) NEUROSECRETORY NEURONS IN VITRO. J.C.R. Randle, C.W. Bourque and L.P. Renaud. Neurosciences Unit, Montreal Gen. Hosp. Res. Inst. and McGill University, Montreal, Canada H3G 1A4.

Intracellular recordings of rat SON neurons in perfused explants of hypothalamus (cf. Bourque & Renaud, J. Neurosci. Methods 7:203, 1983) reveal a high frequency of spontaneous hyperpolarizing post-synaptic potentials (psps). These spontaneous psps rise to peak in 3-5 msec; their decay is roughly exponential with a mean time constant of 16.8 msec, 1.6-fold longer than the cell time constant for decay of a hyperpolarizing pulse. These may arise from the anteroventral third ventricular (AV3V) region where focal stimulation evokes graded hyperpolarizing psps that peak in 3-10 msec and decay exponentially with a mean time constant of 32.9 msec. Both spontaneous and evoked psps are reduced or abolished by tetrodotoxin (10^{-6} M) or 15mM $MgCl_2$. In recordings with potassium acetate (KAc)-filled electrodes the mean psp reversal potential (Epsp) is -72mV. Reversal potentials for spontaneous and evoked psps agree within 1 mV. Recordings obtained with KCl electrodes reveal a mean Epsp of -45mV and replacement of medium NaCl with Na Glucuronate induces a positive shift of Epsp indicating a chloride dependence of the psps. Conductance changes associated with spontaneous psps are consistently near 0.65 nS. Maximal conductance values for evoked psps vary widely between cells (range 1.5-20 nS). Bicuculline methiodide (BMI, 10^{-6} -10^{-4} M) reversibly abolishes both spontaneous and evoked psps. Pentobarbital (10^{-4} M) prolongs both spontaneous and evoked psps, increasing the time constant of decay 2-3 fold.

These data suggest that the majority of this spontaneous synaptic activity is inhibitory (ipsps) and may be mediated by a GABA-activated chloride conductance. GABA (10^{-5} -10^{-3}M) induces 5-20nS increases in input conductance in SON neurons. In about 60% of cells recorded with KAc electrodes, GABA induces a membrane hyperpolarization (reversal potential near -75 mV, in agreement with Eipsp) which, at high doses of GABA can be followed by a depolarization (reversal potential near -50 mV). In the remaining cells recorded with KAc electrodes, and in all cells recorded with KCl electrodes, GABA induces a membrane depolarization with a reversal potential near -40mV. These effects of GABA are blocked by BMI and mimicked by muscimol, properties of a GABA-A receptor-mediated process.

We conclude that in the hypothalamic explant, GABA-mediated ipsps form the majority of spontaneous synaptic activity. In vivo, synchronization of this powerful inhibitory input may have an important influence on SON neuronal firing patterns. Supported by the MRC and FRSO.

49.4 EFFECTS OF CHRONIC ETHANOL ADMINISTRATION ON SYNAPTIC MEMBRANE NA^+-CA^{2+} EXCHANGE ACTIVITY. E. K. Michaelis, M. L. Michaelis, E.W. Nunley*, and N. Galton*. Dept. of Human Development and Center for Biomedical Research, Univ. of Kansas, Lawrence, KS 66046.

We have recently reported that ethanol and other short chain alcohols inhibit the activity of the Na^+-Ca^{2+} exchange system in brain synaptic plasma membrane vesicles. This transport system is sensitive to ethanol at low concentrations (IC_{50}≈ 15 mM) when the activity is measured at physiological temperatures.

The present studies were undertaken to determine whether chronic ethanol administration leads to alterations in this Na^+-Ca^{2+} antiporter system that might be indicative of an adaptive response to alcohol. Sprague Dawley rats were maintained for 3 weeks on a diet that contained 8% ethanol or sucrose substituted for ethanol in controls. Following the drug treatment, animals were sacrificed, the brains removed, and highly purified synaptic plasma membranes isolated as described earlier (J. B. C. 258, 6101, 1983). The Na^+-dependent Ca^{2+} transport activity in the two plasma-membrane fractions obtained was measured at various Ca^{2+} concentrations. Membrane vesicles (≈10-20 μg protein) were loaded internally with 150 mM NaCl and diluted 20-fold into an incubation medium containing either 150 mM KCl ($+Na^+$ gradient in → out) or 150 mM NaCl ($-Na^+$ gradient), plus the indicated concentrations of isotopically-diluted $^{45}CaCl_2$. Incubations were carried out for 8 sec at 24°C. Membranes from each pair of control and ethanol-treated animals were processed and tested together. Results of these experiments revealed that the 3-week ethanol regimen brought about a significant increase in the Na^+-dependent Ca^{2+} transport activity only in the membrane fraction enriched in synaptic junctional complexes. These membranes showed a near doubling in the specific activity of the antiporter in alcohol-treated compared to the control animals. The kinetic constants determined from computer fitting of the data to the Michaelis-Menten equation were: K_{act} for Ca^{2+} = 28 μM and 31 μM and the V_{max} = 3.1 and 4.3 nmol/mg protein for control and alcohol-treated animals respectively. Changes in the kinetic parameters were reversible as the Na^+-Ca^{2+} exchange activity in these membranes from animals maintained on alcohol for 3 weeks and then withdrawn for 1 week was indistinguishable from that of membranes from control animals. Thus it appears that this Ca^{2+} antiporter which is quite sensitive to the presence of ethanol in vitro is also sensitive to the chronic in vivo presence of this agent, and treated animals make a reversible adaptation in their neuronal cell membranes to compensate for its effects. (Supported by grants AA 04732 and AG 04762 and the Ctr. Biomed. Res., Univ. of KS.)

49.5 MODULATION OF EVOKED SYNAPTIC POTENTIALS BY ADENOSINE AND CAFFEINE. R.W. Greene* and H.L. Haas* (SPON: D. Weinreich). Neurochirurgische Universitätsklinik, CH 8091 Zürich, Switzerland.

Adenosine and caffeine affect the same neuronal membrane properties but in the opposite manner. These include membrane potential and resistance, the long lasting after hyperpolarization and accommodation of firing. The adenosine dose-response curve of the population EPSP is shifted to the right by caffeine (Haas & Greene, Pflügers Arch. 402,244; Greene et al., Brit. J. Pharm., 1985; J. Physiol., 1985). We have now examined the effects of adenosine and caffeine on the intracellularly recorded EPSP-IPSP evoked by stimulation of the stratum radiatum in CA 1 neurones in rat hippocampal slices. Adenosine (20 µM) reduced this compound potential by 50% with less than a 2 mV hyperpolarization of the membrane potential, in confirmation of Siggins & Schubert (1981) and Segal (1982). This could result in part from an adenosine evoked increase in gK (Ca) which may reduce conduction of the EPSP from dendrites to soma. Caffeine (50 µM) increased the EPSP 15-28% with less than a 1 mV depolarization of the membrane potential. The time course of the EPSP-IPSP sequence was unchanged. These observations are consistent with a mechanism of action for caffeine as an antagonist of adenosine, which is normally present in the CSF in an electrophysiologically active concentration. A late potassium dependent IPSP component follows stimulation of the stratum radiatum. We found this component insensitive to caffeine (100 µM), in contrast to the adenosine actions. Therefore, adenosine is unlikely to be the transmitter mediating this IPSP. The late IPSP was not enhanced by adenosine.

49.6 PHARMACOLOGY OF RABBIT LUMBAR SYMPATHETIC GANGLIONIC TRANSMISSION; AN INTRACELLULAR ELECTROPHSYIOLOGICAL STUDY. W.H. Percy* and J. Krier, Department of Physiology, Michigan State University, East Lansing, Michigan 48824.

Experiments were performed to investigate synaptic transmission in, and the pharmacological and electrophysiological properties of, cells in a mammalian lumbar sympathetic ganglion. The lumbar sympathetic chain from above the L3 ganglion to below the L5 ganglion with associated inferior splanchnic nerves (ISN) and rami communicantes (RC) was removed en bloc from rabbits killed by cervical fracture. Intracellular electrophysiological recordings were made in vitro from the L4 ganglion. Stimulating electrodes were positioned on any of RC or ISN above, below or entering the ganglion or on the lumbar chain above or below L4. The mean transmembrane potential (TMP) was 55.4 ± 2.3 mV (n=30). No cells studied exhibited spontaneous action potentials. Fast EPSPs with or without associated action potentials were seen following electrical stimulation of the chain above (n=15), RC (n=11), chain below (n=5) and ISN (n=3). These responses were abolished by superfusion of the ganglion with either hexamethonium (10^{-4} M) or tetrodotoxin (5×10^{-6} M) but were unaffected by atropine (10^{-6} M). Slow EPSPs of 3-12 mV amplitude and 2-30s duration were seen following stimulation of the chain above (n=6), RC (n=3), ISN (n=2) and chain below (n=1). These responses were atropine (10^{-6} M)-resistant. In 2 cells slow EPSPs led to spontaneous firing of action potentials. Antidromic action potentials were recorded from 5 cells and occurred without correlation to the locus of electrical stimulation. McNeil A-343 (5×10^{-6} M), an M1 agonist, caused an 8 mV depolarzation with action potential discharge in 2 cells. Substance P (5×10^{-6} M) caused depolarization ranging between 4 and 20 mV in 8 cells. In 2 cells this was associated with action potential discharge. In conclusion, cells in the rabbit L4 ganglion receive synaptic input consisting of either or both fast and slow EPSPs following electrical stimulation of axons in the lumbar sympathetic chain, ISN and WR. Cells have nicotinic, muscarinic M1 and substance P receptors, but only the role of the former in physiological function has been deduced. (AM 29920)

49.7 ACTIONS OF PHORBOL ESTERS ON SYNAPTIC TRANSMISSION AND MEMBRANE CURRENTS IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. R.A. Nicoll, D.V. Madison, R.C. Malenka and R. Andrade. Depts. of Pharmacology and Physiology, University of California, San Francisco, CA. 94143.

Protein kinase C (PKC) is a calcium and phospholipid-dependent kinase which has been implicated in the transduction of extracellular signals at a variety of receptors, and is selectively activated by phorbol esters. We have examined the actions of 4β-phorbol-12, 13-dibutyrate (PDBu), which directly activates PKC, to determine what effects this kinase might exert on synaptic transmission and on membrane currents in the hippocampus. We have used standard extra- and intracellular recording and single-electrode voltage clamp techniques in the in vitro hippocampal slice preparation.

PDBu had marked effects on synaptic potentials evoked by orthodromic electrical stimulation in the slice. Using extracellular recording of field potentials we observed that PDBu caused increases in both the population spike, recorded at the pyramidal cell layer, and in the population EPSP, recorded in the stratum radiatum. Intracellular recording revealed that PDBu caused large increases in the amplitude of the fast monosynaptic EPSP, but had little consistent effect on the fast IPSP. Both the slow IPSP, which may result from activation of $GABA_B$ receptors (Newberry and Nicoll, J. Physiol 360, 161,1985), and the slow muscarinic EPSP, elicited by repetitive electrical stimulation in stratum oriens (Cole and Nicoll, J. Physiol. 352, 173, 1984), were reduced by PDBu. These effects could be accounted for by a postsynaptic action, since the hyperpolarizing action of the selective $GABA_B$ agonist, baclofen, and the depolarizing action of carbachol, were reduced during PDBu application. In some cells in the presence of PDBu, epileptiform bursts and presumed spontaneous EPSPs were observed.

We have also examined the effects of PDBu on a number of membrane currents in pyramidal cells. The slow afterhyperpolarization (AHP) and its underlying current (I_{AHP}) were blocked by PDBu, resulting in a decrease in accommodation. I_M and I_Q were unaffected by PDBu. In conditions designed to block K^+-currents, we have identified a slowly activating current which is turned on by hyperpolarizing commands from holding potentials of less than -50mV. This current is carried primarily by Cl^- ions and is abolished by PDBu.

Analogs of phorbol, which have in other systems been shown to be ineffective in activating PKC, produce none of the effects described above.

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49.8 SYNAPTIC ACTIONS OF DIMETHYLSULFOXIDE. J. G. McLarnon*, D. M. J. Quastel, and D. A. Saint*. Department of Pharmacology & Therapeutics, The University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

At mouse and frog neuromuscular junctions concentrations of dimethylsulfoxide (DMSO) in excess of 1% have actions, completely reversible upon withdrawal, resembling those of alcohols and acetone, to increase the frequency and amplitude of miniature end-plate potentials (MEPPs). Using a two electrode point voltage-clamp at the mouse junction the subsynaptic action is seen to consist in a prolongation of miniature end-plate currents in association with a reduction in maximum size of the currents. Presynaptically, the increase in frequency of MEPPs (f-MEPP) occurs either in 5 mM K^+ or when control f-MEPP is raised using 10 or 15 mM K^+; the multiplication of f-MEPP by a given concentration of DMSO is similar whether or not base-line f-MEPP is raised, and remains when Ca^{2+}-dependent release of transmitter is blocked using Cd^{2+} (0.5 mM). DMSO differs from acetone and alcohols in that it does not block conduction of action potentials and end-plate potentials can be recorded in the presence of up to at least 10% DMSO. In raised Mg^{2+}/low Ca^{2+} solution, DMSO causes a parallel multiplication of quantal content of end-plate potentials (EPPs) and of f-MEPP. Facilitation phenomena are apparently unaffected by DMSO; the multiplication of f-MEPP by DMSO reduces the number of iterations needed for study of stimulus-evoked modification of spontaneous transmitter release. At junctions blocked using d-tubocurarine the increase in EPP amplitude is associated with an increase in "early tetanic rundown" in keeping with an increase of fractional release of available transmitter. These results are consistent with an effect to increase quantal transmitter release without alteration of Ca^{2+} influx into nerve terminals, perhaps by increasing the effectiveness of internal Ca^{2+}, and inconsistent with the notion that spontaneous and stimulus-evoked quantal release are governed by different mechanisms.

(Supported by grants from the Muscular Dystrophy Association of Canada, and the Medical Research Council of Canada.)

49.9 CALCIUM-INSENSITIVE QUANTAL ACETYLCHOLINE RELEASE AT THE NEUROMUSCULAR JUNCTION. M.T. Lupa* and N. Tabti*. (SPON: R.K. Josephson). Dept. of Pharmacology, University of Lund, S-223 62 Lund, Sweden.

Del Castillo and Katz (J. Physiol., 124:560, 1954) based their explanation for quantal acetylcholine (ACh) release, the vesicle hypothesis, on the close correspondence in both amplitude and rise time between the spontaneous miniature end-plate potentials (mepps) and the nerve-evoked end-plate potentials seen in conditions of low ACh release. In 1957, Liley (J. Physiol., 136:595, 1957) observed that there were occasional mepps which were much larger and slower-rising than the normal "fast" mepps, could also be blocked by curare, and which did not fit into the Gaussian amplitude and rise-time distributions of the normal population. Molgó and Thesleff (Proc. Roy. Soc. Lond. B., 214:229, 1982) noticed that this type of release dominated at botulinum toxin (BoTx) poisoned muscles, and was insensitive to many procedures which increase intracellular Ca^{2+} concentrations, such as 20 mM K^+, 8 mM Ca^{2+}, and 0.2 mM ouabain. This type of ACh release could be selectively increased in both normal controls and BoTx-poisoned muscles by the drug 4-aminoquinoline (4-AQ), without affecting the normal "fast" population.

Kim et al. (Kim, Y., Lömo, T., Lupa, M.T. and Thesleff, S., J. Physiol., 356:587, 1984) called these Ca^{2+}-insensitive mepps "slow" mepps for their long rise time, and found that they were released at a rate of 0.04 Hz at normal untreated endplates, accounting for 5% of the total mepp population, with variability between fibres. 1-2 days after BoTx poisoning, the "fast" mepps disappear, and about 5-10 days later the "slow" mepp frequency increases dramatically to approximately 0.5 Hz. As the muscle recovers and the epps become larger the "fast" mepps gradually reappear and the "slow" mepps gradually decrease to control values. Interestingly, the frequency of "slow" mepps reached higher values in partially paralysed than in completely BoTx-paralysed muscles.

We have concluded that the "slow" mepps represent a type of discrete spontaneous ACh release which is not stimulated by increased intracellular Ca^{2+}, and therefore cannot contribute to nerve-evoked release and synaptic transmission. It is a type of release present at normal neuromuscular junctions, and prominent in many pathological conditions of the neuromuscular junction (BoTx poisoning, murine muscular dystrophy, regenerating synapses). Speculatively, it may have a role in recovery of normal synaptic transmission, or function in maintenance of a trophic relationship at the synapse.

49.10 GLUTAMATERGIC SYNAPTIC RECEPTOR-SPECIFIC AFFERENT PATHWAYS TO SECOND ORDER VESTIBULAR NEURONS IN THE FROG. S.L. Cochran and W. Precht*. Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland.

Glutamatergic synaptic receptors are thought to consist of at least two types: those activated best by N-methyl-D-aspartic acid (NMDA) or by kainic (KA) and quisqualic (QUIS) acids. This classification is based on demonstrations of differential blockade of acidic amino acid-induced excitations by various 'glutamate' antagonists. We find that each of these two receptor types is selectively activated by different afferent pathways to second order vestibular neurons of frogs (Rana temporaria). EPSP's (recorded in vitro) in these neurons appear to be exclusively 'glutamate'-mediated in that high concentrations of kynurenic acid (KENYA; 1-5 mM) in the bath totally and reversibly block the chemically-mediated component of these EPSP's, regardless of their source of origin. Lower concentrations (0.05-0.25 mM) of KENYA, however, have a differential effect on these EPSP's: EPSP's evoked from electrical stimulation of the contralateral VIIIth nerve and from spinal stimulation, the longer latency component of EPSP's evoked from ipsilateral VIIIth nerve stimulation, and bicuculline-induced rhythmic excitations of these cells are abolished. Similarly, depolarizations of these neurons induced by bath-applied NMDA are reduced. The short latency component of EPSP's evoked by ipsilateral VIIIth nerve stimulation and QUIS-induced depolarizations are resistant to these low concentrations. γ-D-glutamylaminomethylsulfonic acid (GAMS; ∿1 mM), which is reported to be a more effective KA/QUIS than NMDA receptor antagonist, more strongly reduces short latency, ipsilateral VIIIth nerve-evoked EPSP's, while only slightly reducing the contralaterally-evoked EPSP's or the rhythmic excitations. On the other hand, D-2-amino-5-phosphonovaleric acid (APV; 50 μM), a relatively specific NMDA receptor antagonist, reversibly abolishes the contralateral input, the long latency ipsilateral input, and the rhythmic excitations. These findings support the NMDA vs. KA/QUIS receptor classification scheme and indicate that polysynaptic pathways which mediate excitation from the ipsilateral VIIIth nerve, the pathways mediating commissural excitation, and many other excitatory synapses on these cells act through NMDA receptors. VIIIth nerve afferents mediate excitation, however, through KA/QUIS receptors. Because VIIIth nerve afferents terminate largely on the cells' somata and principal dendrites, while commissural afferents terminate on the more peripheral dendrititure, it is speculated that KA/QUIS receptors are somatic and proximal dendritic, while NMDA receptors are peripheral dendritic in location.

49.11 **ABAMECTIN BLINDS THE EYES OF THE BOLLWORM MOTH WHILE OTHER SENSORY SYSTEMS ARE UNAFFECTED.** **H. R. Agee* (SPON: C. Leonard), Insect Attractants, Behavior, and Basic Biology Research Lab, ARS, USDA, Gainesville, Florida 32604.**

It was discovered by electrophysiological techniques that the compound eye of the bollworm moth, Heliothis zea (Boddie), was totally blinded by abamectin (common name for avermectin) when 0.1 microgram of abamectin in a saline solution was injected into the abdomen of the moth or when the moth feeds ad lib. on a 5% sucrose-water containing 200 micrograms of abamectin per ml. Abamectin (#MK-936) is a product of Merck Sharp and Dohme Co., Edgerton et al. (Antimicrob. Agents. Chemother. 15:369, 1979). Injected moths were toally blind, as measured by the electroretinogram, within 2 hours. Sixteen hours after ingestion of the abamectin, the moths were blind. The compound eyes of these nocturnal moths have secondary pigment cells that move to shield the receptors from excessive light during the day and move to expose the receptors to maximum light at night. After introduction of abamectin into the moths, the shielding pigments move to and remain in the day-adapted position, regardless of the light conditions.

Abamectin is a GABA agonist that blocks action potential transmission across the neuromuscular junctions (Friz et al., Proc. Natl. Acad. Sci, USA, 76:2062, 1979. With higher dosages, abamectin also causes total paralysis of skeletal muscles, which makes it an especially useful tool for immobilizing insects when inserting microelectrodes and making stable single-cell electrophysiological recordings. However, sensory inputs from the acoustic, olfactory, chemoreception, and mechanoreceptor systems remain fully functional. Sensory and motor traffic in the central nervous system of abamectin-treated moths appear identical to that of normal moths, except for the absence of visual inputs. The heart continues to beat with a lower volume and rate for 2-4 days after moths are treated with abamectin.

50.1 KAINIC ACID NEUROTOXICITY IN THE DEVELOPING RAT HIPPOCAMPUS. T. M. Cook and K. A. Crutcher, Dept. of Anatomy, Univ. Utah Sch. Med., Salt Lake City, Utah, 84132.

Kainic acid (KA), an excitatory neurotoxin, produces a specific pattern of neuronal death in the adult rat hippocampal formation (HF). The CA3/CA4 pyramidal cells are most sensitive followed by CA1, the dentate granule cells and CA2. Several studies, however, have failed to obtain significant pyramidal cell damage in developing rats following IP or ICV injections of KA (Ben-Ari et al., Dev. Brain Res., 1984; Nitecka et al., Neurosci., 1984; Wolf and Keiloff, Dev. Brain Res., 1984). In a study examining the aberrant ingrowth of mossy fibers into CA1 (Cook and Crutcher, in press) we also observed that 1.0 ug of KA did not produce significant CA3/CA4 pyramidal cell loss (target cells of the granule cell axons) in five day old (PN5) rats. Higher doses however, did destroy pyramidal cells. Since the vulnerability of neurons differs markedly between CNS regions and doses of KA required to produce cytotoxicity may approach the LD50 dose for KA (1-5 ug), we sought to determine the susceptibility of developing hippocampal neurons to intra-hippocampal injections of KA. Sprague-Dawley rats were injected with KA (1.0, 2.5, 5.0 or 7.5 ug) on PN days 3-7 or 9. The injections were made via a microdispenser directly into the dorsal hippocampus. The animals were sacrificed at survival times ranging from 9-30 days. Serial transverse (16 um) sections were taken through the HF and stained for Nissl substance. The CA3 pyramidal cells were counted on a single section through the middorsal HF at the level of the injection site. The mean CA3 cell count for noninjected controls (PN30) was 601. The lowest dose of KA (1.0 ug) produced little CA3 cell loss ($\bar{X}$=580) in PN5 pups. This same dose, however, produced a 40% loss of CA3 cells in the PN 6, 7 and 9 pups. Greater than 50% CA3 cell loss was observed following 2.5 ug KA at PN 5-9. At 5 ug PN3 and 4 pups exhibited greater than 50% CA3 cell loss. The 5.0 ug, dose produced an increasingly greater loss of CA3 pyramidal cells in the PN5 (65%), PN6 (69%), PN7 (80%) and PN9 (100%) animals. None of the pups survived the 7.5 ug dose. These results indicate that KA cytotoxicity is observed in the HF of developing rats, but at doses significantly higher than required in adults. The extent of pyramidal cell loss as a function of age and dose indicates that two factors are probably involved in KA neurotoxocity. One factor is the direct cytotoxicity on the pyramidal cells, presumably mediated by postsynaptic receptors. The second factor is the development of excitatory (presumably mossy fiber) innervation to the CA3 subfield. The interaction of KA with an excitatory input has previously been proposed to account for the selective toxicity of KA. Our results support this hypothesis and clarify the parameters needed for producing pyramidal cell loss in the developing rat HF. Supported by NIH Grant #NS17131.

50.2 DESTRUCTION OF PERIPHERAL SYMPATHETIC NEURONS IN MACAQUE MONKEYS TREATED WITH GUANIDINIUM ADRENERGIC NEURON BLOCKING AGENTS. M.A. Palmatier*, R.E. Schmidt*, and E.M. Johnson, Jr. (SPON: H.B. Clark) Depts. of Pharmacology and Pathology, Wash. Univ. Sch. of Med., St. Louis, Mo. 63110.

Guanadinium adrenergic neuron blocking agents, the prototype being guanethidine, have been used extensively in the treatment of clincial hypertension. Guanacline, another guanadinium antihypertensive agent, was used clinically in the late 1960's. Some of the patients who received guanacline developed severe orthostatic hypotension and remained hypotensive for at least 18 months after guanacline treatment was stopped. There have been no reports of the subsequent clinical status of these patients nor papers describing the histology of their peripheral sympathetic ganglia. We have synthesized guanacline and the saturated analog of guanacline. We are using these three guanadinium compounds in an attempt to elucidate the mechanism by which the functional sympathectomy was produced by guanacline in the hypertensive patients.

Monkeys, (Macaca fasicularis or Macara nemestrina) were treated with 20 mg/kg guanacline, guanethidine, or the saturated analog of guanacline for 30 days or 12 weeks. The peripheral sympathetic ganglia, examined at the LM and EM level, from monkeys treated with guanacline, guanethidine or the saturated analog of guanacline for 30 days show neuron loss when compared to sympathetic ganglia from untreated control monkeys. There is also a perivascular and parenchymal infiltrate of heterogeneous mononuclear cells including lymphocytes, and immunoblastic and plasmacytoid cells in the sympathetic ganglia of treated monkeys. Peripheral sympathetic ganglia from 12-week-treated monkeys have a similar histologic appearance to the ganglia from 30-day-treated monkeys, although the neuron loss is more severe. A dose of guanacline comparable to that used clinically (2 mg/kg) for 30 days or 12 weeks also resulted in the loss of sympathetic neurons and a similar mononuclear infiltrate. No histologic changes are seen in dorsal root or nodose sensory ganglia or the adrenal medulla of monkeys treated with these guanadinium compounds.

The histologic appearance of the peripheral sympathetic ganglia from the treated monkeys is identical to the appearance of sympathetic ganglia from rats treated with guanacline, guanethidine, or the saturated analog of guanacline. The sympathetic neuron loss in rats treated with these compounds has been shown to be immune-mediated. We propose that the sympathetic neuron loss in guanadinium compound-treated monkeys is also immune-mediated and speculate that the functional sympathectomy produced by guanacline in patients reflected a similar process resulting in the loss of sympathetic neurons. (Supported by NIH grants HL-20604, AM-19645 and 5T32GM07805-04).

50.3 TWO MECHANISMS UNDERLYING GLUTAMATE NEUROTOXICITY IN CORTICAL CELL CULTURE. D. W. Choi. Department of Neurology, Stanford University School of Medicine, Stanford, CA 94305.

The central neurotoxicity of glutamate (glu) is a possible link in the pathogenesis of several diseases including Huntington's disease and epilepsy, but its mechanism is unknown. One possibility, first proposed by Olney, is that this toxicity is a direct consequence of neuroexcitation ("excitotoxicity"), perhaps due to intracellular volume overload (NaCl/water influx). More recently, evidence has been accumulating that an influx of extracellular Ca may serve as a common pathway in the injury of several cell types by several toxins; however, as reported by Rothman (Neurosci. Abstr. 10:24) and by Olney et al. (ibid.), glutamate can kill neurons in the absence of extracellular Ca.

The phenomenon of glu neurotoxicity was examined in dissociated mouse cortical cell culture. Brief (5 minute) exposure of mature cortical cultures to 0.5 mM glu was followed immediately by neuronal swelling and increased granularity (phase-contrast microscopy), and over the next day by widespread neuronal disintegration. Replacement of extracellular Na or Cl attenuated the early neuronal swelling, but resulted in only minor reduction in the late neuronal loss. Exposure of cultures to 100 mM K produced immediate neuronal swelling similar to that produced by glu.

Removal of extracellular Ca, on the other hand, did not block early swelling, but did markedly reduce ultimate neuronal loss; elevation of extracellular Ca accentuated the loss produced by threshold (.05 mM) concentrations of glu. Exposure of cortical cultures to the Ca ionophore A23187 (10-70 microM) produced little in the way of immediate morphological changes, but was followed by widespread late disintegration of neurons (and many non-neuronal cells). Increasing the time of glu exposure to 30 minutes increased late neuronal cell loss in cultures exposed to glu in the single absence of either Na or Ca; however in the absence of both Na and Ca, cultures were largely intact even after such prolonged exposure.

These observations are consistent with the hypothesis that the toxicity of glu on cortical neurons can be separated into two components that can be distinguished by differences in time course and in ionic dependence. The first component occurs early, is dependent on extracellular Na or Cl, can be mimicked by high K, and is thus possibly "excitotoxic". The second component occurs late, is dependent on extracellular Ca, and is possibly mediated by a transmembrane influx of Ca through glu-activated channels and/or voltage dependent channels. While either component alone is ultimately capable of producing irreversible neuronal injury, at lower exposures to glu the Ca-dependent mechanism predominates. (Work supported by BRSG RR5353 and by a grant from the Wills Foundation).

50.4 ENHANCEMENT OF MPTP NEUROTOXICITY BY METABOLISM TO MPP AND MPPYRIDONE. R. D'Amato*, J. Nye, C. Pronsky* and S.H. Snyder. Johns Hopkins Univ. Sch. of Med., Dept. of Neuroscience, Baltimore, MD 21205.

Metabolic studies of liver microsomes have demonstrated the production of MPP+ and MPPyridone (N-Methyl-4-Phenyl-2-Pyridone) when MPTP is incubated in the presence of NADP (Biochemical and Biophysical Research Communications, 125, No. 2, 1984). We have synthesized the MPPyridone from MPP+ and ^{3}H-MPP+ to study the toxic effects of this compound and to investigate its binding or uptake properties. Preliminary results in mice reveal that the pyridone metabolite is neurotoxjc as measured by loss of dopamine uptake (^{3}H-mazindol binding) sites and tyrosine hydroxylase activity in the striatum. Further studies in PC12 cells using enzyme markers (LDH, tyrosine hydroxylase), mazindol binding and dopamine uptake have demonstrated that MPP+ is 100 times more neurotoxic than MPTP. The pyridone also shows enhanced neurotoxicity. Both MPP+ and its metabolite MPPyridone are taken up by catecholamine neurons which may explain their enhanced selective toxicity. Ongoing studies examine the effects of inhibitors of MAO and amine uptake on the toxicity of the metabolites.

50.5 CHARACTERIZATION OF COVALENT BINDING OF AN MPTP METABOLITE TO RAT, MOUSE AND MONKEY BRAIN IN VITRO. S. Pintus, A. Bocchetta, M. Del Zompo* and G.U. Corsini. Section on Immunopharmacology, NINCDS, NIH, Bethesda, Maryland 20205. *Clinical Pharmacology, University of Cagliari, Cagliari, Italy, 09100.

We have recently demonstrated that MPTP is converted by MAO in vitro to a reactive intermediate which binds covalently to one or more proteins in rat and monkey brain. Different lines of evidence support the concept that the generation of the covalently bound metabolite in rat, mouse and monkey brain tissue homogenates is enzymatic. When [methyl-^{3}H] -MPTP was incubated at 37°C with rat brain homogenates, an increasing amount of radioactivity was recovered in tissue proteins after exhaustive extraction with organic solvents. At 10^{-5}M MPTP the time course of formation was linear for 2 hours, reaching the amount of 71 picomoles bound per milligram of protein. The rate of formation was of 22.88 pmoles/mg protein/min. The enzyme activity was greatly reduced at 0°C and was virtually absent in preboiled homogenate or under nitrogen. There was a variation in enzyme activity in different subcellular fractions and brain regions. The highest activities were seen in the P_2 fraction and in the frontal cortex, hypothalamus and caudate, while the P_1 and S_2 fractions, the pons, midbrain and cerebellum exhibited the lowest activities. MAO inhibitors almost completely prevented covalent binding of radioactivity. Deprenyl and pargyline were the most potent, while clorgyline was far less. Incubations with [methyl-^{14}C]-MPTP and [2,6-$^{3}H_2$]-MPTP resulted in the formation of similar amounts of binding, indicating that neither the ^{3}H label nor the N-methyl group was lost during the metabolic oxidation. If the tissue homogenate was incubated with [methyl-^{14}C]-MPP^{+}, the final product of MAO dependent MPTP metabolism, no radioactivity was recovered after exhaustive extraction indicating that the intermediate which binds covalently to tissue proteins is generated during the MPTP oxidation. We suggest that the finding of a chemically reactive metabolite of MPTP in brain might play a role in its neurotoxicity.

50.6 CYTOTOXIC CHANGES IN CULTURED DOPAMINE NEURONS TREATED WITH MPP+. C. Mytilineou, Dept. of Neurology, Mount Sinai Sch. of Med., New York, N.Y. 10029.

The parkinson inducing agent, MPTP, can be metabolized in vivo by monoamine oxidase to 1-methyl-4-phenylpyridine (MPP+) which could be responsible for MPTP induced toxicity. We have shown that MPTP treatment results in degeneration of dopamine (DA) neurons in cultures of embryonic rat midbrain. We, therefore, examined whether MPP+, is also toxic to DA neurons in explant and dissociated cell cultures. The toxicity of MPP+ was assessed by measuring the 3H-DA uptake by the cultures, as well as by catecholamine (CA) histofluorescence and tyrosine hydroxylase (T-OH) immunocytochemistry. Cultures were established from the ventral midbrain area of rat embryos between the 14th and 16th day of gestation. On the 7th day in vitro MPP+ was added to the feeding medium. After 7 days of treatment with 10 uM MPP+, uptake of 3H-DA was reduced to 4% of control values, while 2.5 uM MPP+ caused a reduction to 35% of control (Mytilineou et al., Neurosc. Lett., in press). Histofluorescence, after loading the CA neurons with alpha-methylnorepinephrine, demonstrated a complete absence of fluorescing neurons and fibers 7 days after treatment with 10 uM MPP+. With the lower concentration (2.5 uM), most of the fluorescing axonal outgrowth had degenerated, but some fluorescing neurons and processes remained within the main explant. The higher susceptibility of neuronal processess to the toxicity of MPP+ is consistent with recent evidence indicating that MPP+ uses effectively the amine uptake pump. In order to visualize the entire DA neuron during the analysis of MPP+ toxicity, we used dissociated cultures stained with antibodies against T-OH. Treatment with 10 uM MPP+ for 24 hours, which produces a 34% reduction in 3H-DA uptake, resulted in morphological changes to the T-OH positive neurons, which could be grouped in 3 general categories. (1) Neurons almost entirely devoid of axonal or dendritic processes; (2) trunkated neurons with short processes restricted to the immediate vicinity of the soma and (3) neurons with appearance indistinguishable from the controls. In general, the fibers present were smooth with less prominent varicosities. In addition to these changes, which could be considered as events leading to neuronal degeneration, several neuronal somata had lost the normally smooth appearence of their membrane and exhibited many filopodia-like protrusions. The possibility of an attempt for new growth by the MPP+-damaged neurons is of interest, in view of the extensive amounts of large fluorescent varicosities present in the vicinity of the substantia nigra of monkeys treated with MPTP. (Supported by NIH grant NS 18979).

50.7 PROTON MAGNETIC RESONANCE STUDIES OF BRAIN AND SPINAL CORD OF DEVELOPING RABBITS INTOXICATED WITH TRIETHYL TIN. A.V. Lorenzo, F.A. Jolesz*, J.K. Wallman* and P.W. Ruenzel*. Dept. of Neurosurgery and Radiology Children's, and Brigham and Woman's Hosp and Physiology and Biophysics, Harvard Med Sch, Boston, MA 02115.

Myelinogenesis and tissue dehydration are characteristic features that occur concomitantly during perinatal brain development in most mammalian species. Coincident with these changes there is in the developing postnatal rabbit brain a marked reduction (approx 50%) in the proton MR average relaxation times, T_1 and T_2. It is not clear if these changes in proton MR parameters are attributable to myelin accumulation or dehydration.

To resolve this issue groups of rabbits, 1 to 90 days of age, were injected ip with 1mg/kg/day triethyl tin (TET) or saline. At day 5 they were killed, the brains removed and weighed. Cortex, white matter, midbrain, pons-medulla, cervical and lumbar spinal cord, and multifidus muscle were sampled. These were placed in tared vials and dessicated to constant weight to determine water content, or in test tubes to measure relaxation times with an IBM PC 10 Minispec at 10 MHz. T_1 was determined with an inversion recovery pulse sequence; and a 20 spin echo CPMG sequence was used to determine T_2. Some animals were perfused with aldehydes and tissues were processed for light and electron microscopy.

As early as 24 hours hind limb weakness, poor planting reflex and tremor were evident in TET treated younger but not older animals. Weight gain was slower and brain weights lower in TET treated than in normal littermate controls. Both in control and TET treated animals tissue water content decreased with age. However, except for cortex and muscle, water content was significantly higher in TET treated than in control animals. Interestingly, the highest content was observed in white matter, pons-medulla, cervical and lumbar spinal cord of the youngest animals whose brains were least myelinated. Light and electron microsocopy revealed extensive vacuolation of myelinated areas and splitting of myelin interlaminal sheaths by large fluid filled vacuoles.

At all ages and for all tissues, except cortex and muscle, T_1 and T_2 were of longer duration in TET treated animals than in respective controls. As with water content these changes were most prominent in spinal cord segments followed by white matter, pons-medulla and midbrain obtained from the youngest animals. In older TET treated rabbits MR relaxation times were most prolonged in white matter and less so in spinal cord segments. The close regional and temporal relationship between the proton MR relaxation times and the pathologic increase in tissue water of brain suggests that alterations in proton MR parameters during development are related to changes in content and/or mobility of brain water. Supported in part by NIH Grant HD15304, Valleley Family Fund, and The Ingraham Fund.

50.8 ATTENUATION OF TMT INDUCED NEUROTOXICITY BY CHRONIC, CONTINUOUS ADMINISTRATION OF SCOPOLAMINE AND MUSCIMOL J.J. Kinsora*, M.E. Smith*, J. French, D.G. Robertson* and J.G. Marriott (Spon:D.Boyd). Warner-Lambert/ Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105

Trimethyltin (TMT) selectively damages the CA1 and CA4 pyramidal cell layers of the hippocampus. EEG studies have shown that the developing neuronal injury is correlated with a persistant increase in hippocampal 4-6 Hz theta activity. The anticholinergic scopolamine and the GABA agonist muscimol can block the appearance of TMT induced increases in hippocampal theta. Chronic, continuous administration of scopolamine and muscimol were evaluated for their effects on TMT related pyramidal cell loss.

Adult male, Long-Evans rats (240 gm) received either chronic scopolamine(n=6) or muscimol(n=6) at 0.1 mg/kg/day via osmotic minipumps implanted subcutaneously in the nape of the neck. Another 6 rats were sham operated. After 24 hr recovery, all rats received TMT (3 mg/kg PO) daily for 3 consecutive days. Fourteen days post TMT, all rats were anesthetized and perfused fixed with formalin. Coronal sections through the hippocampus were cut at 50 u or 5 u and stained with either cresyl-violet or iron-hematoxylin, respectively. The number of cells in the pyramidal layers were estimated by light microscopy (50X).

Animals treated with TMT showed body weight, temperature and behavior changes characteristic of previous reports. Rats given TMT alone lost 85-90 % of the hippocampal CA1 pyramidal cells with some evidence of CA4 injury. Two of the 6 rats given TMT and chronic scopolamine demonstrated comparable neuronal damage. The remainder of the TMT/chronic scopolamine rats and all of the TMT/chronic muscimol rats showed only 5-10 % CA1 cell loss with no apparent damage to the CA4 region.

Chronically administered scopolamine and muscimol protected hippocampal neurons from typical TMT damage. Since these agents attenuate TMT induced increases in hippocampal theta, the present data support the contention that the prolonged occurrence of theta activity following TMT, may have excitotoxic consequences in hippocampal neurons.

50.9 VENTRAL ROOT AXONOPATHY ASSOCIATED WITH THE NEUROFIBRILLARY DEGENERATION OF LOWER MOTOR NEURONS IN EXPERIMENTAL ALUMINUM ENCEPHALOMYELOPATHY. L.C. Triarhou, J. Norton*, O. Bugiani* and B. Ghetti. Depts. of Pathology, Psychiatry, and Medical Genetics, Indiana University Med. Ctr., Indianapolis, IN 46223, and Istituto Neurologico 'C. Besta', 20133 Milano, Italy.

When metallic aluminum (Al) is injected intracisternally in adult rabbits, lower motor neurons of the spinal cord undergo neurofibrillary degeneration. It has been previously reported that the PNS of rabbits injected with Al may also show pathologic changes (Bugiani, O. and Ghetti, B., Neurobiol. Aging, 3: 209, 1982). In the present study, we examined the ventral roots that originate in the cervical enlargement and the corresponding motor neurons in Al-treated animals to clarify the modality and extent of axonal pathology, and to relate the latter to the severity of perikaryonal involvement. Eight rabbits received 0.15 ml of a 1% Al slurry each into the cisterna magna. They were perfused through the heart with aldehydes at 14-62 days post-injection. Spinal cords and spinal roots were processed, embedded in Epon, and examined by qualitative and quantitative morphological methods.

In animals that developed a rapidly progressing motor weakness, neurofibrillary degeneration was widespread in the spinal cord, involving the lateral motor nucleus as well, and the incidence of ventral horn neurons with neurofibrillary tangles was 66-81%. In the remaining animals, motor signs were mild and developed slowly, and the lateral motor nucleus was relatively spared. In the former group, a severe axonopathy of the ventral spinal roots was observed. The axonopathy was characterized by neurofilamentous axonal swellings and myelin attenuation. At the C6 level, the overall fiber-diameter distributions in animals with axonopathy differed significantly from the average distribution in controls ($P<0.0001$). Using individual comparisons, a significant excess or shortage was detected in several fiber-size classes ($P<0.05$). However, in one animal with severe qualitative changes in the roots, the distribution of fiber diameters did not differ significantly from controls. The variability of histograms could be better understood through the analysis of individual axons in serial sections, which showed that axons that appeared normal at a given level showed signs of axonopathy several micrometers distally. Moreover, there was a highly significant difference between the variability of diameter in fibers traced serially in treated animals and controls ($P<0.0005$). The presence of myelin of normal thickness proximally to the axonal swellings supports the view that the peripheral axonopathy is unifocal or multifocal. The findings of this study demonstrate that PNS changes in the Al-induced encephalomyelopathy are related to the axonopathy of motor neurons. Our observations may be relevant to the pathophysiology of lower motor neuron disease in humans.

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50.10 THE SUSCEPTIBILITY OF VARIOUS INTERMEDIATE FILAMENTS TO AGGREGATION BY 2,5-HEXANEDIONE: PARALLELS WITH THEIR SUSCEPTIBILITY TO AGGREGATION SECONDARY TO DISRUPTION OF MICROTUBULES. H.D.Durham*. (SPON: G.Karpati). Dept. Neurology & Neurosurgery, Montreal Neurological Inst. & McGill University, Montreal, Canada, H3A 2B4.

2,5-hexanedione (2,5HD), the neurotoxic metabolite of n-hexane and methyl n-butyl ketone, induces both focal accumulation of neurofilaments (NF) in axons and juxtanuclear aggregates of intermediate filaments (IF) of the vimentin-type in cultured human skin fibroblasts. It has been proposed that 2,5HD prevents the association of IF with microtubules (MT), thereby disrupting NF transport. If this is correct, only IF-subclasses which depend on MT for their cellular distribution should be affected and the IF-aggregates formed should resemble those induced secondary to depolymerization of MT in the presence of colchicine. Therefore, the effect of 2,5-HD was investigated in PTK1 cells, which contain two distinct networks of IF: vimentin-IF whose distribution is colchicine-sensitive and keratin-IF which are insensitive to colchicine. The distribution of both networks of IF was examined in the same cell by double-label immunohistochemistry using antisera specific against vimentin and keratin. In cultures exposed to 6mM 2,5HD for 15 days, aggregates of IF were observed which were labeled by anti-vimentin, but not by anti-keratin. No aggregation of keratin-IF was observed even after exposure of the cells to 2,5HD (2 to 8 mM) for up to 8 weeks.

PTK1, a cell line derived from marsupial kidney, was approximately 3-fold less sensitive to 2,5HD than were human skin fibroblasts. Also, IF pathology in non-neuronal cells was not reported in studies of 2,5HD on organotypic cultures of mouse spinal cord-DRG-skeletal muscle (Veronesi et al. J. Neuropathol. exp. Neurol. 42:153, 1983), although the concentration and duration of exposure which induced focal accumulation of NF in these neurites was similar to that which aggregated IF in cultured human skin fibroblasts (Durham et al. Muscle & Nerve 6:631, 1983). We have confirmed this species difference between human and mouse, i.e. in the susceptibility of IF distribution in non-neuronal cells in culture to alteration by 2,5HD, using human and mouse skin fibroblasts, fibroblast-like cells from mouse spinal cord and 3T3, a cell line derived from mouse fibroblasts. Differences between human and mouse cells were also observed in the response of their IF to depolymerization of MT. IF aggregates were found in human skin fibroblasts, but not in 3T3 cells, after 24 hr exposure to colchicine (5uM).

50.11 FINE STRUCTURE OF CAT CNS AFTER SOMAN EXPOSURE K.C. Sikora-VanMeter, W.G. VanMeter and R.C. Wierwille. Dept. Pharmacol. and Exptl. Therap., Sch. Med., Univ. Maryland, Baltimore, MD 21201; USAMRICD, APG, MD 21010 and Dept. Vet. Physiol. and Pharmacol., Iowa State Univ., Ames, IA 50011

Male or spayed female cats weighing 2.0 to 3.5 kg were given single subcutaneous injections of 5 µg, 10 µg, 15 µg, 17.5 µg, 18.75 µg or 20 µg/kg SOMAN. Cats treated with 5 µg or 10 µg/kg failed to show convulsions for 24 hours at which time they were anesthetized with DIAL (80 mg/Kg ip.), and fixed by whole body perfusion with 5% phosphate buffered glutaraldehyde. Selected tissue samples were taken from the brain and spinal cord, postfixed in OsO_4, dehydrated and embedded in Poly-Bed 812/Araldite mixture. Neuronal as well as glial elements of supraoptic nucleus (SON), hippocampus (HPC), caudate nucleus (CN) and the anterior horn of lumbar segment 7 (L-7) of the spinal cord were examined for changes in fine structure. Cats receiving the other doses were anesthetized either on the appearance of convulsions or after 60 minutes, in the absence of convulsions. These animals as well as the controls were then processed as described above. All animals showed effects of treatment. However, these effects are dependent on dose, duration of exposure and vary with the selected sites. Thus, the SON and HPC of all treated animals show invaginations of the nucleus, vacuolization of nuclear membrances and multiple nucleoli in neuronal nuclei. Neurons in the SON, HPC and L-7 of cats given 15 µgm/Kg or larger doses of SOMAN show increased amounts of rough endoplasmic reticulum and well developed Golgi complexes which indicates increased metabolic activities in these neurones. Also, HPC CA3, and L-7 neurones show increases in mitochondria and lysosomes. On the other hand, changes in fine structural elements of the CN were not observed. A rank ordering of the selected sites according to degree of change and dose shows the SON most effected followed by HPC, and L-7. Finally, the influence of the duration of exposure is demonstrated in an initial increase of metabolic acitivity followed by early signs of degeneration such as dense lamellar bodies, vacuolization, accumulation of mitochondria and increase in electron density in neuronal as well as some glial elements.

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50.12 AMINO ACID ANALYSIS AND HPLC CHARACTERIZATION OF THE ISOFORMS OF THE METALLOTHIONEIN-LIKE PROTEIN IN RAT BRAIN. M. Ebadi, D. Babin*+ and S. Swanson*. Dept. of Pharmacology, Univ. of Nebraska College of Medicine and Dept. of Biochemistry*+, Creighton University School of Medicine, Omaha, Nebraska 68105.

Previous studies from our laboratory identified in rat brain a metallothionein-like protein whose synthesis was stimulated by Zn^{2+} and Cu^{2+}, but not by Cd^{2+}. The zinc-stimulated protein incorporated a large quantity of S^{35} cysteine and was blocked by actinomycin D.

The zinc-stimulated metallothionein-like protein was further purified by ion exchange chromatography on DEAE Sephadex A-25 columns. The proteins, which were separated with a linear gradient using Tris acetate buffer (0-200 mM, pH 7.5), produced two isoforms, eluting at 75 mM and 137 mM, respectively. The comparative HPLC profiles of zinc-treated rat hepatic metallothionein and metallothionein-like proteins from rat brain are very similar (Fig. 1).

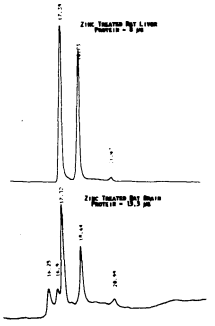

Fig. 1. Comparative HPLC profiles of Zn^{2+}-stimulated hepatic metallothionein and metallothionein-like protein from rat brain.

Metallothionein-like protein isoform I possesses a Mr of 6200, consists of 60 residues of 12 cysteine and no histidine, arginine, leucine, tyrosine, or phenylalanine. Metallothionein-like protein isoform II possesses a Mr of 6300, consists of 61 residues of 3 cysteine and no histidine, arginine, leucine, tyrosine, or phenylalanine. The function(s) of metallothionein-like protein in the brain is not clear and remains to be determined. The lack of binding of Cu^{2+} and Cd^{2+} may be interpreted to indicate that the metallothionein-like protein in the brain is not involved in metal detoxification. Since zinc in physiological concentrations stimulates a number of pyridoxal phosphate-dependent reactions, and in pharmacological doses inhibits an extensive number of SH-containing enzymes and receptor sites for neurotransmitters, we postulate that the metallothionein-like protein in the brain may have function(s) associated with zinc homeostasis and perhaps events related to synaptic functions. (Supported in part from a grant from USPHS-NS-08932).

51.1 **FORMATION OF PRESYNAPTIC SPECIALIZATION INDUCED BY LATEX BEADS IN SPINAL CORD CULTURES.** **H. Benjamin Peng, D.R. Markey, W. Muhlach and E. D. Pollack.** Dept. of Anatomy and Inst. Study Dev. Disabilities, Univ. of Illinois at Chicago, Chicago, Illinois 60612.

The development of the synapse is initiated by an interaction of the pre- and the postsynaptic elements. In the development of the neuromuscular junction (NMJ), the postsynaptic differentiation is triggered by the nerve-muscle contact. Recently we showed that basic polypeptide-coated latex beads can also induce the postsynaptic development in cultured Xenopus muscle cells. Previously, R.W. Burry (Brain Res. 184:85, 1980) showed that Sepharose beads coated with poly-basic compounds also cause the formation of apparent presynaptic elements in cultures of rat cerebellum neurons. In this study, we examined whether a similar presynaptic differentiation can be elicited in spinal cord cultures. Explant cultures were prepared from spinal cords isolated from the lumbar region of Nieuwkoop and Faber stage 50 tadpoles or from stage 22 embryos. After the neurite outgrowths were established, latex beads (1-4.5 μm in diameter) coated with polyornithine were applied to the cultures. Following an incubation period of 1-2 days, the cultures were fixed and processed for light and electron microscopy (LM and EM). For LM, we permeabilized the neurites with saponin and labeled them with a monoclonal antibody (mab 48) specifically against the synaptic vesicles (Matthew et al., J. Cell Biol. 91:257, 1981; a gift from Drs. W. Matthew and R. Burry). Immunofluorescence showed that the mab 48 antigens were concentrated at 60% of the bead-neurite contacts. The rest of the neurite showed very little labeling. Cultures treated with negatively charged (polycarboxylate) beads did not show labeling at the bead contacts. However, labeling was also detected at varicosities along the neurites cultured on polylysine substrate. EM studies revealed the following features: (1) Clusters of 50-60 nm clear vesicles were prominent at the neurite-bead contacts. The vesicles were densely packed at the contact and larger clusters were often composed of smaller 0.1-0.5 μm subclusters. (2) A class of large (90-100 nm) dense-cored vesicles was also seen in the bead-induced terminal, although they were not concentrated at the immediate contact area. (3) Basement membrane did not form at the bead-neurite contact. This is in contrast to our previous observation that this structure does form at the bead-muscle contact. (4) Coated pits were occasionally seen at the contact area.

Our LM study with mab 48 strongly suggests that the vesicles at the neurite-bead contacts are synaptic vesicles. These results thus indicate that basic polypeptide-coated beads can induce a presynaptic-type specialization along the neurite. This offers a model system to study the differentiation of the nerve terminal in the NMJ formation. (Supported by NIH grant NS-16259 and MDA)

51.2 APPARENT CONVERSION OF THE GATING PROPERTIES OF ACETYLCHOLINE RECEPTORS AT RAT SOLEUS ENDPLATES IN VITRO. S.M. Schuetze and S. Vicini*. Dept. Biol. Sci., Columbia Univ., New York, NY 10027 and Fidia Research Laboratories, 35031 Abano Terme (Pd), Italy.

The apparent mean channel open time (τ) of acetylcholine receptors (AChRs) decreases 4- to 5-fold at developing rat endplates. During the first three weeks after birth, ACh-activated channels are converted from a slow, embryonic type (τ=5 msec at 21°C) to a fast, adult type (τ=1.2 msec). Individual endplates contain both types of AChRs during this transition period, and as a result they have miniature endplate currents (MEPCs) with doubly-exponential decay phases. The relative amplitudes of the fast and slow exponential components indicate the relative activities of adult-type and embryonic-type AChRs.

We have used MEPC analysis to see if channel conversion occurs at endplates in neonatal muscles maintained in vitro. Using soleus muscles taken from 5 to 15 day old rats, we followed the kinetics of MEPC decays at 97 individual endplates for 2-24 hr each. On the average, the size of the slow decay component decreased from 49.5% of total MEPC amplitude initially to 36% of total MEPC amplitude 6.6 hr later. The fast and slow time constants did not change. Noise analysis studies revealed a similar progressive decrease in the relative amount of slow channel activity in perijunctional regions. There was no indication that these changes were due to deterioration of the preparation. Since the overall amplitude of the MEPCs remained roughly constant, it is unlikely that slow, embryonic-type AChRs simply become silent with time. A conversion of AChR channel properties seems more likely.

The frequency and extent of this apparent channel conversion varied with the initial state of the endplate. Endplates that had virtually all embryonic-type or all adult-type AChRs at the start of the experiment rarely showed channel conversion. Endplates that started with a mixture of channel types, suggesting that they had already initiated channel conversion in vivo, were most likely to show changes in MEPC decays in vitro. The major difference between channel conversion in vivo and apparent channel conversion in vitro is that the latter process is about 10-20 times faster. This in vitro system should prove useful in investigating the mechanism of channel conversion. Preliminary experiments indicate that high concentrations of sugars (200 mM dextrose or sucrose) inhibit the process, but we do not yet know whether this is a direct effect of the sugars or an indirect effect of the increased osmolarity.

51.3 THE ANEURAL DEVELOPMENT OF THE ACETYLCHOLINE RECEPTOR IN THE PRESENCE OF AGENTS WHICH BLOCK PROTEIN SYNTHESIS AND GLYCOSYLATION. C.G. Carlson*, R.J. Leonard and S. Nakajima. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Cultured muscle from Xenopus embryos offers an ideal preparation for the developmental analysis of the acetylcholine receptor (AChR) since (1) two classes of receptor (high and low conductance) with different mean open times develop in the absence of neural influence, and (2) the open time of the low conductance class decreases by about 50% between developmental Stages (St) 22 and 35. In addition, the proportion of high conductance events ('junctional' type) gradually increases between St 28 and 43. In an attempt to determine whether these changes occur before or after translation or insertion of the AChR into the plasma membrane, muscle cultures from myotomes dissected from St 15-18 embryos were treated with cycloheximide (Cy-100 μg/ml) or Tunicamycin (TM-5 μg/ml) at St 22 to 29. The physiological properties of the two classes of receptor were then examined at St 35-42 utilizing the cell-attached patch clamp technique.

When Cy was added to cultures as early as St 24 or as late as St 28, the proportion of high conductance events subsequently examined at St 37 to 43 was reduced from control levels of about 50% to between 5 and 12%. Many cells appeared to completely lack high conductance events. Neither the developmental change in open time nor the conductance of the low conductance event were altered by Cy. Similar results were obtained with TM which, when added as early as St 22, blocked the appearance of the high conductance event, but left unaltered the developmental change in the open time of the low conductance event. Since Cy is known to block protein synthesis at the translation stage and TM blocks protein glycosylation and insertion of the receptor into the plasma membrane (Rotundo and Fambrough, Cell, 22: 595-602, 1980), these data suggest that no post translational or post-insertional mechanism exists for the conversion of low conductance to high conductance events. Nevertheless, the possible development of a converting enzyme which could act on receptors in the plasma membrane has not been excluded. The failure of TM to block the developmental reduction in open time of the low conductance event, however, suggests that this reduction normally occurs subsequent to protein glycosylation, or after insertion of the AChR into the plasma membrane. (Supported by NIH grants NS08601 and T-32-GM-07211, and an MDA grant).

51.4 THE LOCALIZATION OF ACETYLCHOLINE RECEPTORS ON MUSCLE CELLS CONTACTED BY SINGLE NEURONS IN CULTURE. M.W. Cohen and E. Wilson*. Dept. of Physiology, McGill Univ., Montreal, Quebec.

Previous studies have indicated that competent spinal cord (SC) neurites which innervate muscle cells in culture induce the development of sites of high acetylcholine receptor (AChR) density along the path of contact. However, the capacity of a single competent neuron to interact with muscle cells in this way has not been assessed since the possibility of polyneuronal innervation of the muscle cells was previously not excluded. In the present study cultures prepared from dissociated SC and myotomal muscle cells of Xenopus embryos were analyzed for only those cases where the muscle cells were contacted exclusively by the neuritic arborization originating from a single soma. SC cells were added to 2-3 day old muscle cultures and the cultures were examined for AChR localization up to 6 days later by combined phase contrast and fluorescence microscopy after staining with tetramethylrhodamine-labelled α-bungarotoxin.

So far eight competent SC neurons, containing neuritic arborizations 400-1200 μm in total length, have been analyzed. Each neuron contacted four to seven muscle cells, and on each of these muscle cells AChRs were localized along the path of contact. For each neuron the length of contact exhibiting AChR localization was 20-40% of the total length of contact. The neuritic arborization of individual neurons contained as many as seventeen segments (the portions of neurite between the soma and a branch point, between two branch points, or between a branch point and the end of the neurite). These neuritic segments varied in length from 5 μm to more than 100 μm. More than 85% of them exhibited AChR localization along their path of contact with muscle. Likewise more than 85% of the contacts formed by terminal neuritic segments had sites of high AChR density. In addition, 75% of the ends of terminal neuritic segments also exhibited AChR localization at their sites of contact with muscle.

These results indicate that all muscle cells in this culture system can interact with competent SC neurons to localize AChRs at the sites of contact and that most, if not all, of the neuritic segments are capable of participating in this interaction. The absence of AChR localization at contacts formed by a few of the neuritic segments does not necessarily mean that these segments were incapable of inducing AChR localization. Rather individual muscle cells may have a limited capacity to develop sites of high AChR density and in the case of some terminal neuritic segments the contact may have been too immature for AChR localization to be detected. The fact that AChR localization occurred at the great majority of contacts involving terminal segments, and even their end points, suggests that the capacity to induce AChR accummulation is a feature of the entire neuritic arborization to the limit of its growth. (Supported by MRC).

51.5 **AChR/AChE-AGGREGATING FACTOR FROM TORPEDO ELECTRIC ORGAN IS ANTIGENICALLY SIMILAR TO MOLECULES CONCENTRATED IN THE SYNAPTIC CLEFT AT NEUROMUSCULAR JUNCTIONS.** Noreen E. Reist, Ralph M. Nitkin, Justin R. Fallon, Bruce G. Wallace, U.J. McMahan. Dept of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Molecules tightly adherent to the synaptic cleft basal lamina in skeletal muscle direct the aggregation of acetylcholine receptors (AChRs) and acetylcholinesterase (AChE) on regenerating muscle fibers. As part of a series of studies aimed at characterizing the AChR- and AChE-aggregating molecules, we have extracted a factor from a basal lamina fraction of Torpedo californica electric organ, which causes both AChRs and AChE to form aggregates on cultured myotubes. Thus far we have purified the factor several thousand fold and have made two different monoclonal antibodies (6D4 and 2F6) against it. In the study described here, we sought to determine if the antibodies recognize molecules at the neuromuscular junction of skeletal muscles, as would be expected if the electric organ factor were similar to the AChR- and AChE-aggregating molecules in the synaptic basal lamina.

We used muscles from electric rays and observed the distribution of molecules recognized by the antibodies by indirect immunohistochemistry. In frozen cross sections fluorescence microscopy revealed that both monoclonal antibodies heavily stained the neuromuscular junctions. Staining was also observed along axons and in the walls of muscular arteries. Thus, the monoclonal antibodies recognize molecules associated with more than one tissue component in muscle, but on muscle fibers such molecules are concentrated at neuromuscular junctions. To learn whether or not epitopes recognized by 6D4 are extracellular, intact muscles were fixed, incubated with the antibody, and examined in whole mounts. Under such conditions the antibodies could interact only with the extracellular matrix and the external surface of plasma membranes. Fluorescence microscopy revealed that all of the structures labeled in cross sections were also stained in the whole mounts, including neuromuscular junctions. Some whole muscle preparations incubated with 6D4 and HRP-conjugated second antibody were sectioned and examined by electronmicroscopy to determine the distribution of 6D4 epitopes at neuromuscular junctions. HRP reaction product occupied much of the synaptic cleft, including the synaptic portion of the myofiber basal lamina; no labeling was detected beyond the synaptic cleft. Thus, epitopes recognized by 6D4 at neuromuscular junctions are concentrated in the synaptic cleft and are a part of or adjacent to the synaptic basal lamina.

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51.6 METAMORPHIC TURNOVER OF TRIGEMINAL NEUROMUSCULAR CONTACTS IN RANA PIPIENS. K. Alley. Dept. of Oral Biology, Ohio State Univ., Columbus, OH 43210.

During metamorphic climax the cranial architecture of larval anuran amphibians is dramatically changed to the adult form. As part of this adaptive process the larval jaw apparatus, used in an aquatic filter-feeding habitat, is retooled to accommodate the predatory life style of the adult. Gross changes in cranial morphology are accompanied by a complete degeneration of the larval myofibers and the regeneration of a replacement population during a second wave of myogenesis. Moreover, previous investigation has shown that the same trigeminal motoneurons innervate both the pre- and postmetamorphic musculature (Alley and Barnes, J. Comp. Neur. 218: 395, 1983).

This investigation analyzed the morphologic and molecular events associated with the rewiring of the preterminal axons during the transition from larval to adult myofibers. A combination of ^{125}I-α-bungarotoxin autoradiography, light and electron microscopy was used to assess the reactivity of the pre- and postsynaptic components of the myoneural junctions in the adductor jaw muscles of metamorphic leopard frogs. Specifically, I wanted to determine whether the entire end plate complex is destroyed with the larval myofiber, or if the preterminal axon is separated from the muscle prior to the onset of myodegeneration and recycled to innervate the adult myofibers.

Both light and electron microscopic observations indicate that the presynaptic component of the NMJ remains in contact with the larval myofibers throughout the entire degenerative process. In fact, axon terminals were frequently observed contacting the empty basal laminar tubes long after the sarcoplasm was digested. These contacts exhibited many of the characteristics of normal myoneural junctions. Bungarotoxin labeling of acetylcholine receptors (AChR) provides further support for the stability of the larval NMJs. The distribution and number of AChRs remains unchanged even in myofibers undergoing degenerative changes.

Although the ultimate fate of the preterminal axon is still unknown, the current observations suggest the following conclusions: 1). innervation of the newly formed adult myotubes is by sprouting of the trigeminal axons and not recycling of the larval terminals, 2). the basal lamina of the larval myofibers is capable of maintaining the synaptic ending in the absence of the postsynaptic receptors, and 3). a stable neurotrophic relationship does not prevent the degeneration of the larval muscle fibers.

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51.7 REGULATION OF THE STABILITY OF SYNAPSES. D.G.Puro. Bascom Palmer Eye Institute, University of Miami, Miami,FL

The turnover of synapses appears to play an important role in the development of specific neural circuits. Here, I report the use of a cell culture system in which it is possible to quantitate the on and off rates of synapse formation, to identify transient and stable synaptic pairs and to influence the stability of synapses.

Striated muscle cells were used as postsynaptic targets for cholinergic retinal neurons. Rat myoblasts were cultured in 35mm dishes for 5 days under conditions which limited fusion and, thus, myotube length. Retinal cells from embryonic day 8 chicks were trypsin-dissociated; $5x10^4$ cells were added to a muscle culture. By plating a low density of retinal cells onto short myotubes, innervated myotubes received synaptic input from only one neuron. Neuron-muscle synapses were detected by the presence of spontaneous synaptic input demonstrated by intracellular recordings from muscle cells.

Between 12 and 36 hrs of coculture, the percent of randomly sampled muscle cells with synaptic input increased from 0 (0/280) to 7.6 (26/342). For the next 24 hrs, the overall percent ofinnervated muscle cells remained stable. However, a significant turnover of synapses was discovered during this time. Specifically, of the myotubes shown to be innervated at 36 hrs of coculture, only 67% (14/21) of these myotubes remained innervated 8 hrs later.Conversely of 60 muscle cells found to be without innervation at 36 hrs of coculture, 6 became innervated within 12 hrs. Thus, despite a constant net percent of innervation, synapses were turning over.

Not all retina-muscle synapses were turning over at the same rate. Although a third terminated within 8 hrs, the other two-thirds remained for at least 16 hrs. This suggested two populations- one of transient synapses, the other of stable synapses. In a search for a functional correlate for this difference, the potency of glutamate-evoked transmission across retina-muscle synapses was assessed at 36 hrs of coculture. Responses were classified as weak (2-3 fold increases in the frequency of psp's) or strong (≥5 fold increase). Over an 8 hr period, none (0/10) of the muscle cells with weakly evocable input remained innervated. In contrast, 90% (9/10) of the muscle cells with strong evoked input remained with synapses. Thus, a stable and a transient population of synapses could be identified.

Attention was also directed to the period in culture, 60 to 96 hr, when the net percent of innervation decreased from 7.6 (25/326) to 1 (1/110). Could this termination be regulated? When cocultures were exposed to 50 μM glutamate from 48 hrs of coculture, 6.7% (6/90) of the sampled myotubes were innervated at 96 hrs of coculture. Glutamate exposure did not influence the on rate of synapse formation. Rather, glutamate appeared to maintain a population of synapses.

In summary, the turnover of synapses can be quantiated and regulated in a cell culture system.

51.8 SYNAPSE FORMATION BETWEEN JUVENILE AND ADULT APLYSIA NEURONS IN VITRO. S. Schacher and M. S. Flaster. Center for Neurobiol. & Behav., Dept. of Anat. and Cell Biol., Columbia Univ., College of P&S, and N.Y.S. Psychiatric Instit., New York, NY 10032.

We are interested in examining whether adult CNS neurons can re-establish their normal chemical connections in vitro. We have previously shown that both juvenile and adult neurons isolated from the abdominal ganglion of Aplysia regenerate extensive neuritic processes in cell culture (Schacher, S. & Proshansky, E., J. Neurosci., 3:2403, 1983). Juvenile cells reliably form appropriate chemical synapses (Camardo et al., J. Neurosci., 3:2614, 1983). Here, we report that adult cells form chemical connections reliably only in the presence of juvenile cells.

The cholinergic interneuron L10 and target cells L2-L6 (LUQ cells) were isolated with portions of their original axons from juvenile (1-3 gm) and adult (30-50 gm) animals, co-cultured in various combinations, and assayed for the presence of chemical synapses using standard intracellular recording methods. As described previously (Camardo J. et al., J. Neurosci., 3:2614, 1983), when both pre- and postsynaptic cells were derived from juvenile animals, the typical dual component IPSP was recorded in over 85% of the cultures (13 of 15). The PSP amplitude averaged 5 mV (2-10 mV range) and the properties of the PSP were identical to that seen in the intact ganglion. In contrast, adult-adult cultures formed the dual component IPSP in only 1 of 10 cultures and had an amplitude of 1 mV. In two other cultures only repetitive firing in L10 produced a small response in the target cells.

Chemical connections by adult cells are greatly enhanced in the presence of juvenile cells. Adult L10-juvenile LUQ cultures formed dual component IPSPs in all cases (5 of 5) with an average amplitude of 5 mV (2-10 mV range). Juvenile L10-adult LUQ cells formed normal connections in 2 of 3 cultures (1.5 mV average). In the presence of juvenile LUQ cells, adult L10 cells formed appropriate chemical connections with adult LUQ cells in all cases (N=3) with an average amplitude of 3 MV. Preliminary evidence suggests that smaller cell body input resistance in adult LUQ cells compared to juvenile cells cannot account for the reduced level of connectivity in adult cells. In addition, ACh sensitivity on isolated juvenile and adult LUQ cell bodies and distal neurites revealed no significant differences. We tentatively conclude that there is a deficit with regard to some aspect of synapse formation in adult presynaptic and/or postsynaptic cells that is overcome in the presence of juvenile cells. We are now studying the role played by cell-cell interactions in the enhancement of synapse formation in older cells.

51.9 CHEMICAL NEUROTRANSMITTERS CAN INDUCE AND STRENGTHEN ELECTROTONIC SYNAPSES. D.C. Spray, J.C. Saez, M.V.L. Bennett, J.A. Kessler+. Depts. Neuroscience and Neurology+, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Cultured superior cervical ganglion (SCG) neurons from fetal or neonatal rats form electrotonic synapses when cultured in a defined medium (DM) but do not in ordinary serum containing medium (Higgins and Burton, J. Neurosci. 7: 2241, 1982; Kessler et al., PNAS 81: 6235, 1984). The coupling is partially attributable to components uniquely present in DM (insulin and selenium). However, coupling is also induced by membrane permeant cAMP derivatives; endogenous cAMP is suppressed by serum which accounts for its inhibitory effect on electrotonic synapse formation (Kessler et al., ibid.). Further work has shown that expression of electrotonic synapses in insulin-containing medium is blocked by the protein synthesis inhibitor cycloheximide (2 ug/ml) and the mRNA synthesis inhibitors actinomycin D (1 ug/ml) and camptothecin (2 ug/ml). Dopamine and the cholinergic agonist carbachol induce electrotonic synapse formation within 12 hrs of application (dopamine and acetylcholine are prominent neurotransmitters in SCG). For dopamine at concentrations of 10 nM to 10 uM the lowest dose is most effective, perhaps due to receptor down-regulation or desensitization. For carbachol, the effect increases with increasing doses over a similar concentration range. Incidence of coupling induced by 10 uM carbachol is similar to that induced by 1 mM db-cAMP; when both drugs are added together the incidence is unchanged. Junctional conductances (g_j) measured in carbachol or db-cAMP treated coupled pairs are similar; g_j is approximately doubled when both drugs are present. Presumably carbachol and db-cAMP act upon the same subpopulation of neurons. We conclude that chemical neurotransmitters normally present in sympathetic ganglia can induce formation of electrotonic synapses and strengthen coupling between cells. Supported in part by McKnight Foundation Development Award (DCS).

51.10 **EXPERIMENTAL REDUCTION OF SEROTONIN CONTENT DURING EMBRYOGENESIS ALTERS MORPHOLOGY AND CONNECTIVITY OF SPECIFIC IDENTIFIED HELISOMA NEURONS.** **I.I. Goldberg* and S.B. Kater. (SPON: G.J. Mpitsos). Dept. of Biology, University of Iowa, Iowa City, IA 52242.**

In this investigation, we begin to test the hypothesis that during embryogenesis, neurotransmitters play a role in regulating development of the nervous system. 5,7-dihydroxytryptamine (5,7-DHT) was used to experimentally reduce the serotonin content of embryos during a defined window of embryogenesis. The developmental consequences of this embryonic perturbation were subsequently assessed in developed snails.

To reduce levels of serotonin, embryos staged between 45% and 60% of total embryonic development were incubated in 0.5 mM 5,7-DHT for 24 hours, and then returned to normal rearing conditions for the remainder of development. HPLC with electrochemical detection was performed to determine the magnitude and timecourse of a 5,7-DHT - induced change in serotonin content. Whole embryo serotonin levels were lowered by 42% within 3 days following treatment. This decrease was transient, with no difference between treated and untreated embryos by 6 days post-treatment (embryonic stage 95%) nor upon maturing into adult snails. In addition, the cerebral neuron C1, the only serotonergic neuron which innervates the buccal ganglion, survived the embryonic treatment and was physiologically normal in the adult. Thus, the perturbation of serotonin levels was achieved during an embryonic window spanning the latter half of embryonic development.

Mature snails were examined morphologically and electrophysiologically to assess the effects of embryonic perturbations in serotonin levels. The morphology of buccal neuron 19 (B19), as determined by Lucifer Yellow injection, was grossly abnormal in 3 of 14 animals treated with 5,7-DHT, while normal in all of the controls. In addition, 7 of the 14 experimental B19's were aberrantly dye-coupled to one or several neighboring somata, in comparison to 1 case of aberrant dye-coupling in control B19's. The morphology of buccal neuron 4, which was also examined as a test of specificity, appeared normal in all of the 5,7-DHT - treated and control preparations. Furthermore, only a single example of aberrant dye-coupling was observed in the treated preparations, while the controls had none. Electrotonic coupling measurements were taken to more precisely determine whether the observed changes in frequency of dye-coupling represented changes in synaptic connectivity. In treated preparations, the electrotonic coupling coefficient for bilateral pairs of B19 was 52% greater than that recorded in control preparations. This effect was neuron-specific as the same treatment did not affect the strength of the electrotonic synapse between bilateral buccal neuron 4's. Therefore, both morphological and electrophysiolgical data demonstrate that a transient reduction in serotonin concentrations during embryonic development evokes a neuron-specific increase in electrical synaptic connectivity.

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51.11 **THE FORMATION OF TRANSIENT CHEMICAL SYNAPTIC CONNECTIONS BY IDENTIFIED NEURONS OF HELISOMA.** **P.G. Haydon and S.B. Kater. Department of Biology, University of Iowa, Iowa City, IA 52242.**

The nervous system of Helisoma exhibits extensive neuroplastic capabilities that can be studied in detail and with great precision due to the ability to identify as individuals its component neurons. A reliable experimental perturbation, axotomy, evokes neurite outgrowth and subsequently the formation of novel electrical synaptic connections (Bulloch and Kater, Science. 212: 79, 1981). Such changes in connectivity are transient. The nervous system forms a series of novel electrical synaptic connections and then by selection processes the majority of these electrical connections are broken. We now report that normally unconnected identified neurons will also form transient chemical synaptic connections during such neuroplastic periods. The esophageal nerve trunks were crushed in vivo in order to axotomize identified neurons 4 and 5 of the buccal ganglia. Such animals were allowed to recover and were maintained for up to 8 days following surgery. Subsequently, buccal ganglia were acutely isolated and the connectivity of the test neurons was determined by pairwise intracellular recording. Identified neurons 4 and 5 of the buccal ganglia are not synaptically interconnected in the mature, unperturbed nervous system. Axotomy, however, evoked the growth of new neurites from these neurons and the reliable formation of a transient excitatory chemical synapse from neuron 5 to neuron 4. Shortly after its formation this chemical synapse was broken and neurons 4 and 5 returned to their normal unconnected state. The elimination of this chemical synaptic connection does not reflect a generalized withdrawal of chemical synaptic inputs to neuron 4. Previously characterized extant excitatory chemical inputs to neuron 4 were maintained during the same time period that the excitatory input from neuron 5 was broken. Thus these data demonstrate that neurons not normally interconnected are, in fact, capable of forming chemical synaptic connections with one another, and that after sampling such a connection the nervous system is capable of selectively eliminating synapses in order to restore its preferred connectivity.

For further examination of the mechanisms involved in the initial formation and subsequent elimination of chemical synapses, the resolution afforded by cell culture is required. We therefore isolated as individuals neurons 4 and 5 and plated them as pairs into cell culture. Here they extended neurites and formed an excitatory chemical synapse from neuron 5 to neuron 4. Thus neurons that will form chemical synaptic connections in vivo will form the same specific synapse in cell culture where we can study with a high degree of resolution those mechanisms involved in synaptogenesis.

Supported by NIH grant NS18819.

51.12 EXTENT OF MULTIPLE INNERVATION OF CEREBELLAR PURKINJE CELLS BY CLIMBING FIBERS IN ADULT RATS AFTER DIFFERENT SCHEDULES OF POST-NATAL X-IRRADIATION. J. Mariani, P. Benoit*, M.D. Hoang* and N. Delhaye-Bouchaud*. Dep. de Biologie Moléculaire, Institut Pasteur, and Lab. Neurophysiologie Ontogénétique, Univ. P. et M. Curie, Paris, France.

The multiple innervation of cerebellar Purkinje cells (PCs) by climbing fibers (CFs), that exists transiently in the normal developing rats, can be experimentally maintained in adult rats the cerebellum of which has been degranulated by repetitive postnatal X-irradiation between birth and postnatal day 14 (see review in Benoit et al., Dev. Brain Res., 14, 310, 1984). It has been previously shown (Delhaye-Bouchaud et al., Neurosci. Lett., 9, 51, 1978) that irradiation restricted to the first postnatal week was sufficient to prevent most of the CF-PC synapse elimination; since the latter occurs essentially between postnatal days 5 and 10 and given that postirradiation effects last 2-3 days, the question arose to know whether it is possible to further delimit a "critical period" of irradiation within the first week.

A quantitative estimate of the extent of multiple innervation of PCs was made in adult rats that had been irradiated according to 4 different schedules :
In group 1, rats received 200r on day 0 (birth) 4,5,6,7 and 150r on day 3 (total dose 1150r).
In group 2, rats received 200r on day 0, and 150r on days 3,4,5,6,7 (total dose 950r).
In group 3, rats received 200r on days 4,5,6,7 (total dose 800r).
In group 4, rats received 200r on days 1,2,3 (total dose 600r).
The rats were anesthetized, paralysed, artificially respired, and the CF pathway was electrically stimulated at the level of the inferior olive or in the cerebellar white matter. Intracellular recordings of spontaneous and evoked PC responses were performed in the medial part of lobules VI, VII and VIII. The number of afferent CFs was estimated by the number of steps in the CF postsynaptic excitatory potential. The location of all the recorded cells was ascertained on sagittal histological sections.

The mean number of CFs impinging on a given PC was similar in group 1 (0 → 7 days, n = 124 cells) and in group 3 (4 → 7 days, n = 102) ie 3.1 ± 0.1 (sem). By contrast, in group 4 (1 → 3 days, n = 174), the mean number of CFs was only 1.27 ± 0.04. These results strongly suggest that the critical period of irradiation is from postnatal day 4 to day 7. In group 2, the mean number of CFs per PC was significantly lower than in group 1 (m = 2.25 ± 0.11, n = 113), which is similar except for lower doses between days 4 to 7, suggesting that the dose delivered should also be taken into account. These results establish that a close correlation exists between the elimination of supernumerary CF-PC synapses and the settlement of granule cells during a limited period of time.

51.13 CEREBELLAR AXON DEVELOPMENT: FEATURES OF THE EXUBERANT STATE. E. Gregory and C.A. Mason, Dept. of Pharmacology, New York University School of Medicine, New York, NY 10016.

During cerebellar development, climbing fibers (CF) branch exuberantly onto multiple Purkinje cells and then, after withdrawal of excess branches, focus on single Purkinje neurons. A second type of cerebellar axon exuberance and synapse elimination is the transient projection of mossy fiber (MF) branches into the Purkinje cell zone. These branches have the form of CF branches and contact both Purkinje and granule neurons (Mason and Gregory, 1984). To study the developmental histories of these different exuberant afferents in cerebellum, and to compare their projection, form, and fine structural relationships, axons were labeled by inserting a HRP-coated microelectrode into fiber tracts in fresh slices of mouse cerebellum. After brief rest periods, sections of slices were processed with DAB for light and electron microscopy. At postnatal day (P)6-8, axons with CF-like branches (fine with small tapered endings) project over many adjacent Purkinje cells. The growing tips associate with Purkinje cell somatic spines and insert themselves into the soma, forming simple synapses. By P9-12, 'nids' or perisomatic nests are common. Nids occur on axons at the over-branched stage, but single CF's support only one nid. Conversely, individual Purkinje cells can be contacted by multiple CF's during the over-branched stage, but never by more than one nid. Nid terminals contact Purkinje somatic spines but do not insert themselves into the soma. Axons with primarily MF features at P6-8 have large growth cone-shaped boutons that are central to synaptic glomeruli. MF boutons give off filopodial branches, many of which reach up to Purkinje cells and have small CF-like growing tips. These tips make elementary synapses and adherent junctions onto Purkinje cells; profiles apposed to these filopodial branches commonly have coated vesicles. By P14-21, MF terminals have few short filopodia and are confined to the granule layer. Thus, while axon exuberance is mainly observed in the Purkinje cell zone, CF and MF branching patterns and cell-cell contacts differ. The nature of synapses made by exuberant branches may indicate 'acceptance' by the postsynaptic cell of the appropriate input. (Supported by NIH Grant NS15182 and Irma T. Hirschl Foundation).

ENDOCRINE EFFECTS ON DEVELOPMENT AND BEHAVIOR

52.1 STEROID-INDUCED CHANGES IN ACTION POTENTIAL WAVEFORMS OF AN ELECTRIC ORGAN. A.H. Bass and S.F. Volman. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

We are interested in the effects of gonadal steroid hormones on the generation of action potential (spike) waveforms by excitable cells. We now report that steroids induce dramatic changes in the duration of spikes produced by the cells (electrocytes) of the electric organs of the mormyrid fishes of Africa.

For some mormyrids, androgenic steroids induce changes in the Electric Organ Discharge (EOD) waveform of females and juveniles that mimic the changes natural males undergo during sexual maturation. Specimens of Brienomyrus (sp. 2) undergo a 2-3 fold increase in EOD duration when treated with 17α-methyl-testosterone. Cholesterol has a weak, though non-significant, effect. Testosterone-induced changes in EODs are accompanied by nearly a two-fold increase in the duration of spikes generated by the electrocytes excitable membrane.

All cells were studied from intact fish. Following anesthetization, animals were spinalectomized, turning off the EOD-generating mechanism and immobilizing the trunk. The tail, which contains the electric organ, was isolated in a Ringer's-filled chamber and the electrocytes were exposed. The animal was revived and cells were impaled with two intracellular 3M KCl-filled microelectrodes. Each electrode was used alternately as a stimulating and then a recording electrode, so that spikes were often recorded from more than one position in the cell.

Our study includes 9 Testosterone (T)-treated (11-28 days), 3 Cholesterol (C)-treated (10-18 days) and 12 Untreated (U) specimens. We recorded 206 individual spikes from 138 cells (76-T, 26-C, 36-U). Duration is measured at 50% of peak-to-peak amplitude. The average spike duration is 0.370 ms (n=117, SE=0.009 ms) for T-, 0.187 ms (n=42, SE=0.007 ms) for C-, and 0.137 ms (n=47, SE=0.004 ms) for U-treated animals.

Mormyrid electrocytes are wafer-shaped cells with well-defined anterior and posterior faces (see Bennett and Grundfest, Bioelectrogenesis, 1961). In 16 cells, the intracellular spike could be split into two spikes, each one probably representing the spike generated by each face; their duration is also about two-fold greater in T-treated, compared to C- and U-treated animals.

Together with morphological and biochemical data (see Bass et al., Neurosci. Abstr., 1984), these new physiological results emphasize that mormyrid electric organs have evolved a sensitivity to gonadal steroid hormones. While changes in spike duration may relate in part to the electrocyte's passive electrical properties, it seems more likely they also depend on changes in ionic conductances.

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52.2 DEVELOPMENT OF ANDROGEN ACCUMULATION IN A FOREBRAIN NUCLEUS THAT MEDIATES SONG LEARNING IN ZEBRA FINCHES. S.W. Bottjer & A.P. Arnold, Dept. of Psychology & Lab. of Neuroendocrinology, UCLA, CA 90024.

MAN (magnocellular nucleus of the anterior neostriatum) is a forebrain nucleus that is uniquely involved with the development of learned song behavior in zebra finches (Bottjer et al., 1984, Sci.). The normal ontogeny of this nucleus includes the loss of half of its original contingent of neurons; this loss occurs during the time juvenile birds are learning the motor pattern of song production (Bottjer, et al., 1985, J. Neurosci). In adult males, a large proportion of cells in MAN accumulate androgens (Arnold et al., 1976, JCN), thereby raising the possibility that hormones are importantly involved in song learning and in cell loss from MAN.

The purpose of this experiment was to examine the characteristics of androgen accumulation in MAN during song development. 7 juvenile male zebra finches with a mean age of either 24 (n=5) or 60 (n=2) days were gonadectomized; 24 hours later they were injected intramuscularly with equal molar doses of tritiated dihydrotestosterone (^{3}H-DHT; 20ng/g body weight, specific activity = 123-135uCi/mmole). 90 minutes later the animals were killed and the brains were processed for thaw-mount autoradiography. Sections were stored for 4 to 8 weeks in dessicated boxes at 4°C, developed, fixed, and stained with thionin. A Poisson criterion was used for quantitative assessment of cell labeling.

Data in Table 1 show that a much lower percentage of cells in MAN accumulate DHT or its metabolites in juvenile birds that are just beginning to produce song-related vocalizations (24 days) than in older birds in which the basic motor pattern of song is typically established (60 days). However, because the number of neurons in MAN is approximately twice as large in younger birds (Bottjer et al., 1985) these results suggest that (1) 24-day males may possess the full complement of androgen-accumulating cells in MAN that will be preserved into adulthood, and (2) a population of cells that does not accumulate androgens may be selectively lost from MAN during vocal development. Table 1 also shows that the intensity of labeling (density ratio of labeling in cells relative to background) is much higher in older than in younger males, indicating that the ability of these cells to concentrate androgen increases during this phase of song learning. This result suggests that the number of androgen receptors are increasing, that receptors are better able to bind hormone, or that there is a developmental change in steroid metabolism. In any case, the increase in cellular concentration of DHT that occurs while birds are learning to produce song is compatible with the notion that androgens influence song learning.

TABLE 1 ($\bar{X}$ ± S.D.)

age	% labeled	density ratio	size labeled cells
24 days	21.4 ± 4.6	2.5 ± 0.3	241.0 ± 20.4 um^2
60 days	52.5 ± 7.8	5.8 ± 1.3	235.5 ± 7.8 um^2

52.3 ANDROGEN-INSENSITIVE SNB MOTONEURONS CAN BE SPARED BY ANDROGEN FROM DEATH DURING DEVELOPMENT. S. Marc Breedlove. Department of Psychology, University of California, Berkeley, CA 94720.

The bulbocavernosus and levator ani (BC/LA) muscles of male rats are innervated by the spinal nucleus of the bulbocavernosus (SNB). Both the BC/LA and the SNB are present in newborn female rats, but without androgen both motoneurons and targets die. We have eliminated supraspinal neural afferents as a site of androgen action for sparing the SNB system (Soc.Neurosci.Abstr. 10 453 '84), and have shown that neonatal androgen can spare the BC/LA following complete removal of the lumbo-sacral spinal cord containing SNB motoneurons (R.B.Fishman, this volume). We now report that in mice, androgen need not act upon SNB motoneurons themselves in order to spare them from death during development.

Testicular feminization (tfm) is a recessive mutation on the X chromosome which, when combined with a paternal Y, produces a fetus with testes and testosterone, but which develops a female external phenotype because its tissues lack androgen receptors. Male carriers of the sex-reverse (Sxr) mutation produce sperm with an X chromosome which has an attached Y fragment. Embryos from such sperm develop testes because of the Y fragment, and testicular secretions produce a mouse with a normal male phenotype, despite an XX genotype.

We bred tfm carrier mice with Sxr carrier males. Some of the offspring clearly carried both traits: coat color markers indicated an XX genotype, yet they had an enlarged clitoris, hypospadias and abdominal testes. Such an intersex phenotype is never seen with tfm or Sxr mutations alone, and can be explained as an XX mouse with the Sxr mutation, which develops testes and testosterone, but only partially responds to the hormone due to the tfm gene. These mice, with a tfm gene on one X, are partially androgen-insensitive because of random-X inactivation. In the developing blastocyst of XX individuals, each of the cells which will give rise to the somatic tissues begins to express only 1 X chromosome. Thereafter, all cells clonally derived from these cells continue to express only that particular X chromosome. Thus a proportion of the cells in these mice expressed the X with tfm and were therefore androgen-insensitive. Because they were partially androgen-insensitive, these mice only partially responded to their own testicular androgen: hence the intersex phenotype.

Two tfm/Sxr mice retained BC/LA muscles into adulthood and had more SNB cells than do normal females. If androgen spares SNB cells by interacting with motoneuron receptors, then one would expect these SNB cells to be androgen-responsive. But if androgen acts at some other site to spare SNB cells, then some SNB cells could be androgen-insensitive, i.e. tfm. In fact, androgen autoradiography with 3H-methyltrienolone revealed a total of 132 SNB cells, none of which accumulated hormone, even though other nearby motoneurons did. Apparently androgen can spare dying motoneurons to form the SNB even if the cells are themselves incapable of interacting with the hormone. Androgen must interact with another cell population, perhaps the muscles, to spare the SNB system.

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52.4 DEVELOPMENTAL CHANGES IN THE PULSATILE LH PATTERNS OF OVARIECTOMIZED, IMMATURE GUINEA PIGS. M.D. Loose* and E. Terasawa, Neurosciences Training Program & Wisconsin Reg. Primate Research Center, University of Wisconsin, Madison, WI 53715-1299.

Our previous finding that pulsatile LHRH infusion into immature female guinea pigs resulted in precocious vaginal opening and first ovulation (Biol. Reprod. 30; Suppl. 1:71, 1984), suggests maturational changes in LHRH release occur prior to puberty. To examine this possibility, the ontogenic pattern of pulsatile LH release, which presumably reflects developmental changes in LHRH release, was investigated in ovariectomized female guinea pigs. Three groups of females at 46.6±0.3 days (47D, n=7), 32.7± 0.3 days (33D, n=7), and 24.3±0.6 days of age (24D, n=7) were evaluated, since first vaginal opening and ovulation of female guinea pigs in our colony are usually seen at 45-60 days of age. All animals were ovariectomized 12 days prior and received a jugular catheter 2 days prior to the experiment. Blood samples (0.4ml) were collected through the catheter at 5 min intervals for 180 min and equal volumes of red blood cells and a plasma protein fraction were infused after samplings. Plasma LH estimated with RIA is expressed in ng/ml. Either LHRH (20ng) or saline was injected through the catheter after sampling at 120 min and 150 min. Results are: 1) Basal LH levels in the 24D group (212±27) were lower than those in the older age groups (33D, 382±37, $p<0.01$; 47D, 322±35, $p<0.05$); 2) the mean LH level during the first 120 min in the 24D group (326±65) was much lower than that in the 33D (672±67, $p<0.01$) and 47D (642±77, $p<0.05$) groups; 3) the number of LH pulses during the first 120 min in the 24D group (2.6±0.8) was significantly smaller ($p<0.05$) than that in the older groups (33D, 4.7±0.3; 47D, 4.3±0.3); 4) peak value of LH pulses in the 24D group (862±76) was lower ($p<0.05$) than that in the 33D group (1154±105), but not in the 47D group (1190±148); 5) there was a positive correlation ($r=0.899$, $p<0.01$) between peak LH values and body weights in the animals of the 24D and 33D groups; 6) the amplitude of the LH pulses was not different between groups (24D, 640±91; 33D, 710±85; 47D, 742±128); 7) LH release induced by LHRH injection was similar in all groups (24D, 1027±190; 33D, 1045±195; 47D, 940±248). The results indicate that 1) an elevation of mean LH, due to increases in basal LH, LH pulse frequency and peak LH, occurs during the early pubertal phase, approx. between 24 and 33 days of age, and it remains high through the midpubertal phase, 2) increased release of LHRH from the hypothalamus may be responsible for this elevated LH release during the early phase of puberty, since the changes are independent of the ovary, and the pituitaries of the youngest females respond to LHRH in a similar manner as that of older females. It is concluded that an increase in LHRH release may occur during the early pubertal stage, several weeks prior to the age at first ovulation. (Supported by NIH grants RR00167 and HD11355).

52.5 MANIPULATION BY GONADAL STEROIDS OF THE SEXUALLY DIMORPHIC MET-ENKEPHALIN FIBER POPULATION IN THE ANTERIOR PORTION OF THE PERIVENTRICULAR NUCLEUS. R.E. Watson, Jr., G. Hoffman and S.J. Wiegand. Department of Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

We have recently identified a distinct sexual dimorphism in the density of methionine-enkephalin (m-ENK) immunoreactive (ir) fibers in the anterior portion of the periventricular nucleus (Pea) (Bleier et al., JCN 212:116, 1982) in the rat (Watson and Hoffman, Anat. Rec. 211:211A). This region exhibits a cytoarchitectonic sexual dimorphism as well in which the density of cellular packing is greater in the female than in the male (Bleier et al., 1982). Immunoperoxidase studies performed upon tissue from male-female pairs of rats revealed that in the female there exists a dense band of m-ENK ir fibers, approximately 100 um wide, over the Pea. This dense fiber plexus is apparent over the anterior-posterior interval between the medial preoptic nucleus (Bleier et al., 1982) and the sexually dimorphic nucleus of the preoptic area (Gorski et al., Brain Res. 148:333, 1978) and is present at all stages of the estrous cycle. In contrast, in the male, the density of m-ENK ir fibers is not appreciably increased over that present in more laterally situated regions in the preoptic area.

Gonadal steroids exert both an organizational and activational effect upon the presence of the m-ENK dimorphism. Administration of testosterone propionate (200 ug/day s.c. on postnatal days 2,3,4,5) to genotypic female rats resulted in the elimination of the m-ENK band at adulthood. This response appears to be a function of the action of aromatizable androgen since comparable dosing with dihydrotestosterone (and vehicle alone) produced no alteration in the typical dense band of m-ENK ir. Castration of the male rat on the day of birth with subsequent treatment on days 2,3,4,5 with testosterone (200 ug/day), dihydrotestosterone (200 ug/day), or vehicle alone (0.05 ml/day) resulted in the typical male m-ENK distribution pattern.

Regarding the expression of activational effects of the gonadal steroids, ovariectomy of the young adult female resulted in the elimination of the m-ENK ir band within 2 weeks post surgery while castration of the male rat produced no discernable alteration of m-ENK ir in the region. Replacement of estradiol in the female via Silastic capsules implanted s.c. brought about re-expression of the m-ENK ir band within 2 weeks. Estradiol implants in the male castrated at adulthood were without effect. Thus, the sexual dimorphism of m-ENK fibers in the Pea requires activation by estrogen in order to be expressed. These observations are of significance in the context of opiate effects upon various sexually differentiated functions, including, particularly, the regulation of gonadotropin secretion.

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52.6 AN OVARIAN-INDEPENDENT AFTERNOON EPISODE OF PROLACTIN (PRL) SECRETION OCCURS DURING FEMALE SEXUAL DEVELOPMENT: AMPLIFICATION BY ESTRADIOL-17β. H.F.Urbanski* and S.R. Ojeda* (SPON: N. McArthur). Dept. of Physiology, Univ. Texas Hlth. Sci. Ctr., Dallas, Texas 75235.

Previous investigations have demonstrated that mean plasma PRL levels increase during sexual development of the female rat and that the changes occur preferentially in the afternoon. The aims of the present study were (a) to characterize the nature of this enhanced level of secretion and (b) to determine the extent to which it is controlled by a central, ovarian-independent, mechanism. Animals were maintained under a 14L:10D photoperiod (lights on from 05:00 to 19:00h) and blood samples collected every 5 min for 4h via an intraatrial cannula. The preovulatory PRL surge occurred at approximately 15:30h, reaching peak plasma levels of 600ng PRL-RP-3/mL When juvenile rats were ovariectomized and PRL secretion examined 2 days later a similar midafternoon episode of PRL release was detected but this was considerably smaller (ca. 1/10th) than that observed in the intact animals at first proestrus. Administration of estradiol -17β (E_2) at different doses (s.c., via Silastic capsules) at the time of ovariectomy greatly enhanced the PRL secretory episode. This effect was produced even by small E_2 doses which generated preovariectomy plasma E_2 levels. More significantly, when a separate group of rats was ovariectomized neonatally and subsequently bled every 5 min for 5h either 20, 30 or 40 days later, the PRL mini-surge persisted in the majority of these animals regardless of the length of the postovariectomy period.

The results suggest that the increase in plasma PRL levels observed in the afternoons of prepubertal development reflect an episode of secretion which is initiated by a centrally-originated, ovarian-independent, mechanism. They further suggest that the preovulatory PRL surge itself is initiated by this central mechanism and that the signal strength is markedly amplified by ovarian E_2. The ovarian independence of the underlying primary signal is emphasized by the finding that mid-afternoon PRL mini-surges persisted even 40 days after ovariectomy.

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52.7 BEHAVIORAL EFFECTS OF NEONATAL AND ADULT EXPOSURE TO GONADAL HORMONES IN A MARSUPIAL. B.H. Fadem* (SPON: H. Edinger). Dept. of Psychiatry, UMDNJ-NJ Med. Sch., Newark, NJ 07103.

Gonadal hormones present during development and in adulthood affect a variety of behavioral patterns associated with reproduction in mammals. One of these is scent-marking behavior which is sexually dimorphic in many species. In these studies, the effects of gonadal hormones administered neonatally and in adulthood on scent-marking and related behavior were examined in the gray short-tailed opossum (Monodelphis domestica). The gray opossum is a small, South American marsupial which is a practical species for laboratory research (Fadem et al., Lab. Anim. Sci. 32:405, 1982).

Scent-marking in gray opossums occurs with greater frequency in males (Fadem & Cole, Anim. Behav., 33:730, 1985) and only males possess a suprasternal scent gland. High levels of scent-marking were induced in ovariectomized adult females with both estradiol benzoate (EB) and testosterone propionate (TP). Scent-marking behavior was not eliminated although scent gland size was significantly reduced eight weeks following castration of adult males.

Because the reproductive system is in an undifferentiated stage at birth, marsupials are a particularly good model for studying the effects of early exposure to gonadal hormones on mammalian sexual differentiation. As evidence for the sensitivity of the newborn marsupial to gonadal hormones, EB administered on days 1 and 3 of postnatal life, blocked testis development and resulted in feminized genitalia in genetic male gray opossums (Fadem & Tesoriero, Anat. Rec. 211, 1985). Similar sensitivity of the developing marsupial brain and hence behavior to early hormone treatment is therefore likely. Studies of scent-marking and related behavior in neonatally hormone-treated gray opossums will be discussed.

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52.8 MATING BEHAVIOR IN HYPOGONADAL FEMALE MICE WITH GNRH CELL-CONTAINING BRAIN GRAFTS. M.J.Gibson,G.Kokoris,A.J.Silverman and E.A.Zimmerman.Dept.Med.,Mt.Sinai Sch.ofMedicine,New York,NY10029 and Columbia College Phys.&Surgeons,New York,NY 10032.

Due to a genetic deficiency in hypothalamic GnRH, the hypogonadal (Hpg) mutant adult female mouse is sexually immature and infertile. When GnRH cell-containing implants,from normal fetal preoptic area(POA),are placed in the IIIrd ventricle of Hpg females, the mice respond with increased LH and FSH, ovarian and uterine development,and continuous vaginal estrus. 7 of 10 such mice became pregnant when housed overnight with normal males (Science225:949'84).

This study examined the characteristics of Hpg/POA mice in mating tests with normal males. The females were compared with normal females in proestrus(PE), for their ability to elicit mounting from males,and in their lordosis response. In addition,blood samples from the orbital plexus were obtained at various times before and after mating in Hpg/POAs and on days of the cycle in normals. Mice were housed in 12L:12D(lights off:1200hr). Two-hr mating tests were conducted in the male's home cage during 1300-1700hr.

			Latency (in minutes) to:		
Group	N	#♂ mounts	1st♂ mount	♀ lordosis	♂ ejaculation
Normal	15	33.5±8.5 (n=13)	20.4±5.2 (n=13)	45.5±10.5 (n=10)	77.2±8.7 (n=5)
Hpg/POA	21	19.8±5.3 (n=20)	18.2±4.0 (n=20)	21.9±3.1 (n=10)	47.8±10.8 (n=10)

The Hpg/POAs appear to elicit the male's first mount attempt as effectively as the PE normal mice, although males mounted more frequently with normal females. The 10 of 21 Hpg/POAs that showed lordosis were responsive sooner than the normals that responded in the 2-hr tests. Furthermore, the males ejaculated sooner with the responsive Hpg/POAs than with normal PE females. Plasma levels in normal females at 1200hr were: on diestrus(n=7) 1.22±0.21ng/ml; on PE(n=10) 16.82±4.78ng/ml, and on estrus(n=8), 1.44±0.33ng LH/ml. In Hpg/POAs in constant estrus, LH levels at 1200hr were 0.87±0.09 ng/ml(n=10).Following the male's ejaculation,LH levels in Hpg/POAs were:at 30 min(n=6) 1.18±0.17ng/ml; at 60 min(n=4) 1.05±0.17ng/ml and at 120 min(n=7), 0.94±0.09mg/ml.

Hpg females with successful POA grafts appear to show normal or increased receptivity in restricted behavioral tests. However, we have not yet been able to detect significant increases in plasma LH following mating in the constant estrus females. An LH rise is expected, since our previous finding of pregnancy following one overnight mating session implied reflex ovulation. Additional time points will be studied.

Supported by NIH Grant HD 19077 to MJG.

52.9 BRAIN REGION SPECIFICITY IN ANTIESTROGEN INHIBITION OF LORDOSIS IN FEMALE RATS. R.L. Meisel, G.P. Dohanich, B.S. McEwen, and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Prior studies have demonstrated that estradiol stimulation of neurons in the ventromedial nucleus of the hypothalamus (VM) is a sufficient estrogen treatment condition for the display of sexual receptivity (i.e. lordosis) in female rats (Rubin & Barfield, Endocrinology, 106:504). In the present experiments, we tested the possibility that the interaction of estradiol with its receptors in VM neurons is necessary for lordosis in female rats.

In the first experiment, ovariectomized female rats were bilaterally implanted with cannulae aimed at the VM, preoptic area (POA), or medial amygdala (AMY). All females received subcutaneous implants of 5mm Silastic capsules containing a 5% mixture of estradiol diluted with cholesterol, followed 48 h later by an injection of 500 ug progesterone. Behavioral testing was conducted 4-5 h after progesterone, and a lordosis quotient (LQ), the percentage of male mounts that elicited lordosis, was calculated. A 1% crystalline dilution of the high affinity antiestrogen monohydroxytamoxifen (TAM) was delivered to the VM, POA, or AMY 24 h prior to estradiol treatment with matched groups of control females receiving empty implants. An additional group of VM females received TAM implants 12 h after estradiol. We also tested the effects of VM implants of a second antiestrogen, LY 156758 (LY). Application of TAM or LY to the VM 24 h prior to estradiol significantly reduced lordosis responding compared with control females (TAM - LQ = 46 vs. 89; LY - 50 vs. 90). TAM had no effect on lordosis when delivered to the POA or AMY 24 h prior to estradiol. TAM applied to the VM 12 h after estradiol was also without effect.

In a second experiment, we again found that VM implants of 1% TAM (LQ = 52) significantly reduced lordosis responsivity relative to control females (LQ = 85). Increasing the concentration of TAM to 10% essentially eliminated lordosis responding. Furthermore, even at this higher concentration of TAM, we found evidence of site specificity in that TAM-treated females with both cannula tips in or adjacent to the VM had lower levels of lordosis (LQ = 15) than did TAM-treated females with one or both cannulae outside the VM (LQ = 68), or control females (LQ = 85). A recovery test was given the following week, and the females previously treated with 10% TAM showed normal levels of lordosis (LQ = 75).

Thus, selectively blocking estradiol receptors specifically in VM neurons can reversibly antagonize the effects of systemically-delivered estradiol on lordosis behavior.

[Monohydroxytamoxifen was the generous gift of Dr. A.H. Todd of ICI Ltd. LY156758 was the generous gift of Dr. J. Clemens of Lily Research Laboratories.]

52.10 ATTENUATION OF THE PERIOD OF SEXUAL BEHAVIOR IN FEMALE GUINEA PIGS BY THE PROGESTIN RECEPTOR ANTAGONIST, RU 486. T.J. Brown* and J.D. Blaustein (Spon: Paul Herron). Div. of Neuroscience and Behavior, Dept. of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

In estrous-cycling guinea pigs, as well as in many other rodent species, the sequential secretion of estradiol and progesterone activates a period of sexual behavior (heat) of a limited duration. Although the activation of sexual behavior by estradiol and progesterone, characterized by the lordosis response, is thought to be mediated by mediobasal hypothalamic (MBH) intracellular progestin receptors, little is known of the mechanism by which the behavior terminates. Previously we have hypothesized that the termination of sexual behavior results from the loss of progestin receptors from MBH cell nuclei. We have shown that hormonal manipulations that delay heat termination also delay loss of MBH nuclear progestin receptors. In order to determine if accelerated nuclear receptor loss results in attenuation of the period of sexual behavior, we tested the effect of RU 486, a progesterone antagonist, on heat termination. We have shown previously that RU 486 prevents the initiation of sexual behavior when administered at the time of progesterone, and acts to decrease the concentration of available progestin receptors in MBH cytosol. Ovariectomized guinea pigs were treated with 10 ug estradiol benzoate. Forty h later, animals received 100 ug progesterone followed 4 h later by an injection of RU 486 or vehicle. A third group was treated identically except that RU 486 was given at the time of progesterone treatment. Animals were tested hourly for presence of the lordosis response. RU 486 injected along with progesterone prevented the initiation of sexual behavior, and when injected 4 h after progesterone, caused heat termination to occur 3-4 h earlier than in vehicle-injected animals.

We have found that RU 486 administration to estradiol-treated guinea pigs causes accumulation of progestin receptors in the nuclear extract; however, this accumulation can be detected only when assay conditions are used that promote exchange of RU 486 progestin receptor complexes (15 °C incubation rather than 0 °C). Therefore, we can use our routine assay conditions (at 0 °C) to measure primarily receptors that are occupied by progesterone. In order to confirm that RU 486 decreased (progesterone-occupied) nuclear progestin receptor levels when injected 4 h after progesterone, animals treated as in the behavioral experiment were killed 6 or 10 h after progesterone injection (2 or 6 h after RU 486), and nuclear progestin receptor levels were measured. RU 486 treatment resulted in lowered nuclear concentrations of MBH progestin receptors at both times, a finding that was confirmed by Scatchard analysis. These results support our hypothesis that the termination of the period of sexual receptivity in female guinea pigs is the result of loss of progestin receptors from MBH nuclei.

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52.11 FACILITATION OF MATING BEHAVIOR IN THE FEMALE RAT BY AN LHRH FRAGMENT, AC-LHRH 5-10: SITE SPECIFICITY AND ONSET OF ACTION. C.A. Dudley and R.L. Moss. Dept. Physiol. UTHSCD Dallas, TX 75235

Third ventricular infusions of Ac-LHRH 5-10 had previously been shown to facilitate mating behavior in the estrogen (EB) primed, ovariectomized (OVX) female rat (Neuroendocrinol. 86: 486, 1983). The present study assessed CNS-site specificity of this behavioral effect, and compared the magnitude and onset latency of the lordotic enhancement produced by Ac-LHRH 5-10 with that obtained by the entire LHRH decapeptide. Female rats were OVX and cannula were bilaterally implanted in the ventromedial hypothalamus (VMH; n=20), the midbrain central gray (MCG; n=20), the medial preoptic area (MPOA; n=15), or the cerebral cortex (CC; n=14). Animals were injected with EB (1-3µg at 0hr) and infused with either Ac-LHRH 5-10 (100ng/0.5µl saline/side) or saline (0.5µl/side) at 46hr. Mating behavior tests were conducted at 15, 45, 90,and 180 min post-infusion. The same procedure was followed for testing LHRH (100ng/0.5µl saline/side), except that the LHRH trials were counterbalanced and that LHRH was not tested in the CC.

Saline/Ac-LHRH 5-10 [$\bar{X}$ Lordosis-to-mount ratios (L/M)]

	15min	45min	90min	180min
VMH	.28/.38	.32/.61	.39/.85*	.39/.81*
MCG	.34/.44	.39/.74	.45/.78*	.41/.85*
MPOA	.06/.42	.18/.76*	.17/.84*	.39/.92*
CC	.16/.16	.21/.21	.20/.22	.28/.29

Saline/ LHRH [$\bar{X}$ Lordosis-to-mount ratios (L/M)]

VMH	.39/.66	.52/.86*	.54/.80	.60/.83
MCG	.36/.77*	.37/.82*	.36/.93*	.34/.96*
MPOA	.24/.42	.38/.30	.35/.32	.37/.37

*difference between peptide and saline L/M <.05; two-tailed t test.

In both the VMH and MCG, Ac-LHRH 5-10 significantly enhanced mating behavior at 90 and 180 min post-infusion. LHRH infused into the VMH produced a more rapid (45min) enhancement of lordotic responding than the fragment, however the LHRH effect was detected only at this one time point. Infusion of LHRH into the MCG quickly facilitated behavior (15 min) and maintained a significant elevation throughout the testing period. In the MPOA, Ac-LHRH 5-10 facilitated sexual receptivity at 45, 90, and 180 min whereas LHRH failed to enhance mating at this site. No change in mating behavior was observed in the CC following infusion of Ac-LHRH 5-10. An overall index of the magnitude of lordotic enhancement was obtained by summing the differences between the mean L/Ms obtained following drug and saline treatment. This manipulation revealed that Ac-LHRH 5-10 and LHRH were equally effective in the VMH; LHRH produced enhancement superior to that of Ac-LHRH 5-10 in the MCG; and Ac-LHRH 5-10 was more effective than LHRH in the MPOA. Supported by HD11814.

52.12 EFFECTS OF CASTRATION AND TESTOSTERONE REPLACEMENT ON TRAINED CIRCLING BEHAVIOR AND BRAIN DOPAMINE IN THE MALE RAT. P.C. Doherty, J. Lin* and M.J. Baum. Dept. of Nutrition and Food Sci., Massachusetts Institute of Technology, Cambridge, MA 02139.

Previous studies have shown that concentrations of dopamine (DA) in the nucleus accumbens and septum are decreased in male rats castrated for 30 days or more (Alderson and Baum, Brain Res. 218:189, 1981). The present studies were undertaken to determine if these deficits in DA content could be related to functional deficits in dopaminergic activity. Thus, the effects of castration or castration and testosterone (T) replacement on the ability of male rats to display trained circling behavior, an activity involving both the mesolimbic and nigrostriatal DA pathways, were assessed according to the methods of Yamamoto and Freed (Nature 298:467, 1982). Adult male Long-Evans rats that had been castrated or sham-castrated for thirty days or longer were deprived of water for 24h and trained for 7 days to circle in a clockwise or counterclockwise direction to receive a 10% sucrose solution on a continuous reinforcement schedule. The animals were subsequently retrained for 6 days and on the seventh day were allowed to circle for 0, 20 or 70 minutes. Castrated animals displayed significantly less circling behavior at both the 20 and 70 min time periods than did sham-operated controls. To determine if these differences could be ascribed to an effect of castration on the acquisition of the circling behavior, adult male Sprague-Dawley rats were trained to circle and subsequently were castrated, castrated and given a T implant or sham-castrated. Beginning 24 days later, the animals were retrained and thirty days after castration, the animals were allowed to circle for 0 or 20 min after which time the animals were sacrificed and the caudate, accumbens, septum and preoptic area-anterior hypothalamus (POA-AH) were removed for analysis of DA and dihydroxyphenylacetic acid (DOPAC) levels using HPLC with electrochemical detection. No significant effects of castration or T replacement were observed on the rate of circlng or DA concentations in these animals. Concentrations of DA and DOPAC were increased in the caudate in response to circling, as were DOPAC concentrations in the septum and accumbens; however, there were no significant effects of gonadal status or circling on lateralization of DA or DOPAC levels in any of these areas. Interestingly, there were also no significant differences in DOPAC/DA ratios in the POA-AH in these animals. This area has been reported to be sensitive to the effects of castration and T replacement on the rate of decline of DA concentrations afer α-methyl-p-tyrosine treatment. These results show that while castration may affect the acquisition of trained circling behavior, it has no significant effects on the display of this behavior, nor on the lateralization of DA within the central nervous system. (Supported by MH-00392 and HD-06333).

52.13 ANDROGEN RECEPTORS IN THE CEREBRAL CORTEX OF FETAL FEMALE RHESUS MONKEYS. S.A. Sholl* and S.M. Pomerantz. Wisconsin Regional Primate Res. Ctr., University of Wisconsin, Madison, WI 53715.

Previously, we demonstrated the presence of androgen receptors in the brain of the fetal rhesus monkey (Pomerantz et al., *Endocrinology* 116:83, 1985). The present study extends this finding by characterizing and comparing binding activities of both dihydrotestosterone (DHT) and methyltrienolone (R1881) in the fetal female cerebral cortex (Day 125-135 postconception). Cytosolic extracts were prepared in a buffer consisting of 10 mM Tris-HCl, 1.5 mM EDTA, 10% (v/v) glycerol, 1 mM mercaptoethanol and 25 mM $NaMO_4$ (pH 7.4). Extracts were incubated with 0.075-16 nM ^{3}H-DHT or ^{3}H-R1881 at 4°C for 16 hr. Separation of receptor-bound and free hormone was achieved on Sephadex LH-20 columns. A computer-fit of Bound/Total versus Total-ligand binding curves for ^{3}H-R1881 yielded a $K_a \approx 4.3 \times 10^9$M (n=4) and a receptor concentration of 5.5×10^{-15} moles/mg protein (n=2). Competition with DHT (2×10^{-6} M) or triamcinolone (TA, 10^{-5} M) reduced binding by 85% and 86%, respectively. Computer analysis of ^{3}H-DHT binding curves yielded a similar K_a ($\approx 7.0 \times 10^9$ M) and receptor concentration (1.7×10^{-15} moles/mg protein) (n=1). Competition with R1881 (10^{-6} M) was greater than 99%. In a separate series of experiments, the competition obtained when ^{3}H-R1881 was incubated with DHT (2×10^{-6} M) was greater than that observed when ^{3}H-R1881 was incubated with DHT when both the DHT-competed and uncompeted samples contained additional TA (10^{-5} M). This reduced competition suggests that TA may interfere with the binding of R1881 to the androgen receptor. Further analysis of receptor-binding on DEAE cellulose columns (KCl gradient) revealed multiple peaks (0.05-0.3 M KCl) with either ^{3}H-R1881 or ^{3}H-DHT as ligand. Similar elution profiles were obtained for the two ligands. Throughout the KCl-gradient competition with either R1881 or DHT significantly reduced ^{3}H-DHT binding while TA had no effect. In contrast, ^{3}H-R1881 binding was reduced by all three competitors. In summary, the findings verify the existence of a specific androgen receptor(s) in the cerebral cortex of female rhesus monkeys. Its presence may be important in terms of the influence of potential teratogenic agents or for normal developmental change.

53.1 TEMPERATURE COMPENSATION IN THE NERVOUS SYSTEM OF THE GRASSHOPPER, SCHISTOCERCA AMERICANA. C.I. Miles*(SPON: R.B. Pinter). Dept. of Zoology, University of Washington, Seattle, Wa. 98195.

Small terrestrial ectotherms like grasshoppers experience wide variations in body temperature as they move through thermally complex environments. Such variations can affect the performance of the nervous system, as individual insect neurons are known to be sensitive to temperature.

The temperature sensitivities of mechanosensory hair neurons were examined in an earlier study (Miles, C.I., J. exp. Biol. in press). Of these neurons, wind-sensitive head hairs were by far the most temperature sensitive. These mechanoreceptors occur in five fields on each side of the head, on its dorsal surface. Their sensory neurons have inputs to an identified interneuron in the brain, the Tritocerebral Commissure Giant, (TCG). Inputs from four of the fields are excitatory to the TCG, while those of one are inhibitory (Bacon & Mohl, J. comp. Physiol. 150:439-153, 1983).

Temperature differences of up to 5°C were recorded between the head cuticle near the wind-sensitive hairs and the brains of animals placed in thermally complex environments. The effects of such differences on the response of the TCG to stimulation of the head hairs were examined. If the temperatures of head hairs were increased while that of the TCG did not change, TCG output showed little sensitivity to temperature. Thus, in spite of great increases in the firing frequencies of its inputs, the TCG's output remained essentially constant. If, however, the temperature of the TCG itself increased while that of its inputs did not change, the interneuron's output in response to stimulation of either excitatory only or both excitatory and inhibitory inputs was dependent on its temperature.

When temperatures of both the TCG and its mechanosensory inputs increased together, the TCG's output displayed a sensitivity to temperature when only the excitatory inputs were stimulated. While substantial, this sensitivity was not as great as that of the sensory neurons themselves. If, however, both excitatory and inhibitory inputs to the TCG were stimulated, the temperature dependence of the interneuron's output was greatly reduced. Reducing the strength of stimuli applied to its excitatory inputs alone had a similar effect.

There are thus at least two levels of information processing in the head hair/ TCG system where temperature compensation occurs: One is at the level of receiving sensory inputs; temperature induced increases in inhibitory input can counteract increases in excitatory input. Another involves the generation of interneuronal output; if the temperature of the TCG does not change, its output remains constant despite marked increases in its inputs. The neuronal basis for these compensations is under investigation.

53.2 GABA-MEDIATED INHIBITION IN THE ANTENNAL LOBES OF THE MOTH *MANDUCA SEXTA*. T.A. Christensen, B.R. Waldrop and J.G. Hildebrand. Dept. of Biol. Sci., Columbia Univ., New York, NY 10027.

Electrophysiological studies of neurons in the primary olfactory centers, the antennal lobes (ALs), in the brain of *Manduca sexta* have revealed that inhibitory synaptic interactions play a prominent role in the processing of olfactory information in this species. Projection neurons (PNs), which relay information from the ALs to higher centers in the protocerebrum, generally show a biphasic inhibitory/excitatory response when the antennal nerve (AN) is stimulated by electrical shock [Harrow & Hildebrand, *Soc. Neurosci. Abstr. 8*:528 (1982)]. Many of these cells, including the male-specific, pheromone-responsive PNs, are inhibited synaptically when the ipsilateral antenna is stimulated with natural odors.

The ALs synthesize and contain relatively large amounts of γ-aminobutyric acid (GABA)[Maxwell *et al., Comp. Biochem. Physiol. 61C*:109 (1978); Kingan & Hildebrand, *Insect Biochem.*, in press], which is known to be a major inhibitory neurotransmitter in many species of invertebrates and vertebrates. Moreover, recent immunocytochemical studies in our laboratory have shown that GABA is present in many intrinsic, local interneurons (LNs) in the ALs of *Manduca* [Hoskins *et al., Soc. Neurosci. Abstr. 10*:688 (1984)]. We are studying PNs to test the hypothesis that the observed synaptic inhibition is GABA- and chloride-mediated.

Injection of hyperpolarizing current reverses the IPSP evoked by shocking the AN, suggesting that the IPSP may be due to an increased conductance for chloride or potassium ions. Treatment of the preparation with picrotoxin or bicuculline reversibly blocks the IPSP. Preliminary results indicate that injection of GABA into the AL neuropil can hyperpolarize the PNs and that the response to GABA reverses near the apparent resting potential. We are assessing the role of chloride ions in the IPSP in PNs by means of chloride-substitution experiments. If the IPSP is mediated by chloride, then a decrease in the chloride concentration gradient across the cell membrane will shift the reversal potential of the IPSP in a depolarizing direction. To perform such experiments, we inject chloride (using KCl-filled electrodes) into test PNs or superfuse the preparation with low-chloride saline solution. In still other experiments, we examine the simultaneous effects of application of GABA and alteration of chloride concentration gradients.

These electrophysiological studies, together with the aforementioned neurochemical investigations, promise to advance our limited understanding of GABA-mediated synaptic inhibition in the insect central nervous system.

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53.3 HEARING IN A PRAYING MANTIS. D. Yager* and R. Hoy. Section of Neurobiology & Behavior. Cornell University, Ithaca, NY 14853.

To observe the prominent eyes of the praying mantis is to acknowledge the obvious: it is a highly visual animal. Only recently, however, has the almost complete dominance of vision in the mantis's sensory world begun to be questioned. We now report that the mantis, Mantis religiosa, possesses an acute and well-developed auditory sense. Ours is the first report of hearing in this group of insects.

We demonstrated an auditory capability in the mantis by making electrical recordings from its ventral nerve cord. First, extracellular recordings from the connectives between the pro- and meso-thoracic ganglia revealed, 1) as many as four action potential classes that responded to sound, 2) that the auditory response encompasses a broad frequency range from 10 to 80 kHz, and 3) that, as in crickets and some moths, mantids are by far most sensitive (55-60 dB SPL) at frequencies between 25 and 45 kHz. Second, intracellular recordings were made from two types of auditory neurons whose physiological properties paralleled those described from extracellular recordings. The anatomy of one of these neuronal types was revealed by lucifer yellow staining.

Perhaps the most surprising aspect of hearing in mantises is the ear itself. Contrary to the usual pattern of paired, laterally placed acoustic receptors, the mantis possesses a single, midline ear. Externally the ear appears as two membranes facing each other within a groove situated between the metathoracic legs. Internally, each membrane is backed by a large tracheal sac; the sacs behind the two membranes are connected to each other. Associated with each sac is a small receptor organ which is connected to the metathoracic ganglion by a nerve root containing about 20 axons, 3-5μ in diameter. As might be suggested by the anatomy of the ear, we have found no indication that the mantis auditory system can distinguish right from left at frequencies as high as 40 kHz.

In summary, the praying mantis can hear. It does so using an unorthodox single ear located in the animal's midline. The auditory system is almost exclusively sensitive to the ultrasonic frequencies of hearing. Thus far, the behavioral significance of audition in mantids remains a mystery.

53.4 THE INFLUENCE OF SOUND DIRECTION ON ENCODING OF STIMULUS TEMPORAL PATTERN IN THE CRICKET TELEOGRYLLUS OCEANICUS. G.S. Pollack. Dept. Biol., McGill Univ., Montreal, Quebec, Canada H3A 1B1.

Crickets are able to recognize the calling song of a conspecific using the temporal pattern of the stimulus as a cue. Teleogryllus oceanicus orients towards a song model with the temporal pattern of the conspecific song in preference to a model of heterospecific song when both stimuli are presented simultaneously from loudspeakers on opposite sides of the animal. The ability to discriminate between simutaneously presented patterns implies that they are represented separately within the nervous system. This might be made possible by the directional characteristics of the auditory system. The present work examines the relationship between sound direction and the fidelity with which temporal patterns are encoded in the central nervous system.

Recordings were made from omega neurons (ON), a pair of interneurons in the prothoracic ganglion which have been implicated in the processing of directional information. Each ON is most sensitive to sound on its soma-ipsilateral side, although it can also be excited by soma-contralateral sound. Responses were recorded to presentation of conspecific and heterospecific song models from opposite sides of the animal, both one at a time and simultaneously. The fidelity with which ON encoded the temporal pattern of the song on the soma-ipsilateral side was determined by cross-correlation analysis of the PST histogram describing its spiking response. ON faithfully encoded both song models when they were presented one at a time. Encoding of the soma-ipsilateral song was only minimally disturbed by simultaneous presentation of the soma-contralateral song when the broadcasting loudspeakers were at angles of 90°, 60° or 30° to the left and right of the midline, but was severely disrupted when the speakers were at 15°.

Preliminary behavioral experiments accord well with these findings. In a tethered flight assay, crickets stimulated simultaneously with both patterns reliably oriented towards the conspecific pattern when the loudspeakers were at angles of 90°, 60° or 30°. This ability deteriorated when the speakers were at 15°, even though crickets could reliably orient towards the stimulus when it was presented alone from this angle.

Considerable attention has been focused on the directional properties of the cricket's auditory system. This work has been concerned primarily with the problem of how the cricket is able to locomote to the sound source. The present findings suggest that another important behavioral role of directionality may be to permit the cricket to distinguish stimulus temporal patterns under less than ideal, but perhaps not unnatural, acoustic conditions. Supported by the Natural Sciences and Engineering Research Council of Canada.

53.5 IDENTIFICATION AND CHARACTERIZATION OF INHIBITORY INPUTS IN THE CRICKET CERCAL AFFERENT SYSTEM. G.A. Jacobs, C. Redfern* and J.P. Miller. Dept. of Zoology, UC Berkeley, Berkeley, Ca. 94720

The directionally sensitive response properties of wind sensitive interneurons in the terminal abdominal ganglion of the cricket result from a combination of monosynaptic excitatory inputs and polysynaptic inhibitory inputs. At present, these inhibitory interneurons are unidentified. We have undertaken a series of experiments aimed at identifying these neurons and characterizing their effects on identified wind sensitive interneurons in the cricket cercal afferent system. Interneuron 10-3 receives polysynaptic inhibitory inputs which can be activated by stimulating selected classes of sensory afferents on either cercus. The ion that mediates the IPSP is most likely chloride since the IPSP recorded in the cell body reversed near the resting potential of the interneuron and injection of chloride ions eliminated the IPSP evoked by wind stimuli. As a first step in the anatomical identification of these inhibitory interneurons, we have stained the terminal ganglion with a commercially available antibody directed against γaminobutyric acid (GABA) (Immunonuclear) which is the putative inhibitory transmitter. Terminal ganglia were fixed, embedded in paraffin and serially sectioned at 10 microns. The sections were incubated in the antibody at a dilution of 1:1000 and processed using a modified PAP technique. This technique revealed approximately 80 neurons that exhibited GABA-like immunoreactivity in the terminal ganglion. The cell bodies of these neurons fall into two size classes: those with soma 80-100 microns in diameter and 20-50 microns in diameter. The cells are arranged in bilaterally paired clusters that are located on both dorsal and ventral surfaces of the ganglion. We are currently using a double label technique to identify individual putative GABAergic neurons by injecting wind sensitive neurons with lucifer yellow followed by sectioning and staining for GABA-like immunoreactivity. (Supported by NSF Grant BNS 82-02416 to JPM.)

53.6 MORPHOLOGICAL AND PHYSIOLOGICAL EXAMINATION OF A PAIRED DORSAL GROUP OF INTERNEURONS IN THE THORACIC GANGLIA OF THE COCKROACH. A.J. POLLACK* AND R.E. RITZMANN. Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106.

Several insect circuits clearly employ many interganglionic interneurons to integrate sensory information and direct motor behavior. In an attempt to understand complex orientation behavior in the cockroach, we are conducting a long term study directed at characterizing important groups of interganglionic interneurons in that animal.

Cobalt backfills were performed on the thoracic T2-T3 connectives. Most of the somata filled in the metathoracic ganglion were ventral (67%) and in specifically located, reproducible groups. Two bilateral groups with dorsal somata were also filled. One of these, the dorsal posterior group (DPG), could be easily located by backfilling only the lateral 1/2 to 1/3 of the connective, which contains the more lateral and dorsal tracts of axons travelling through the thoracic ganglia (Gregory, 1974). In T3 these DPG cells emerged as two distinct groups. DPG-I cells were more anterior but still posterior to the ganglion A-P midline. DPG-II cells were even more posterior; in fact, they appeared in the first abdominal ganglion which is fused to the third thoracic ganglion. In addition to grouped somata, the DPG cells all had neurites that crossed the midline in common tracts and axons restricted to two longitudinal tracts; the lateral dorsal and dorsal intermediate tracts (LDT and DIT, respectively).

More specific morphological information was obtained by intracellular penetration of DPG cells with dye-filled microelectrodes. Lucifer yellow fills showed fine detail in wholemounts and sections, and an overlay-template system of grids for soma and tract positions allowed cell-to-cell comparisons of location and reproducibility. The detailed morphology of five cells in DPG-I and at least one in DPG-II have been documented. At least three of these cells have large axons in the connectives, making them useful targets for further intracellular analysis.

Numerous physiological recordings were made while the intracellular electrodes were in place. All T3 DPG cells were found to be activated by wind directed at the cerci and by tactile stimulation of the metathoracic legs. In addition, in double penetrations of a GI and a DPG, vGI stimulation caused DPG excitaton while dGI stimulation did not. Upon intracellular stimulation of the DPG cells, weak but definite excitation of motor neurons was often seen. This work is being continued to determine if the DPG's play a role in integrating wind information from the GI's and directing activity to appropriate motor neurons for the wind mediated escape behavior.

This work was supported by NIH grant 1 R01 NS17411-01 to R.E.R..

53.7 CHARACTERIZATION OF THORACIC INTERNEURONS IN THE COCKROACH: PHYSIOLOGICAL AND MORPHOLOGICAL ANALYSIS. M. Murrain and R.E. Ritzmann. Dept. of Biology, Case Western Reserve Univ., Cleveland, OH 44106.

A large array of sensory structures are located on the leg of the cockroach. These relate information about leg position and movement to the central nervous system, and are used in a variety of ways to help coordinate and fine-tune many behaviors. Of particular interest to us is how this information is used to help coordinate the walking behavior. Macmillan and Kein (Proc. R. Soc. Lond. 218:287,1983) have demonstrated specific reflexes from different leg sensory structures in the locust, which help to time the different phases of walking.

In order to further understand the cellular mechanisms involved in processing of leg sensory information we have begun a study of thoracic interneurons which are sensitive to leg sensory structures. The structures tested were those which have been determined to be involved in intra- and intersegmental coordination and fine tuning of walking (Macmillan and Kien,1983; Wong and Pearson J Exp. Biol. 64:233, 1976). Individual cells were impaled intracellularly, and the responses to stimulation of individual sensory structures analyzed. A group of interneurons, both intra- and intersegmental have been identified by morphology. Also, the arborization patterns of the sensory cells have been determined by a cobalt backfilling technique similar to that used by Hustert, et. al. (Cell Tiss. Res. 216:97,1981).

One of the questions that we would like to ask about these interneurons is whether they have common morphological loci, and, can we match those common loci with the morphological location of the sensory neurons themselves. This gives us predictive capabilities in two situations: if we only know the morphology of a given cell, we can predict whether this cell has a chance of getting input from a specific sensory structure. Also, if we know that a given cell recieves input from a sensory structure, we can predict from its morphology whether this cell gets this sensory input directly or from an intervening interneuron. We have employed a computer system to compile morphological data from many cells, and to aid in finding overlap in three dimensions.

The computer system we developed involves digitizing the morphological data into a computer and reconstructing the cell and ganglion in three dimensions. The system then partitions the ganglion into many small cubes. The cell data is then run through these partitions and counted. Each cube can be analyzed for total number of cells that go through the cube, and those cells that go through with specific physiological charictaristics. Several areas of the ganglion have been found to be common loci for groups of cells, and some of these have been correlated with backfill data. Supported by NIH grant NS17411-01 to R.E.R.

53.8 PHYSIOLOGICAL CHARACTERISTICS OF SENSORY CELLS OF DROSOPHILA WING CORRELATE WITH CENTRAL PROJECTIONS AND DEVELOPMENTAL TIMING. M. H. Dickinson and J. Palka, Department of Zoology, University of Washington, Seattle, WA 98195.

Campaniform sensilla are common sensory structures of insect appendages; they respond to small deformations of the cuticle. Eight such sensilla, appearing identical under light and scanning electron microscope, are found in specific locations on the wings of Drosophila. The single sensory cells associated with these identified sensilla send axons into distinct branching regions and tracts in the CNS. Their projection patterns correlate not with the topographic distribution of the sensilla on the wing, but with the time in development when the neurons differentiate (Wigston, et al., Abst. Soc. Neurosci. 10, 139). We have now found that certain physiological characteristics of the sensory neurons also correlate with the central projections.

We monitor the action potentials (AP's) elicited by deformational stimuli using a high impedance AC amplifier, recording differentially along the length of the wing veins in which the axons lie. Stimuli are delivered by a fine glass probe mounted on a piezoelectric bender. Transverse cuts through the wing and ablations of individual sensilla allow extracellular recordings of single neurons. With these techniques we can unambiguously determine the responses of 7 of the 8 sensory neurons to reproducible stimuli. Three of the neurons are phasic, firing short bursts of AP's at the onset and/or offset of wing deformation. Their axons all project medially. The other four neurons respond to apparently identical stimuli with tonic trains of AP's, and their axons all project laterally. Thus, the projection of these wing campaniform sensilla is organized according to sub-modality. The sensilla presumably function during flight, courtship song, grooming and other behaviors during which strains are set up in the wing cuticle. The phasic cells act as accelerometers, encoding rapid changes in wing deformation, while the tonic cells monitor the magnitude and duration of strain. Their signals are delivered to at least partially segregated regions of the CNS.

As demonstrated previously, the medially projecting neurons differentiate and extend axons early during metamorphosis; we now see that they carry phasic information. The laterally projecting neurons arise during a second wave of cell birth and differentiation; they carry tonic information. The causal factors generating these strict correlations remain to be established.

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53.9 MORPHOLOGY OF THE EYE OF THE GIANT DEEP-SEA ISOPOD *BATHYNOMUS GIGANTEUS*. S. C. Chamberlain, V. B. Meyer-Rochow*, and W. P. Dossert*. Inst. for Sensory Research, Syracuse University, Syracuse, NY 13210 and Dept. of Biological Sciences, University of Waikato, Hamilton, New Zealand.

Bathynomus giganteus is the largest known isopod species. We have examined the eyes of four specimens recovered from depths of 500 to 600 meters near the edge of the continental shelf off Key West, FL. The triangular compound eyes face forward and slightly downward and appear to have significant overlap in visual fields. Each eye contains about 3,500 ommatidia independent of animal size over a range of body lengths from 22.5 cm to 37.5 cm. The packing of ommatidia is not uniform across the retina, but is nearly hexagonal in the central, central dorsal, and medial regions and nearly square in the ventral and lateral regions.

The optical elements for each ommatidium consist of a laminar cornea which is flat externally and convex internally and a bipartite crystalline cone. Seven or eight retinular cells closely appose the cone internally and bear the microvilli of the rhabdom. Below the rhabdom the retinular cells form thin pillars near the periphery of the ommatidium and the central portion along the optic axis is occupied by interstitial cells which contain massive arrays of clear vesicles thought to serve as reflective elements. The arhabdomeral segments of the retinular cells and the interstitial cells sit on a basal limiting membrane. Each ommatidium has two unusual apical extensions of the limiting membrane, each with a cylindrical central core and thin sheets of dense material and collagen fibers which occupy spaces between adjacent interstitial cells up to the level of the rhabdom. Arrays of pigment cells separate adjacent ommatidia, but have relatively weak light screening properties.

Animals were fixed (a) within a week after being brought from depth and exposed to daylight and (b) after two months of maintenance in constant darkness following such daylight exposure. In both cases, the microvilli of the rhabdom were severely disrupted and the retinular cytoplasm contained numerous multivesicular bodies. Exposure to natural daylight appears to cause irreversible structural damage to the photoreceptors of these animals.

53.10 **INTRACELLULAR RECORDINGS OF OPTIC LOBE INTERNEURONS IN THE COCKROACH PERIPLANETA AMERICANA: COLOR OPPONENCY AND POLARIZATIONAL SENSITIVITY.** **K.M. Kelly* and M.I. Mote* (SPON: S. McElligott). Dept. of Biology, Temple U., Phila., Pa. 19122**

Intracellular recordings from spontaneously active optic lobe interneurons were made while stimulating the ipsilateral eye with brief broadfield flashes of monochromatic light from 370-703 nm over a 6 log unit range of intensity. Recorded cells were then injected with Lucifer yellow to reconstruct their anatomy. Response patterns were analyzed by applying the known differences in the spectral sensitivity functions as well as the waveform and timecourse of the receptor potential in the ultraviolet (UV) and green (G) receptor groups in the animal's dichromatic retina. Determining which receptor groups were responsible for input to a recorded cell allowed use of a classification system adopted by Kien and Menzel (1977) which categorizes response properties according to the number of receptor groups involved and the type of input. 70 responses fell into one of the categories of broadband, narrow band and color opponent.

Broadband responses result from either excitatory or inhibitory input from both UV and G receptors. Response magnitudes indicate convergence of the UV and G input at short wavelengths where there is broad overlap in the sensitivity functions of the two receptor groups. This usually occurred with high to intermediate intensity stimulation. Narrow band responses result from either excitatory or inhibitory input from only one color receptor group. Narrow band properties were seen in cells receiving predominantly single channel input with evidence of some antagonistic interactions or single channel input at high intensities and dual channel input at lower intensities.

Color opponent responses result from antagonistic input from more than one color receptor group. Opponency was seen only as UV inhibition and G excitation. In some cases, responses to a fixed wavelength were dominated by one receptor group at low intensities and by the other group at high intensities.

Polarizational sensitivity was tested in 30 neurons by a stationary polarizing filter inserted into the light path and rotated between successive flashes of monochromatic light or rotated through a 90° arc in a clockwise, then counterclockwise, direction during constant monochromatic illumination. There was no apparent change to the stationary e-vector orientation, however, 2 cells appeared to change in firing rate by being inhibited by rotation in one direction and excited by rotation in the reverse direction. **Supported by NIH grant EY00784 to MIM.**

53.11 PROCESSING OF COLOR IN BUTTERFLY VISUAL SYSTEM. G.A. Horridge (SPON: E.E. BALL). Dept. of Neurobiology, Australian Natl. Univ. P.O. Box 475, Canberra. ACT 2601, Australia.

Intracellular recording reveals four types of photoreceptors in the retina of Papillo and other butterflies, but in order to measure the properties of the primary photoreceptors as transducers the indifferent electrode must be immediately outside the cell recorded. With a single electrode and indifferent electrode below the basement membrane of the eye, hyperpolarizations can be recorded with an off-axis stimulus, or at some wavelengths on axis. Whether this negative coupling between primary receptors is significant in vivo has been investigated by repeating the experiments but with intracellular recording from the second-order cells (laminar monopolars). Also several behavioral responses have been tested for color opponency and color-specific behavior. The second-order neurons of the lamina, and the behavioral responses are color-coded, but show no sign of color vision in individual neurons or responses. Color vision is defined as the ability for a neuron or behavior pattern to make a response to wavelength irrespective of contrast or intensity. The second-order neurons can have extremely sharp spectral sensitivity curves but as yet it is impossible to locate the exact origin or the behavioral significance of color-opponency neurons already long known in the protocerebrum of butterflies.

53.PO THE MOST DIRECT PATHWAY FROM PHOTORECEPTORS TO EYE MUSCLE MOTONEURONS IN DAPHNIA IS THROUGH ONE INTERNEURON. T.R. Consi, S.J. Sims*, N. Necles*, K.C. Smith* and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

Computer reconstructions from serial electron micrographs of the Daphnia magna compound eye visual system have revealed that at least two classes of interneurons in the optic ganglion receive direct chemical synaptic contacts from photoreceptor terminals in the lamina. The LS neurons, one of these two classes, have dendrites only in the lamina and send unbranched axons which bypass the medulla and enter the supraesophageal ganglion (SEG) (see Sims & Macagno, J. Comp. Neurol. 233:12, 1985). We have now traced these axons for a few of the LS cells (those which have particularly large axons) and have discovered that they travel to and branch in the same area of SEG neuropil where the eye muscle motoneurons branch. We have also found that at least one of these LS interneurons, ml5, is presynaptic to the pair of motoneurons which are connected to the contralateral ventral eye muscle (see Consi et al., Am. Zool. Abs. 21:956, 1981). Cell ml5 branches in the dorsal half of the laminar neuropil and extends across the midplane, making synaptic connections with similar subsets of photoreceptors within each of the optic cartridges which it innervates. Thus, our anatomical observations reveal that the most direct pathway from the compound eye photoreceptors to the motoneurons which innervate the muscles that rotate the eye is through individual LS interneurons.

Several reasons lead us to propose that this is probably the most important neural pathway mediating the eye flick response (see Consi & Macagno, J. Comp. Physiol. A156:135, 1985). 1. The eye flick is always a ventral rotation, independent of the position of the stimulus in the visual field, and it must therefore utilize the ventral motoneurons. 2. The LS cells that branch in the motoneuron neuropil of the SEG are those with the largest axons and are therefore best suited to produce a fast reflex like eye flick. 3. Cell ml5 receives synaptic inputs from photoreceptors of different spectral classes (see Schehr & Macagno, Soc. Neurosci. Abs. 9:325, 1983), which is necessary in order to explain the complex spectral dependence of the eye flick. Current efforts are addressing the question of whether the spectral sensitivities of the photoreceptors known to connect to ml5 provide sufficient information to explain the wavelength dependence of the eye flick. (Supported in part by NIH Grant NS-14946.)

54.1 MPTP PRODUCES CLASSIC PARKINSONIAN SYNDROME IN AFRICAN GREEN MONKEYS. D.E. Redmond, Jr., R.H. Roth, and J.R. Sladek. Psychiat. & Pharmacol., Yale Sch. Med., New Haven, CT 06510, Dept. Anatomy, Univ. Rochester Sch. Med., Rochester, NY 14642.

MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) in humans, rhesus, and squirrel monkeys produces selective neurotoxicity of the substantia nigra and a syndrome almost identical to Parkinson's disease. Rather than the characteristic resting tremor of parkinsonism in humans, one difference in monkeys has been that, if tremor was observed, it appeared to be a postural or action tremor. The present study was undertaken to characterize simultaneous behavioral, biochemical, and histopathological effects in African green monkeys two months after treatment with MPTP at a time when acute pharmacologic and non-specific neurotoxic effects have diminished.

Three male monkeys were treated with 5 doses of 0.25 mg/kg MPTP over 5 days. Four monkeys served as controls. Two MPTP treated monkeys developed mild tremor acutely after the third or fourth dose; the monkey which was most severely affected was successfully treated with Sinemet (25 mg/kg per day) to overcome the effects of motor deficits on food and water intake. Daily observations were made of each monkey over the entire period. Videotapes were made in a variety of semi-natural conditions to demonstrate deficits and their similarities to idiopathic parkinsonism. The severity of deficits in the three monkeys was correlated with changes in brain determined in several ways. After sacrifice under deep anesthesia, the brains were rapidly removed, chilled, blocked, and sliced into 5 mm sections. Monoamine enzymes, transmitters, and metabolites were measured in brain regions dissected by micropunch from one side of the brain, and the opposite side was freeze dried and prepared for dopamine histofluorescence.

The three MPTP animals showed varying degrees of deficits. One animal was more severely impaired, showing bradykinesia, freezing, and difficulty in initiation of movement; a resting tremor of the head and arms was intensified by stress and certain activities but eliminated by other types of movement. All deficits improved after exercise. The other animals showed much more subtle changes. Videotapes of behavioral effects will be shown. Biochemical and histopathological measures showed changes that were consistent with the motor and behavioral deficits, supporting reports that MPTP produces a close approximation to idiopathic parkinsonism.

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54.2 THREE-DIMENSIONAL COMPUTER ANALYSIS OF MPTP-INDUCED AMINERGIC CELL LOSS IN THE MONKEY. D.M. Bowden, M.F. Dubach*, R. Snyder*, S. Askari*, & D.C. German. Regional Primate Res. Cntr., and Dept. of Psychiat., Univ. of Washington Sch. of Med., Seattle, WA. 98195 and Depts. of Physiol. and Psychiat., Univ. of Texas Health Sci. Cntr., Dallas, TX. 75235.

The meperidine analogue, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), has been reported to induce a parkinsonian syndrome in both human and non-human primates (see Burns et al., Proc. Natl. Acad. Sci., 80:4546, 1983). In the monkey, the syndrome was accompanied by a depletion of neostriatal dopamine (DA) but not of nucleus accumbens DA, and a reduction in substantia nigra zona compacta neurons. Norepinephrine (NE) in locus coeruleus (LC) neurons appeared normal.

The purpose of the present experiment was to quantify regional catecholamine-containing cell loss in the monkey produced by MPTP. Four adult male Macaca fascicularis monkeys were used for this experiment, two controls and two treated with MPTP. Both monkeys received 5 consecutive daily injections of MPTP (0.35 mg/kg, i.v.). Monkey 1 survived for 45 days and Monkey 2 for 21 days. Monkey 1 had a more severe parkinsonian syndrome (akinesia, tremor, etc) than did Monkey 2. A computer graphics system was used to reconstruct the 3-dimensional pattern of DA cell loss in the midbrain (nuclei A8, A9 and A10) and NE cell loss in the LC. A tyrosine hydroxylase (TH) immunohistochemical staining procedure was used to identify these neurons. The midbrain DA neurons were found to have a rostral-caudal extent of 6-7 mm, and the A8 and A9 cells are multipolar and measure up to 18 X 30 μm. The A10 cells are round/oval and measure up to 13 μm in diameter. These cell sizes were smaller in the MPTP-treated animals. The midbrain DA cell complex has an estimated 250,000 cells in the normal animal. The LC has a rostral-caudal extent of 2.5-3.0 mm and the oval multipolar cells measure 20-30 μm in diameter. MPTP produced a severe loss (> 70%) of DA cells in Monkey 1 and primarily spared cells in the A10 and substantia nigra pars lateralis. Monkey 2 had less DA cell loss than Monkey 1, and predominantly in the substantia nigra zona compacta. LC cell loss was minimal in both monkeys, however, the TH staining intensity was more prominent in the MPTP-treated vs. control animals. This latter observation suggests that MPTP, like reserpine, may induce TH activity in the LC. Further examination of the present brain tissue will be conducted to quantitatively determine specific regions of DA cell loss and whether the loss is confined to a particular morphological cell type. Research supported by grants from the Dallas Area Parkinsonism Society, American Parkinson's Disease Association, Biological Humanics Foundation and NIH grants RR-00166 and NS-20030.

54.3 ALZHEIMER DISEASE RELATED ANTIGEN PRESENT IN TANGLES, PLAQUES AND MORPHOLOGICALLY NORMAL NEURONS. B. L. Wolozin*, A. Pruchnicki*, and P. Davies* (SPON: C. Raine). Albert Einstein College of Medicine, The Bronx, NY 10461.

Alzheimer's disease is a neurodegenerative disorder whose pathology is defined by the presence of neuritic plaques and neurofibrillary tangles. We have generated a monoclonal antibody, called Alz-50, which recognizes an antigen present in plaques and tangles, as well as in neurons from Alzheimer brains which are normal by thioflavine immunocytochemistry.

An ELISA assay was used to compare the binding of the antibody to Alzheimer and normal brain homogenates. Using 5% nonfat milk as a blocking agent, Alz-50 recognized an antigen that was present in Alzheimer brain and absent from normal brain or present in much lower amounts. Immunocytochemistry on formalin-fixed tissue showed that the antigen is present in both plaques and tangles in Alzheimer brain. In addition, many pyramidal neurons of the hippocampus and large neurons in the frontal cortex were stained. Only an occasional neuron was stained in sections of normal human brain.

Tissue sections were double-stained with thioflavine and Alz-50 followed by rhodamine-labelled or peroxidase-coupled secondary antibody. This protocol revealed neurons in Alzheimer brains that were stained with antibody but not stained with thioflavine. There were also tangles that were stained with thioflavine but were not stained by Alz-50. Biochemical studies show that the antigen recognized by Alz-50 is not strongly coupled to neurofibrillary tangles. Unlike tangle antigens, this antigen is partially soluble in saline, fully soluble in 5% SDS, and is sensitive to both trypsin and neuraminidase. Preliminary studies suggest that the antigen has a molecular weight of 50kd. These results suggest that formation of plaques and tangles might not represent the only changes in the cell biology of neurons in Alzheimer's disease.

This investigation was supported by NIH training grant T32 GM7288 from the National Institute of General Medical Sciences, and a grant from The McKnight Foundation.

54.4 GLUCOSE AND GLUTAMINE OXIDATION, DNA AND PROTEIN SYNTHESIS IN FIBROBLASTS FROM YOUNG, AGED AND ALZHEIMER DONORS. C.Peterson and J.E.Goldman, Dept.Pathology (Neuropathology) Albert Einstein College of Medicine, Bronx, N.Y. 10461

Cultured skin fibroblasts provide a convenient model to study human aging and age-related disorders, such as Alzheimer's disease (N.Engl.J.Med. 312:1063 (1985)). The present study examined the oxidation of glucose and glutamine, the incorporation of leucine into protein and thymidine into DNA in four different cell lines from young, aged and Alzheimer donors. Fibroblasts were seeded (day 1) in glass scintillation vials (20,000 cell/vial) in Dulbecco's modified Eagles medium supplemented with 15% fetal bovine serum. The media was replaced on day 4 by one that was serum-free and any confluent cultures were discarded. On day 5, the cells were incubated, for the indicated times, in serum-free media that contained either ^{14}C-glucose (4 hr), ^{14}C-glutamine (4 hr), ^{14}C-leucine (4 hr) or ^{3}H-thymidine (24 hr). To terminate the incubation for $^{14}CO_2$ collection the vials were acidified. For protein and DNA synthesis, the media was removed and the cells were washed three times with serum free media before acidification. Protein was determined in the acid insoluble precipitate. All conditions were examined in triplicate at three different passages between 5 and 10.

	YOUNG	AGED	ALZHEIMER
Age (years)	23.2±1.1	63.5±2.1	61.7±3.0
Glucose	100±2	85.5±4.9*	72.9±3.8*
Glutamine	100±3	68.9±2.0*	53.9±3.0*
Thymidine	100±3	52.2±2.3*	69.3±2.3*
Leucine	100±3	78.4±3.2*	102 ±1.1

Values are expressed as percent of young.
*Denotes difference (P<0.05) from young control.

Thus, during Alzheimer's disease oxidative processes may be depressed while biosynthetic ones are slightly elevated when compared to normal aging. These findings suggest that at the cellular level Alzheimer's disease may have some systemic alterations.

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54.5 SOMATOSTATIN LOSS PREDOMINATE IN HYPOMETABOLIC BRAIN REGIONS IN ALZHEIMER'S DISEASE, C.A. Tamminga, K. Tanimoto*, S. Kuo*, A. Kask*, T.N. Chase, Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205 and Maryland Psychiatric Research Center, Baltimore, MD 21228

Several cortical neurotransmitter systems have been identified as abnormal in Alzheimer's Disease, with little evidence to indicate which, if any, may be causally related to the dementia. The use of positron emission tomography with fluorodeoxyglucose (PET-2DG) in the study of human brain disease has provided an opportunity to localize areas in CNS of dysfunctional neural activity in the human patient. Using PET-2DG in subjects with Alzheimer's Disease, maximal decreases in glucose utilization have been identified in posterior parietal cortex, whereas frontal, occipital, and anterior temporal areas are significantly less affected. Therefore, we have analyzed postmortem brain samples from parietal cortex (Brodmann areas 39 and 40) for concentrations of various cortical neurotransmitters and compared them to concentrations in frontal areas (Brodmann areas 9 and 10) from Alzheimer and control brains. Cerebral material was obtained at autopsy from 10 individuals with histologically confirmed Alzheimer's Disease and an equal number of normal controls matched for age and sex. Tissue was kept at -70° C and dissected at -20° C from regions corresponding to Brodmann areas 9, 10, 7, 39, and 40. Analysis of choline acetyltransferase (CAT) was carried out according to the radioenzymatic technique of Bull and Oderfeld-Nowak (J. Neurochem. 18:935,1971). Somatostatin levels were determined using a double antibody radioimmunoassay with a ^{11}Tyr-somatostatin tracer. Catecholamines and their metabolites were quantified using reverse phase high pressure liquid chromatography. The activity of CAT in Alzheimer brain was diminished by a mean of 40% relatively uniformly throughout cortex, with decreases in frontal regions indistinguishable from reductions in parietal areas. Levels of catecholamines and metabolites in Alzheimer tissue including NE, DA, and HVA were unchanged from control levels. Somatostatin concentrations, unlike any of the other transmitters quantified, were similar to normal values in the relatively spared frontal cortex (102% of normal values), whereas levels were significantly reduced in parietal samples (45% of normal values). Thus, we propose that this selective loss of somatostatin in the parietal cortex may underlie not only the reduction in glucose utilization predominantly localized to this area, but symptoms of the dementia as well. Treatment of Alzheimer's disease with somatostatin or an active analogue should be considered.

54.6 THIAMIN-DEPENDENT ENZYMES IN BRAINS AND PERIPHERAL TISSUES FROM ALZHEIMER PATIENTS. G.E. Gibson, K.-F.R. Sheu*, J.P. Blass, A. Baker*, K.C. Carlson*, J. Dale*, B. Harding*, P. Perrino*. Cornell Univ. Med. Coll., Burke Rehabilitation Ctr., White Plains, NY 10605.

Thiamin deficiency is a major etiological factor in the mental impairment that accompanies Wernicke-Korsaff syndrome, which, like Alzheimer's disease, is accompanied by severe recent memory loss and neuronal cell loss in the nucleus basalis. Furthermore, decreases in the activities of the thiamin-dependent enzymes, pyruvate dehydrogenase (PDHC), transketolase (TK) and 2-ketoglutarate dehydrogenase (KGDH) accompany behavioral deficits during experimental thiamin deficiency. Thus, the activities of these enzymes were determined in the brains from patients that died with Alzheimer's disease, in cultured skin fibroblasts and in various blood elements from living patients diagnosed as having Alzheimer's disease. In brains from Alzheimer patients compared to controls (% decrease; n of control, n of Alzheimer, respectively) TK declined in frontal cortex (-51%; 8,9), caudate (-54%; 3,5), occipital cortex (-50%; 6,7) or midtemporal cortex (-51%; 4,3). The decreases in KGDH were larger: frontal cortex (-87%; 9,8), caudate (-75%; 3,5), occipital cortex (-92%; 6,7) and midtemporal cortex (-100%; 4,3). Studies with mouse brain demonstrated that TK was stable for two days at room temperature, whereas KGDH declined by approximately one-third. Previous studies demonstrated decreases in PDHC in Alzheimer brains that correlated with deficits in choline acetyltransferase. Alterations in cultured skin fibroblasts or various blood cells were considerably less. In cultured skin fibroblasts (7 control lines; 6 Alzheimer lines at 4 different passage numbers), TK declined 15% ($p<0.05$) and KGDH declined 23% ($p>0.05$). Variable decreases (0-50%) of PDHC occurred in fibroblasts. Analysis of blood platelets revealed no change in the activity or heat denaturation curves of TK or KGDH. In red blood cells, basal and methylene-blue-stimulated flux through the pentose shunt, which utilizes TK, was normal. In conclusion, three thiamin-dependent enzymes decreased substantially in the brain of Alzheimer patients; these enzymes were much less reduced in peripheral tissues.

54.7 PICK DISEASE: PURIFICATION OF NEURONAL PICK FIBRILS. D.R. Sparkman*, K. Hammon*, S. Johnson*, P. Allison* and C.L. White, III* (SPON: R. Tindall) Depts. of Neurology, Pathology and Internal Medicine, Univ. of Texas Hlth. Sci. Ctr., Dallas, TX 75235.

Pick disease is a rare form of degenerative dementia which resembles Alzheimer disease clinically, but is distinctively different in microscopic pathology. A prominant feature is the presence of numerous swollen neurons which contain an intracytoplasmic inclusion or Pick body. Ultrastructurally this inclusion is composed of numerous fibrils in random orientation, with no limiting membrane. These fibrils were isolated from frozen Pick disease brain tissue, and were found to be insoluble in boiling SDS, even in the presence of a reducing agent. They were isolated by sucrose gradient centrifugation of the SDS insoluble material. Negative staining with uranyl acetate and electron microscopy revealed two types of Pick fibrils (PF). Studies to date suggest that Type I is a 14-16 nm fibril composed of two 6-8 nm protofilaments tightly twisted so as to appear as a straight fibril. Type II is a 28 nm twisted fibril where the two protofilaments had relaxed or were hydrated due to the SDS treatment, and reveal obvious twisting with a periodicity of about 165 nm. Various intermediate stages have also been observed. This study demonstrates that PF may be isolated from Pick disease brain by a method similar to that employed for isolation of paired helical filaments (PHF) from Alzheimer brain, but their ultrastructural morphology is distinct from that of PHF. This work was supported in part by ADRDA research grant 84-1482.

54.8 PHENYLETHANOLAMINE N-METHYLTRANSFERASE IN NORMAL AND ALZHEIMER'S DISEASE BRAINS. W. J. Burke, G. L. Marshall* and H.D. Chung*. St. Louis Veterans Administration Med. Center and St. Louis Univ. Med. Sch., St. Louis, MO. 63125

We have recently developed a highly sensitive and specific assay for phenylethanolamine N-methyltransferase (PNMT) in brain. The sensitivity of the assay is due to the use of high specific activity adenosyl-L-methionine S-[methyl-^{3}H] (^{3}H SAM) as substrate coupled with the use of reineckate precipitation of [^{3}H]SAM after the reaction and with the adsorbtion and elution of [^{3}H] epinephrine product from alumina to attain low blank values. The assay has a sensitivity of 30 f moles. When coupled with small homogenizing volumes and removal of naturally occurring inhibitors in the tissue by dialysis, the assay achieves a sensitivity of 50 times that of other brain PNMT assays. With this assay we were able to demonstrate PNMT activity in regions of brain where it had been reported absent or present in only trace amounts. These areas include frontal cortex 39.3 ± 1.8 pmole/h/g; amygdala 50.5 ± 3.1; hippocampus 25.7 ± 2.5; cerebellar cortex 40.9 ± 1.9. In an autopsy proven case of Alzheimer's disease (A.D.), there was a 96% decrease in PNMT in the right and a 67% decrease in PNMT in the left locus coeruleus compared to controls. These results indicate a wider distribution of PNMT in human brain than previously reported and suggest a deficiency of this enzyme in A.D.

54.9 **IMMUNOREACTIVITY OF ANTISERUM TO ALZHEIMER PAIRED HELICAL FILAMENTS IS NEUTRALIZED BY ABSORPTION WITH PICK DISEASE BRAIN.** C. L. White, III*, P. Allison*, J. M. Gause*, S. A. Johnson*, and D. R. Sparkman* (SPON: J. B. Mullen). Depts. of Pathology and Neurology, Univ. of Texas Southwestern Med. Sch., Dallas, TX 75235.

Alzheimer disease (AD) and Pick disease (PD) are two forms of degenerative dementia with distinct pathological features. Light microscopic cellular changes in AD include widespread intraneuronal accumulation of neurofibrillary tangles (NFT) in the hippocampus, neocortex, and subcortical and brainstem nuclei. Classical PD is characterized by the presence of ovoid cytoplasmic Pick bodies (PB) in neurons of the temporal and frontal cortex. Ultrastructurally, the NFT consists of paired helical filaments (PHF), whereas the PB consists of straight fibrils morphologically distinct from PHF. In this study, unfixed brain tissue was obtained at autopsy from a patient who died with pathologically documented AD. PHF protein was isolated from the frontal cortex by the method of Ihara et al. (Nature 1983; 304:727-730). Anti-PHF antiserum was obtained from a New Zealand white rabbit after immunization with purified PHF and Freund's adjuvant, diluted, and applied to paraffin sections of formalin-fixed hippocampus from 5 AD cases, one PD case, and normal control brains. All of these cases had had histopathological documentation of the respective diagnoses. The sections were then developed using the avidin-biotin immunoperoxidase method. Neuronal perikarya in the 5 AD cases showed immunoreactivity in NFT, and the PD case showed immunoreactivity in PB. Morphologically normal neurons showed no immunoreactivity. Absorption of the anti-PHF antiserum with unfixed brain tissue homogenates from either AD or PD cases eliminated the immunoreactivity in both NFT and PB in subsequent staining of tissue from the same regions. Absorption with normal control brain tissue did not affect staining of NFT or PB. We conclude that the structural components of Alzheimer NFT and of Pick bodies have antigenic similarities despite the differences in morphological changes between AD and PD.

54.10 Olfactory Pathways in Alzheimer's Disease (AD): Neuropathological Studies. P.F.Reyes*,G.T. Golden, R.G. Fariello, L.Fagel*, M. Zalewska*,(Spon: R.M. Benjamin). Neurology & Research, VAMC Coatesville PA 19320 and Neurology & Psychiatry , Thomas Jeff. Med. College, Phil. PA 19107.

Dementia of the Alzheimer type (DAT) is the most common cause of progressive dementia among middle-aged and the elderly. Alzheimer's disease (AD) is characterized pathologically by the development of numerous argophilic neuritic plaques (NP) and neurofibrillary tangles (NFT) within the brain.

We have examined formalin-fixed brain obtained at post-mortem from four patients with clinical diagnosis of AD. In all cases, light microscopic examination of serial hematoxline-eosin, luxol fast blue-cresylviolet and Bodian's silver stained tissues demonstrated increased NFTs and NPs in neocortex and hippocampus showing a classical histopathologic morphology and argrophilia. In addition, we examined the olfactory regions including olfactory bulb and tract, anterior olfactory nucleus, prepiriform cortex and amygdala. In one case, NPs and NFTs counts were determined in the prepiriform cortex, amygdala, hippocampus and frontal cortex using a BH-2 Olympus light microscope with 10X microscopic fields the center of which contained the highest concentration of either NPs or NFTs. The density of NPs and NFTs in the prepiriform cortex and amygdala was higher than that observed in the frontal cortex. In all cases studied NFTs and extensive neuronal loss were found in olfactory bulb anterior olfactory nucleus and amygdala. The prepiriform cortex showed NFTs, NPs, extensive neuronal loss and granulovacuolar degeneration.

These changes clearly demonstrate that the olfactory sensory pathway is significantly affected pathologically in Alzheimer's disease. The absence of high concentrations of NPs and NFTs in the frontal cortex suggest that the frontal lobe may not be the optimal site for brain biopsy in all cases of AD. Supported by VA funds.

54.11 ALUMINOSILICATES AND THE GENESIS OF SENILE PLAQUES. J.A. Edwardson*[1], A.E. Oakley*[1], J. Klinowski*[3], R.H. Perry*[1], E.K. Perry*[2] and J.M. Candy*[1]. (SPON: E.T. Hedley-Whyte). MRC Neuroendocrinology Unit[1] and Department of Pathology[2], Newcastle General Hospital, Newcastle upon Tyne NE4 6BE; Department of Physical Chemistry[3], University of Cambridge, Cambridge CB2 1EP.

Using energy dispersive X-ray microanalysis (EDAX) we have shown that aluminium and silicon are co-localized in the central core region of senile plaques, one of the major neuropathological features of Alzheimer's disease. Coincident distribution of these elements was observed in plaque cores isolated from cerebral cortex using protease digestion, treatment with 2% sodium dodecyl sulphate to solubilize proteins, treatment with 4M hydroxylamine to remove collagen, and final purification on a sucrose gradient. Such cores appeared as spherical particles (8-20μm diameter) with a fibrillary ultrastructure and the staining properties of plaque amyloid in situ. However, the protein content was relatively small (<5%) in comparison with the content of Al (4-19%) and Si (6-24%) as measured by EDAX analysis of thin epon-embedded sections. Co-localization of Al and Si was also observed in plaque cores present in formalin-fixed, silver-stained cryostat sections of cortex and in foci of similar dimensions and elemental composition observed in unfixed, unstained tissue.

The close correlation between the distribution of Al and Si suggested the presence of aluminosilicates and this has been confirmed using solid state ^{27}Al NMR with magic angle spinning to analyse plaque cores isolated from Alzheimer brain. The co-localization of Al and Si is a constant feature of senile plaques from both senile and presenile cases of Alzheimer's disease and of the more sparsely distributed plaques from non-demented, aged individuals. Aluminosilicates are a highly reactive class of substances and these findings raise the possibility that their focal deposition may be involved in the genesis or early development of senile plaques.

Abnormalities of calcium homeostasis have been implicated in the accumulation of Al associated with neurofibrillary tangles in the Parkinsonian-dementia complex of Guam and the possibility of such changes being a contributory factor in the deposition of aluminium as aluminosilicates and the development of Alzheimer type pathology is being investgated.

54.12 ALZHEIMER'S DISEASE: MAZE LEARNING DEFICITS AND NEUROPATHOLOGY RESULTING FROM CHRONIC INFUSION OF HEMICHOLINIUM INTO NUCLEUS BASALIS MAGNOCELLULARIS OF THE RAT. B. J. Hurlbut*, J. F. Lubar, R. Switzer, J. Dougherty, M.E. Eisenstadt.Univ. of Tenn., Knoxville, TN 37996-0900

Alzheimer's Disease is characterized by progressive dementia correlated with an insidious loss of acetylcholine (Ach) producing cells in the Nucleus Basalis of Meynert (Mnb). Experimental studies with monkey, cat, and rat have demonstrated a reduction in cortical Ach correlated with lesions in Mnb. Methods utilized to destroy Mnb include electrolyticlesions and acute injection of kainic acid or ibotenic acid. These methods are non-specific to Ach-rich cells, involving the destruction of fibers of passage or all cell bodies at the site of involvement.

In this study, 12 mature male Sprague-Dawley rats were chronically, bilaterally implanted with 30-gauge cannuli terminating in Nucleus Basalis Magnocellularis (Mnm), the homolog of Mnb. The cannuli were connected to two independent Alzet miniosmotic pumps subcutaneously located on the flanks of each animal. Each pump was filled with 214 ul of hemicholinium (HC-3) and 0.9% saline. Over fourteen days, 385 ug/hr of HC-3 were delivered to each Mnm. HC-3 is an agent which interferes with the uptake of choline thus preventing the production of Ach by an affected cell. Twelve control rats were fitted with cannuli and pumps filled with 0.9% saline solution in the same fashion as the rats recieving HC-3. Each HC-3 and control rat was tested in a series of composite T-mazes prior to receiving implants. Learning curves were generated for each rat, and for the HC-3 and saline groups. After receiving an implant, each rat was again tested.

HC-3 rats receiving implants demonstrated a significant deficit in maze-learning ability compared with individual and group performances before receiving the implants. In saline rats there was no significant difference in maze-learning ability before and after receiving implants. The HC-3 group receiving implants demonstrated a significant deficit in maze-learning ability compared with the saline control group. Serial sections through Mnm from 8 control and 8 HC-3 rats were treated with thionine for confirmation of infusion site and cell body destruction; with the cupric silver method of de Olmos for extent of damage in Mnm and concurrent terminal field loss in cortex; and with thioflavin-S for the formation of plaques. All cannuli were located within Mnm. In cupric silver sections, differences in the extent of terminal field degeneration and Mnm cell loss were more apparent in HC-3 animals. However, some damage resulting from cannula insertion interfered with analysis. Plaque formation was not evident in control or HC-3 animals.

55.1 CLASSIFICATION AND LOCALIZATION OF NONCOMPETITIVE INHIBITOR SITES BASED ON TIME-RESOLVED PHOTOLABELING OF THE NICOTINIC ACETYLCHOLINE RECEPTOR BY QUINACRINE AZIDE. M. DiPaola*, P.N. Kao*, R.N. Cox* and A. Karlin. Departments of Biochemistry and Neurology, Columbia University, New York, NY 10032.

We have photolabeled the receptor in membrane from Torpedo californica electric tissue with the photolyzable local anesthetic [3H]quinacrine azide (QA), using a continuous-flow, rapid-mixing device and millisecond-duration irradiation (Cox, R.N. et al. J. Biol. Chem., 260:xxx, 1985). Membrane was mixed with QA and effectors and, 20 ms to 200 ms later, irradiated for 2 ms. Agonists, but not competitive antagonists, induced a transient state of the receptor in which the alpha and beta chains were specifically susceptible to photolabeling by QA. We define specific photolabeling by QA as that obtained in the presence of 1 mM acetylcholine and 0.4 mM hexamethonium minus that obtained in the presence of 0.4 mM hexamethonium, alone. QA concentration was varied from 0.75 uM to 10 uM. The agonist-enhanced labeling of the alpha and beta chains was blocked in the presence of other noncompetitive inhibitors (NCIs) such as proadifen, bupivacaine and histrionicotoxin. Proadifen (50 uM) blocks at least 90% of the specific labeling by 3 uM QA. Saturating concentrations of the NCIs, histrionicotoxin and bupivacaine, however, blocked at most 50% of the specific labeling by 3 uM QA. In contrast, at lower concentrations of QA (1 uM and lower), 100 uM histrionicotoxin blocked 80% to 90% of the specific incorporation. We infer that there are two classes of sites that are specifically (as defined above) photolabeled by QA: both classes bind proadifen, but only one class binds histrionicotoxin and bupivacaine. These NCIs differ in that proadifen is a much more potent promoter of receptor desensitization than either histrionicotoxin (Boyd, N.D., and Cohen, J.B., Biochem., 23:4023, 1984) or bupivacaine (Ikeda, S.R., et al., Molec. Pharmacol., 26:293, 1984). HPLC maps of the CNBr fragments of the alpha chain labeled with QA in the presence and absence of these various NCIs will be presented. Supported by research grants from NIH and MDA and a fellowship to M.D. from The N.Y. Heart Association, Inc.

55.2 LOCALIZATION OF THE CHLORPROMAZINE BINDING SITE ON THE SEQUENCE OF ACETYLCHOLINE RECEPTOR δ-SUBUNIT FROM TORPEDO MARMORATA. J. Giraudat*, M. Dennis*, T. Heidmann*, J.Y. Chang* and J.P. Changeux. (SPON:J. Hirsch). Institut Pasteur, Paris, France; and Ciba-Geigy, Basel, Switzerland.

The permeability response to acetylcholine (ACh) is blocked by a group of compounds referred to as non-competitive blockers (NCBs). Binding experiments with several radioactive NCBs have revealed two types of sites on acetylcholine receptor (AChR)-rich membrane fragments : a) a unique high affinity site, present in one copy per AChR oligomer and common to all NCBs tested; and b) a series of low affinity sites. The high affinity site can be labeled by a variety of photoactivable NCBs. In particular ^{3}H-chlorpromazine (CPZ) covalently attaches to all AChR—subunits; this labeling is prevented in the presence of the NCB phencyclidine (PCP), which binds almost exclusively to the high affinity NCB site.

The availability of complete cDNA sequence for all AChR subunits opens the way for direct identification of the amino acids forming the high affinity NCB binding site using protein chemical techniques. In the present study, the amino acids of the δ-subunit involved in this site were probed with ^{3}CPZ.

AChR rich membranes treated at pH 11 to remove extrinsic proteins were equilibrated with 1mM carbamylcholine, 2.5 μM ^{3}H-CPZ and then U.V. irradiated. Addition of 200 μM PCP reduced CPZ incorporation in the δ-subunit by 80%. AChR subunits were purified by preparative polyacrylamide gel electrophoresis. After reduction and carboxymethylation, the δ-subunit was cut into peptides by a combination of enzymatic and chemical cleavages. On initial analysis by reversed-phase HPLC, 70-75% of the injected radioactivity eluted as a very broad peak. Further purification in several HPLC systems enabled us to resolve four distinct radioactive components. Incorporation of ^{3}H-CPZ in these four peaks was inhibited by the presence of 200 μM PCP during the labeling. Gas phase automated sequencing permitted identification of the labeled peptides by their N-terminal amino acid sequence and provided sequence position of radioactive residues.

55.3 IDENTIFICATION OF THE α-BUNGAROTOXIN BINDING SITE ON THE PRIMARY AMINO ACID SEQUENCE OF THE α SUBUNIT FROM THE TORPEDO ELECTRIC ORGAN ACETYLCHOLINE RECEPTOR. P.T. Wilson*, T.L. Lentz and E. Hawrot. Depts. Cell Biology and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We previously described the binding of α-bungarotoxin (BTX) to proteolytic fragments of the α subunit of the acetylcholine receptor (PNAS 81:2553, 1984). We have now mapped the BTX binding site with respect to Asn 141, the site of N-linked glycosylation. BTX-binding proteolytic fragments that contain Asn 141 bind concanavalin A (Con A) and, after treatment with endoglycosidase H (endo H), demonstrate an increase in electrophoretic mobility during SDS-PAGE consistent with the removal of one N-linked oligosaccharide chain. We therefore tested BTX-binding fragments for sensitivity to endo H. In general, for all proteases used, fragments larger than 30 kd were endo H sensitive and those smaller than 15 kd were endo H resistant. Two large BTX-binding fragments (28 kd: papain; 27 kd: chymotrypsin) were endo H resistant. Since the largest possible endo H resistant fragment residing within the amino terminal side of Asn 141 cannot exceed 16 kd (140 a.a.), the existence of much larger endo H resistant fragments indicates that the BTX binding site must lie on the carboxyl side of Asn 141. The smallest endo H sensitive fragment that binds BTX, a 12 kd (∿103 a.a.) fragment produced by digestion with papain, indicates that the BTX binding site must be within ∿103 a.a. of Asn 141. V8 protease generates a 19 kd BTX-binding triplet that does not bind Con A, and hence does not contain Asn 141. The first V8 cleavage site past Asn 141 is Glu 161. Thus the BTX binding site resides between a.a. 161 and 244. To narrow further the a.a. sequence containing the BTX binding site, a synthetic peptide of 32 a.a. comprising a.a. residues 173-204 and containing the cysteines that are labeled with MBTA (Kao et al. JBC 259:11662, 1984) was tested for its ability to bind ^{125}I-BTX. ^{125}I-BTX bound to the peptide both in a dot-blot assay and after electrophoretic transfer onto positively charged nylon filters. In contrast, no binding of ^{125}I-nerve growth factor to the peptide was detected. Similar to the results obtained with isolated, immobilized α subunit, ^{125}I-BTX binding was competed by BTX and by d-tubocurarine with IC_{50} values of 0.1 μM and 0.1 mM respectively. No binding of ^{125}I-BTX was detected to either insulin or β-endorphin in dot-blot assays. We conclude, therefore, that the major determinant of the BTX binding site resides between Ser 173 and His 204, most likely including a negative subsite near Cys 192. (Supported by NIH GM32629, the PMA Foundation, and NSF 82-03825.)

55.4 PHOTOAFFINITY LABELING OF MAMMALIAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. H. Siegel and R.J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

The curaremimetic neurotoxin, alpha-bungarotoxin (Bgt), was extensively purified and used to determine the molecular composition of the putative nicotinic acetylcholine receptor (nAcChoR) from rat brain. Bgt was covalently modified with the photoaffinity reagent, 4-fluoro-3-nitrophenylazide (Hucho, FEBS Lett. 103: 27, 1979), radiolabeled with iodine-125 and chromatographically repurified. In a related procedure, Bgt or previously purified iodine-125-labeled, monoiodinated Bgt (I-Bgt) was covalently modified with Allison's photoaffinity reagent (Lewis et al., Biochemistry 16: 5650, 1977) and subjected to further purification. Photoaffinity labeled toxin (PAL-Bgt) preparations each exhibited characteristic spectroscopic properties that were sensitive to illumination in the near ultraviolet. Analysis of PAL-Bgt binding to membranes from Torpedo californica electric organ or from rat brain prior to or following photoactivation demonstrated specific, high-affinity, nicotinic interaction at the binding site for Bgt or I-Bgt. The covalent nature of the specific interaction with photoactivated PAL-Bgt and membrane components was shown by comigration of radiolabel with high molecular mass (> 40,000 daltons) material on column chromatography in the presence of sodium dodecyl sulfate (SDS).

Specific binding sites for photoactivated PAL-Bgt were further characterized by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. PAL-Bgt was found to be covalently attached to polypeptides from Torpedo with masses of ca. 40, 50, 60 and 70 kilodaltons, which are comparable to the masses of the subunits of the Torpedo nAcChoR. A similar profile is obtained for polypeptides from rat brain membranes or detergent extracts that are labeled with PAL-Bgt. The PAL-Bgt labeling pattern is also expressed by toxin binding components from either tissue that have been purified by curaremimetic neurotoxin affinity chromatography using carbamylcholine as a specific elutant.

In conclusion, PAL-Bgt conjugates can be used for examination of the molecular composition of neuronal binding sites for curaremimetic neurotoxins. These nAcChoR-like sites appear to express a presumptive subunit pattern that is characteristic of the authentic nAcChoR from peripheral tissues.

Supported by a National ALS Foundation fellowship, NIH grant NS 16821, Epi-Hab of Arizona, Inc. and the Men's and Women's Boards of the Barrow Neurological Foundation.

55.5 ALPHA-BUNGAROTOXIN, BROMOACETYLCHOLINE AND ANTI-NICOTINIC ACETYLCHOLINE RECEPTOR ANTIBODY BINDING SITES ON THE HUMAN MEDULLOBLASTOMA LINE, TE671. R.J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Presumptive nicotinic acetylcholine receptor (nAcChoR) sites on the human medulloblastoma clonal cell line, TE671, have been characterized with respect to their interactions with three classes of specific probes. The properties of these sites are compared to previously described characteristics of toxin binding sites and nAcChoR from other tissues.

TE671 cells in proliferative growth phase express a single class of high affinity binding sites for alpha-bungarotoxin at a site density of 30 fmol per million cells with a dissociation constant on the order of 0.3 nM. The toxin binding site has a distinctive nicotinic cholinergic pharmacology, but exhibits comparatively high affinity for the nicotinic ligand, decamethonium. Consequently, the features of this site are similar to nAcChoR from the electroplax of Torpedo californica. and to toxin binding sites from rat brain, but are different from the toxin binding sites on the rat pheochromocytoma clonal line, PC12.

The nicotinic cholinergic affinity reagent, bromoacetylcholine, reversibly inhibits toxin binding to unmodified TE671 cell membranes. Bromoacetylcholine also irreversibly blocks toxin binding to membranes that have been treated with dithiothreitol. Nicotinic agonists, but not antagonists, block irreversible labeling of toxin binding sites with bromoacetylcholine if they are applied prior to disulfide cleavage and reduction. These interactions of bromoacetylcholine with the TE671 cell toxin binding site are comparable to those with nAcChoR from Torpedo and with toxin binding sites from rat brain and PC12 cells.

Two different polyclonal antisera raised against nAcChoR from the electric tissue of Electrophorus electricus have been tested for their interactions with toxin binding sites. Each of these antisera inhibit binding of toxin to TE671 cells. The potency of this blockade is similar to that for antiserum-mediated inhibition of toxin binding to nAcChoR from Torpedo electric tissue and to toxin binding sites from rat brain or PC12 cells. Each of the antisera also precipitate at least 50% of detergent-solubilized toxin binding sites from TE671 cells. By contrast, under the same conditions these antisera precipitate 0-4% of liganded toxin binding sites from rat brain or PC12 cells, but 100% of nAcChoR from Torpedo.

On the basis of these data, it is suggested that presumptive nAcChoR from a variety of tissues exhibit immunological and pharmacological heterogeneity. The genetic or post-translational bases of this heterogeneity and its functional manifestations are being subjected to further study.

55.6 BIOCHEMICAL CHARACTERIZATION OF TWO NICOTINIC RECEPTORS FROM THE OPTIC LOBE OF THE CHICK. M. Schneider*, C. Adee*, H. Betz*, and J. Schmidt. (SPON: I. Spector). Dept. of Biochem., State Univ. of New York at Stony Brook, Stony Brook, NY 11794 and Zentrum für Molekularbiologie, Univ. Heidelberg, 6900 Heidelberg, West Germany.

We have studied nicotinic receptors in the optic lobe of the newborn chick, using ^{125}I-α-bungarotoxin, a specific blocker of acetylcholine receptors in the neuromuscular junction, and [^{3}H]-acetylcholine, a ligand which in the presence of atropine selectively labels nicotinic binding sites in rat brain cortex (Schwartz, R.D. et al., Mol. Pharmacol. 22:56, 1982). We observed that [^{3}H]-AcCh binds reversibly to a single class of high-affinity binding sites (K_D = 2.2x10^{-8} M; B_{max} = 5.7 picomoles/g). 60% or more of these binding sites are solubilized with Triton X-100, sodium cholate, or CHAPS. Solubilization increases the affinity for AcCh and several nicotinic drugs from 1.5 to 7 fold.

The acetylcholine-binding macromolecule ('agonist receptor') in optic lobe resembles the receptor for α-bungarotoxin (toxin receptor) present in the same tissue with respect to subcellular distribution (both receptors are enriched in the synaptosomal fraction); hydrodynamic properties (mobility on Sepharose-4B and velocity sedimentation); lectin affinities (retention on immobilized concanavalin A, wheat germ agglutinin, peanut lectin, and Ulex europaeus agglutinin). Pharmacologically, the two receptors share a nearly identical rank order for nicotinic agonists.

Several fundamental differences however exist between the two receptors: The agonist receptor has a markedly lower affinity for nicotinic antagonists; it is thermally denatured with a T_m of 49°, compared to a T_m of 70°for the toxin receptor; and in a Triton X-114 biphasic system (Bordier, C., J. Biol. Chem., 256:1604, 1981), the agonist receptor prefers the detergent-rich phase while the toxin receptor partitions approximately equally between the detergent-rich and aqueous phases. Complete physical separation of the binding activities was achieved through incubation with agarose-linked acetylcholine; by affinity chromatography on immobilized α-cobrotoxin; and by precipitation with a monoclonal antibody to chick optic lobe toxin receptor (Betz, H. and Pfeiffer, F., J. Neurosci., 4:2905, 1984).

55.7 REGULATION OF ACETYLCHOLINE RECEPTORS ON CHICK CILIARY GANGLION NEURONS IN CELL CULTURE. J.F. Margiotta, D.K. Berg, and V.E. Dionne . Div. of Pharm. and Dept. of Biol., UCSD, La Jolla, CA 92093.

Chick ciliary ganglion neurons possess functional nicotinic acetylcholine receptors (AChRs) in cell culture. Pressure application of 100 µM ACh induces a large increase in membrane conductance which is blocked by 25 µM d-tubocurarine. The ACh sensitivity of the cells can be regulated by the K^+culture conditions: neurons grown in medium containing elevated K^+ concentrations have four-fold lower levels of ACh-induced conductance (tested under standard conditions) than do neurons grown in normal medium. We have examined the time course of the K^+ effect, shown that it is Ca^{++}-dependent, and determined that it does not involve changes in either the open time or conductance of single AChR channels.

The time course of the K^+ effect on ACh-induced conductances was determined by switching cultures from normal medium (containing 5.4 mM K^+) to medium supplemented with K^+ (25 mM final concentration). After 2-72 hrs the elevated K^+ medium was removed by rinsing, and ACh-induced conductances were examined with intracellular recording techniques. The peak ACh response decreased exponentially with a half-time of 10 hr after switching to elevated K^+ medium. The K^+ effect was Ca^{++}-dependent. Omission of Ca^{++} from the K^+-supplemented medium resulted in ACh responses that were identical to those of neurons grown in normal medium.

Results obtained with intracellular recording were confirmed in voltage clamp studies using the whole-cell recording configuration of the patch clamp technique. After 5-7 days in culture, application of 10 µM ACh to neurons grown in normal medium induced sustained inward currents of 52 +/- 10 pA (mean +/- SE, n=9), but only 15 +/- 2 pA (n=9) in neurons grown in elevated K^+ medium. These currents correspond to whole-cell ACh-induced conductances of 800 +/- 100 pS and 200 +/- 40 pS, respectively. Noise spectra obtained by analysis of ACh-induced current fluctuations from the neurons grown in either medium were well-fit by single Lorentzian functions, suggesting that the cells possess a single population of functional AChRs under both growth conditions. The spectra revealed no differences in the burst duration (ca. 5 ms) or single channel conductance (ca. 30 pS) for neurons grown in either condition. Preliminary single AChR channel data from outside-out patches support these conclusions. The mean channel open time (ca. 1.5 ms) and single channel conductance (ca. 40 pS) were indistinguishable for neurons grown with or without elevated K^+ concentrations.

The absence of an effect of K^+ on single channel parameters suggests that chronic exposure to elevated K^+ concentrations regulates ACh sensitivity by reducing the number of functional AChRs on the neurons. (Supported by NS 15344 and NS 12601.)

55.8 CHOLINERGIC MODULATION OF AN ACETYLCHOLINE RECEPTOR-LIKE ANTIGEN ON CHICK CILIARY GANGLION NEURONS IN CELL CULTURE. D.K. Berg, M.A. Smith, J.F. Margiotta, and J.M. Lindstrom. Dept. of Biol., UCSD, La Jolla, CA. 92093; and Salk Institute, S.D., CA. 92138.

The neuronal nicotinic acetylcholine receptor (AChR) has remained elusive due to lack of suitable probes. Recent work has shown that monoclonal antibodies (mAbs) to the "main immunogenic region" (MIR) of AChR alpha subunit from muscle and electric organ crossreact with a membrane component on chick ciliary ganglion neurons. The MIR-like component in the ganglion has the surface distribution and biochemical properties expected for the neuronal AChR. It is clearly distinct from the alpha-bungarotoxin binding component in the ganglion which previous work has shown to be different from the synaptic AChR on the cells. Since anti-MIR mAbs do not block AChR function either in muscle or neurons, we have sought additional evidence for the relationship of the MIR-like component to the neuronal AChR by testing cholinergic agents for modulation of the component on the neurons in culture.

Exposure to Bgt 3.1, a protein neurotoxin known to reversibly inhibit AChR function on the neurons, causes a 50% reduction in the number of MIR-like sites on the cells after 1 hr, and an 80% reduction after 5 days without competing with anti-MIR mAbs for binding to the component. Cholinergic ligands including d-tubocurarine, nicotine, trimetaphan, carbachol, and ACh protect the MIR-like sites against modulation by Bgt 3.1 both in short- and long-term exposures. Moreover, long-term exposure to carbachol alone induces a 30% decrease in the number of MIR-like sites. Alpha-bungarotoxin has no effect on the number of sites or on the Bgt 3.1-induced modulation. These findings demonstrate that the MIR-like component is subject to modulation through a cholinergic site on the neurons.

ACh sensitivity is altered in parallel with the changes in number of MIR-like sites following chronic exposure to Bgt 3.1, while GABA sensitivity remains unchanged. Co-modulation of ACh sensitivity and the MIR-like component is also seen for trimetaphan protection against the Bgt 3.1 effect, and for carbachol treatment. An exception to co-modulation, however, is observed for neurons grown in elevated K^+ concentrations: the cells express 4-fold lower levels of ACh sensitivity (tested under standard conditions) than do control neurons, and yet have no difference in the number of MIR-like sites.

Previous biochemical and ultrastructural studies, and the co-modulation results described here suggest that the MIR-like component on ciliary ganglion neurons represents the neuronal AChR. While the exception cited above to co-modulation of ACh sensitivity and MIR-like sites does leave questions about the identification, it can also be taken to imply that growth conditions regulate the fraction of neuronal AChRs that are functional. (Supported by NS 12601, NS 11323, MDA, Amer. Heart Asso.)

55.9 SURFACE AND INTRACELLULAR DISTRIBUTION OF AN ACETYLCHOLINE RECEPTOR-LIKE ANTIGEN IN CHICK CILIARY GANGLION NEURONS. M.H. Jacob, J.M. Lindstrom, and D.K. Berg. Dept. of Biol., UCSD, La Jolla, CA. 92093; and The Salk Institute, S.D., CA. 92138.

Ultrastructural studies with monoclonal antibodies (mAbs) to the "main immunogenic region" (MIR) of nicotinic acetylcholine receptor (AChR) alpha subunit from muscle and electric organ have identified a crossreacting component on the surface of ciliary ganglion neurons that is located predominantly in synaptic membrane. Biochemical studies have shown that the MIR-like component in the ganglion has a number of properties expected for the nicotinic AChR on the neurons, and that the component is distinct from the alpha-bungarotoxin binding component in the ganglion. Moreover, cholinergic agents can modulate the number of MIR-like sites on the neurons in cell culture, and in most instances ACh sensitivity is modulated in parallel. Immunohistochemical staining of frozen ganglion sections indicates that a substantial proportion of the MIR-like sites is intracellular. In the present study we show that the intracellular sites are associated with organelles known to be part of the biosynthetic and regulatory pathways for cell surface membrane proteins.

Ciliary ganglia from 15-17 day chick embryos were lightly fixed, saponin-permeabilized, incubated with anti-MIR mAb followed by horseradish peroxidase-labeled secondary antibody, reacted for peroxidase activity, and examined by electron microscopy. Deposits of reaction product were present on most of the rough endoplasmic reticulum, some Golgi complexes, portions of the nuclear envelope, a few coated vesicles, some vacuoles, some multivesicular bodies, the specialized synaptic membranes, and small portions of the pseudodendrite surface membranes. No labeling was associated with other organelles or found in the cytoplasm. The labeling was specific since substitution of nonimmune serum or other mAbs for the anti-MIR mAb failed to generate reproducible labeling in the ciliary ganglion. Also, no labeling was found with anti-MIR mAb in dorsal root ganglion neurons which lack AChRs.

The surface distribution of MIR-like sites was again confined primarily to synaptic membrane on the neurons. The intracellular distribution of the MIR-like component observed here is similar to the biosynthetic pathway described for a number of surface membrane components including the AChR of muscle. Intracellular MIR-like component may represent neuronal AChR undergoing intracellular processing and/or transport to the cell surface. (Supported by NS 12601, NS 11323, The Muscular Dystrophy Association, and The American Heart Association.)

55.10 STRUCTURE OF CHICKEN GENES ENCODING THE NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS AND THEIR VARIANTS. A. Mauron*, P. Nef*, C. Oneyser*, R. Stalder*, C. Alliod* and M. Ballivet*. (SPON: B.W.Fulpius) Dept. of Biochemistry, University of Geneva, CH-1211 Geneva, Switzerland.

The nicotinic acetylcholine receptor (AChR) present at the neuromuscular junction of vertebrates is still the receptor of neurobiological interest whose molecular genetics is best understood. The cDNAs encoding its individual subunits α, β, γ and δ have been cloned first in Torpedo, then in a variety of other organisms and this laboratory has focused on the study of the AChR genes of chicken. We recently published the structure and nucleotide sequence of the γ and δ genes (Nef et al. 1984. Proc. Natl. Acad. Sci. U.S.A. 81, 7975). They were found to be related to each other as well as to the corresponding Torpedo genes. Furthermore, they are very closely linked in the chicken genome. Both coding sequences comprise 12 exons which encode homologous protein domains. A gene encoding the α subunit was obtained as well, whose sequencing now approaches completion. Although it has only 9 exons, these can be derived by exact excision from the 12 exon pattern that obtains for the γ and δ genes.

By screening the same chicken genomic library, a variant α gene, named α2, was discovered and sequenced. It shows more sequence similarity to known α genes than to any other AChR subunits and shows α-specific features, including 4 cysteine residues which are thought to form a box-like structure presumably involved in the binding of cholinergic ligands. The α2 gene has 7 exons: as compared to the α gene, two introns were again excised, thus forming a large exon which encodes the first three putative transmembrane domains. The α2 sequence shows none of the characteristic features of a pseudo-gene and probably represents a functional gene. Transcriptional studies are in progress to determine the function of this variant gene. It is not clear at this point whether the α2 gene encodes an embryonic form of the AChR or one of the several nicotinic AChR-like molecules which have been described in the CNS.

PAIN: CENTRAL PATHWAYS I

56.1 NOCICEPTIVE LAMINA I NEURONS HAVE AXONS THAT ASCEND IN THE DORSOLATERAL FUNICULI (DLF) AND TERMINATE IN THE PARABRACHIAL AREA. J.L.K. Hylden, H. Hayashi*, G.J. Bennett and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

We have physiologically and anatomically characterized a population of lamina I neurons that projects to the parabrachial area (PBA; n. cuneiformis and parabrachial n.). Extracellular recordings were made from 89 lamina I PBA projection neurons in the lumbosacral enlargement of chloralose-anesthetized cats. Half of the cells (44) were antidromically activated from the contralateral PBA; the other half projected to the ipsilateral PBA (40) or to both sides (5). In addition, 8 PBA projection neurons were also activated from the contralateral thalamus. The majority of physiologically characterized projection neurons responded exclusively to noxious stimulation of their cutaneous receptive fields (92%); only a few wide-dynamic-range neurons were observed. Conduction velocities for antidromic activation from the PBA ranged from 1 to 18 m/s.

Following physiological characterization, several neurons were impaled and injected with HRP. Intracellularly stained PBA projection neurons (n=14) had a mean cell body diameter of 13 to 30 μm and extensive dendritic arbors which were oriented in the rostrocaudal axis (850-1900 μm in length) and either remained within lamina I or extended dorsally into the white matter. Nine cells had well stained axons and 5 of these had at least one collateral.

Projection neurons were also labelled following an injection of wheat germ agglutinin-HRP (0.2 μl, 25%, 72h) into the PBA. Many lamina I neurons were retrogradely labelled, bilaterally, in all spinal segments. Bilateral lesions of the DLF at T11 resulted in an 86-94% decrease in the number of labelled lamina I cells below the lesion. Injection of colchicine (6 μl, 1%) into thoracic spinal cord allowed visualization of small and large HRP-containing axons bilaterally in the DLF and ipsilaterally in the anterolateral funiculus.

Our data indicate that there is a large population of nociceptive lamina I neurons with projections through the DLF to the PBA of the midbrain. The similar physiology (Craig and Kniffki J. Physiol., (Lond.), in press) and DLF pathway (Jones et al., Soc. Neurosci. Abstr., 1984) of cat spinothalamic lamina I neurons suggests that a significant number of PBA projection neurons may also project to the thalamus.

56.2 FURTHER EVIDENCE THAT THE ACTIVITY OF WIDE-DYNAMIC-RANGE BUT NOT NOCICEPTIVE-SPECIFIC NEURONS CORRELATES WITH THE ABILITY OF MONKEYS TO DETECT SMALL INCREASES IN NOXIOUS TEMPERATURE. W. Maixner, D.R. Kenshalo, Jr., J.-L. Oliveras, M.C. Bushnell and R. Dubner. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Previous reports from this laboratory (Bushnell et al., J. Neurosci., 1985, in press) have demonstrated that both monkeys and humans detect small increases in noxious heat. The activity of medullary dorsal horn wide-dynamic-range (WDR) but not nociceptive-specific (NS) neurons predicted the monkey's ability to detect near threshold increases in noxious temperature (Kenshalo et al., Neurosci. Abstr. 10:798, 1984). In the present series of studies, we have obtained additional evidence which indicates that the activity of WDR but not NS neurons is correlated with monkeys' ability to detect small increases in noxious temperature.

Two rhesus monkeys were trained to perform a noxious thermal detection task. Monkeys initiated a trial by depressing an illuminated panel button. Following button press, a contact thermode that was positioned on the upper lip increased in temperature from a baseline of 38°C to a noxious (45° or 46°C) temperature (T1). After a random 3 to 9 sec foreperiod (time between button press and onset of T2), the thermode increased an additional 0.2°C to 1.5°C (T2). Monkeys received a fruit juice reward for releasing the button within 2.4 sec of the onset of T2. Single unit activity was recorded from WDR (N=18) and NS (N=9) medullary dorsal horn neurons during the performance of the task. In addition, the median detection latency (MDL) to the onset of T2 was determined.

The MDL was inversely related to the magnitude of T2 irrespective of the preceding T1 temperature. The MDL was also inversely related to the magnitude of peak neuronal discharge produced by the various T2's presented from the two different T1's for both WDR and NS cells. When MDLs and peak neuronal discharge were directly compared, equivalent MDLs were associated with similar peak neural discharge rates for WDR neurons (N=6). In contrast, NS (n=4) neurons did not demonstrate such a strong relationship. In addition, the MDL for a 0.2°C T2 presented from a preceding T1 of 46°C decreased as the foreperiod increased. The peak neuronal discharge response to the 0.2°C T2 was also increased as a function of hold time for WDR (N=18) but not for NS (N=9) neurons.

These data demonstrate that the magnitude of discharge of WDR neurons is a more accurate predictor of MDL than the magnitude of discharge of NS neurons. We conclude that WDR neurons are the critical medullary dorsal horn neuronal population which provides the minimal information required to make fine discriminations in the 45° to 46°C noxious heat range.

56.3 RESPONSE PROPERTIES OF NEURONS IN THE LATERAL CERVICAL NUCLEUS (LCN) OF THE ANESTHETIZED OR DECEREBRATE-SPINALIZED (D-S) CAT. K.C. Kajander and G.J. Giesler Jr., Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

A large number of electrophysiological studies have shown that the majority of neurons at the origin of the spinocervical tract (SCT) respond differentially to noxious cutaneous stimuli. Surprisingly, studies of the LCN, the nucleus in which the SCT terminates, have uniformly failed to find more than a very small percentage of nociceptive neurons. Since these previous studies of the LCN have all been performed in anesthetized animals, the purpose of the present experiments was to compare the response properties of cat LCN neurons in anesthetized and D-S states.

Cats were either anesthetized with urethane or decerebrated and spinalized at the C1-medullary junction. Extracellular single unit recordings were made within the LCN between caudal C1 and C3. Electrical stimulation of the ipsilateral C5 dorsolateral funiculus (DLF) was used as a search stimulus. The criteria for identification of an LCN neuron were: 1) inability to follow reliably DLF stimulation at greater than 50 Hz, 2) action potentials with shape and polarity characteristic of somatic recordings, 3) anatomical localization of recording point within the LCN. On some occasions, successful attempts were made to activate LCN neurons antidromically from either the ventral posterior lateral nucleus of the thalamus (anesthetized cats) or the contralateral C1 ventral funiculus (D-S cats). Responses of each neuron were determined to hair movement, pressure and noxious pinch. In addition, all nociceptive neurons were examined for their responses to a series of ascending innocuous and noxious thermal stimuli.

Receptive fields on the distal portion of the limbs were smaller than those on the proximal aspect of the limbs and trunk in both anesthetized and D-S cats. The sizes and locations of receptive fields were indistinguishable from those previously reported. In anesthetized cats, 25 LCN neurons were examined; only one (4%) responded to noxious stimuli. In D-S cats, 44 LCN neurons have been examined. Of these, 21 (47%) responded either differentially or exclusively to noxious stimuli. The remaining 23 (53%) responded only to innocuous mechanical stimuli. In several nociceptive LCN neurons, responses to noxious stimulation were temporarily blocked by small doses of sodium methohexital, a short-acting barbiturate. It appears that anesthesia importantly affects the capability of LCN neurons to respond to noxious mechanical and thermal stimuli. These findings support a role for the spinocervicothalamic system in nociception in the cat.

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56.4 RESPONSES OF SPINOMESENCEPHALIC TRACT (SMT) CELLS TO THERMAL STIMULI. R.P. Yezierski, H. Hirata and N.A. Olson*. Department of Anatomy, University of Mississippi Medical Center, Jackson, Mississippi 39216.

Recent studies in our laboratory have demonstrated that cat SMT cells can be classified based on their excitatory and/or inhibitory responses to varying intensities of mechanical stimuli. Two classes of SMT cells, i.e. wide dynamic range (WDR) and high threshold (HT), were found to respond best or exclusively to noxious stimuli. The purpose of the present study was to evaluate the responses of these two classes of cells to innocuous and noxious thermal stimuli.

Recordings were made from 25 identified SMT cells located in laminae I and V-VIII of the cat lumbosacral spinal cord. Cells were activated antidromically from stimulating electrodes positioned in the contralateral midbrain. Animals were anesthetized with alpha-chloralose (60mg/kg) and sodium pentobarbital (2mg/kg/hr); expired CO2 and body temperature were maintained within normal limits. Responses to thermal stimuli were recorded while heating/cooling the skin with ascending (35-55°C) or descending (35-5°C) sequences of 20-30 second thermal pulses produced by a Peltier thermal stimulator. The effects of repeated heating and/or cooling on thermal evoked responses were also evaluated.

The results of the present study have shown that WDR and HT SMT cells respond to heating and/or cooling of glabrous skin with thermal thresholds of 50°C and 25°C, respectively. Maximal responses to heating were obtained between 52-55°C and those to cooling between 5 and 15°C. The most consistent responses to thermal stimuli were excitatory although five cells inhibited by mechanical stimuli were observed to have similar responses to noxious heating of the receptive field(s) on the ipsi- and/or contralateral hindlimb. One cell was inhibited by heating the ipsilateral receptive field and excited by the same stimuli applied to the contralateral hindlimb. Two cells were excited by heat stimuli of 50°C and inhibited by stimuli of 52 and 55°C. Four cells were excited by both noxious heating (50-55°C) and cooling (5-25°C). Sensitization was observed for three cells following repeated applications of a series of thermal pulses which increased in a stepwise fashion from 45-55°C.

In conclusion the results of the present study have shown that cat SMT cells can be excited and/or inhibited by heating/cooling the skin within a narrow range of temperatures. The present results will be discussed in relation to the role of the SMT in thermal nociception.

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56.5 **URINARY BLADDER MODULATION OF T_3-T_5 SPINAL NEURONS IN THE CAT.** **T.J. Brennan*, U.T. Oh* and R.D. Foreman. (Spon, R. Person) Dept. of Physiol., Univ. Okla. HSC., Okla. City, OK 73190.**

We have previously demonstrated that feline upper thoracic spinal neurons with somatic receptive fields in the triceps and chest region receive convergent excitatory input from cardiopulmonary (CP) sympathetic afferent fibers innervating the heart. In addition, we have observed that gall bladder distension excited 25% of these cells, inhibited 14%, and had no effect on the remaining neurons. Thus, abdominal input from the gall bladder can influence these upper thoracic spinal neurons.

The purpose of the present study was to determine if the urinary bladder could affect the activity of T_3 to T_5 spinal neurons. Experiments were performed on 8 cats anesthetized with α-chloralose and paralyzed with pancuronium. The upper thoracic segments were searched with microelectrodes for single neurons with somatic receptive fields in the chest and arm region which were excited by the CP sympathetic fibers. Of the 16 cells located in the T_3 to T_5 segments, 15 received convergent excitatory input from the chest and arm and CP sympathetic afferent fibers while 1 cell was inhibited by these stimuli. Urinary bladder distension (UBD) ranging from 30 to 150 cmH_2O inhibited the activity of 8 cells, excited 2 cells, excited then inhibited 1 neuron and had no effect on the remaining 5 cells. Phasic inhibition was usually observed during the onset of small distensions (20-60 cmH_2O) while tonic inhibition occurred with higher distending pressures (30-150 cmH_2O). UBD inhibited excitatory input produced by noxious pinch of the somatic field (n=8), electrical stimulation of CP sympathetic afferent fibers (n=5), and intracardiac injection of the algesic chemical bradykinin (n=3). In 2 experiments, spinal cord transection at the L5-L6 level greatly reduced or eliminated inhibition produced by UBD, suggesting that this inhibition occurs largely via pelvic parasympathetic afferent fibers.

In summary, upper thoracic spinal neurons with input from the chest, arm, and CP sympathetic afferent fibers also received convergent input from the urinary bladder. In contrast to the previously observed input from the heart and gallbladder, stimulation of a more distant visceral organ, the urinary bladder, inhibited (50%) or did not affect (31%) most cells. These results may provide an explanation for the specificity of visceral input to the spinal cord and may also suggest possible mechanisms for localization of visceral pain. (Supported by NIH grant HL22732, HL27260, and HL07430).

56.6 PONTOMEDULLARY RAPHE NEURONS: INTRACELLULAR RESPONSES DURING THE TOOTH PULP-EVOKED JAW OPENING REFLEX, P. Mason, A. Strassman*, and R. Maciewicz (SPON: W.H. Sweet). Pain Physiology Lab, Neurology Service and Dept of Neuroscience, Mass General Hosp and Harvard Med School, Boston MA 02114.

Several lines of evidence suggest an important role for the pontomedullary raphe in modulation of central pain pathways. To determine how raphe cells are activated during a nociceptive behavior, the intracellular responses of raphe neurons were studied during the jaw opening reflex (JOR) elicited by tooth pulp shock in nonparalyzed, lightly anesthetized cats.

Tooth pulp stimulation produces reflex EMG activation of the digastric muscle at a latency of 7-9msec, resulting in jaw opening. Tooth pulp shock that elicits the JOR also produces an EPSP in a subset of raphe neurons. This EPSP consists of an early small depolarization at a latency of 10-12ms followed by a larger depolarization at a latency of 20-30ms. In all cases the EPSP follows digastric EMG activation. These cells also show oligosynaptic EPSPs in response to electrical stimulation of the four paws. No raphe neurons are inhibited by either tooth pulp or peripheral paw shock.

Electrical train stimulation of the midbrain periaqueductal gray region (PAG) suppresses the JOR. Single shock stimulation at the same PAG sites that suppress the JOR evokes monosynaptic EPSPs in the large majority of raphe neurons recorded. In all cases, the threshold for EPSP is below the threshold for suppression of the JOR. The EPSP amplitude is a direct function of PAG stimulus intensity and there is temporal summation of EPSPs evoked by paired PAG shocks. At conditioning-test intervals of 40-90ms, train stimulation of PAG suppresses the tooth pulp-evoked EPSP in raphe neurons. The threshold for EPSP suppression occurs at a PAG stimulation intensity below that required for suppression of the JOR.

The location and morphology of studied neurons were determined by intracellular injection of either HRP or ethidium bromide. Most recordings were made from large or medium-sized raphe neurons in nucleus raphe magnus or rostral nucleus raphe obscurus. Large raphe cells are multipolar in shape with average somatic diameters of 50-90um, and most medium-sized neurons are fusiform in shape with average somatic diameters of 30-40um. The axons of stained cells typically course laterally through the paramedian white matter tracts, then turned caudally to descend through the ventromedial medullary reticular formation to cervical levels.

Immunohistochemical processing of ethidium bromide-labeled cells revealed that few of the electrophysiologically-characterized raphe neurons responding to PAG or tooth pulp shock colocalize serotonin-like immunoreactivity.

56.7 COLLATERAL PROJECTIONS FROM THE PERIAQUEDUCTAL GRAY TO THE THALAMUS AND MEDULLARY RETICULAR FORMATION: A RETROGRADE DOUBLE LABEL FLUORESCENCE STUDY IN THE RAT. C.M. Pechura*, L. Chang* and R.P.C. Liu* (SPON: G.J. Bennett). Dept. of Anatomy, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814-4799.

Both the ascending and descending efferent projections of the midbrain periaqueductal gray (PAG) have been proposed to subserve certain components of stimulation-produced and opiate analgesia. Of particular importance are the midline and intralaminar thalamic nuclei and the medullary reticular formation (MRF). It is not known whether these PAG projections are completely independent of each other or whether some are via axon collaterals which ascend and descend to innervate multiple targets. In order to determine the presence and location of any PAG cells which project to both the thalamus and MRF via axon collaterals, retrograde double label fluorescence techniques were utilized. The tracers 5% Fast Blue (FB) or 2% Diamidino Yellow Dihydrochloride (DY-2HCl) were pressure injected via glass micropipettes into either the medial thalamus or MRF. The thalamic injections were centered in the mediodorsal nucleus and included the central lateral, central medial and habenular nuclei. Reticular formation injections were centered in the n. reticularis gigantocellularis and included n. reticularis magnocellularis. The animals were sacrificed 5-7 days after injections. Frozen, transverse sections (25μ) through the injection sites and serial sections through the PAG were viewed with epifluorescent microscopy. The locations of all double labeled cells were plotted on cross sectional drawings with the use of a microprojector. The distributions of single labeled FB or DY-2HCl cells were plotted in the same manner.

Double labeled cells were located throughout the rostrocaudal extent of the PAG. The heaviest concentration of such neurons was found at intercollicular levels. The vast majority of double labeled cells were observed ipsilateral to the injection sites and were located in the lateral, lateroventral and ventral portions of the PAG. In more caudal regions, double labeled cells were found in the lateral dorsal tegmental nucleus. Few double labeled neurons were seen in the dorsal raphe nucleus.

These data demonstrate the presence of PAG neurons which project to both the medial thalamus and MRF via axon collaterals. Thus, an anatomical substrate exists for simultaneous input from the PAG to both the inhibitory reticulospinal system and the thalamic nuclei proposed to mediate emotional and affective components of pain perception. Such divergent projections from single neurons in the PAG underscore the importance of considering both ascending and descending pathways in the functional sequelae of stimulation-produced or opiate analgesia. (Supported by NSF Graduate Fellowship (CMP) and DoD Grant No. R07058.)

56.8 DIFFERENTIAL EFFECTS OF CORDOTOMIES UPON THALAMIC NEURONAL RESPONSIVENESS IN HEALTHY RATS AND IN AN EXPERIMENTAL MODEL OF CHRONIC PAIN. G. Guilbaud, M. Peschanski, A. Briand and M. Gautron, INSERM U. 161, 2 rue d'Alésia 75014 Paris, France.

Numerous neurons responding to noxious cutaneous stimulation can be recorded in the ventrobasal complex of the thalamus (VB) of healthy rats. In contrast, in an experimental model of chronic pain (arthritic rats), most VB neurons are activated by movement or pressure of the inflammed joints. Changes observed in the spinal neuronal responsiveness in arthritic rats are too limited to explain the striking difference at the thalamic level. The present experiment was therefore designed to test another hypothesis, that afferent ascending pathways involved in the genesis of VB neuronal responses might differ according to the experimental conditions.

Electrophysiological characteristics of the same VB neurons were studied before and after localized lesions of the spinal cord. VB neurons responding to pinches or joint movements of both hindlimbs were recorded extracellularly using glass micropipettes in moderately anesthetized healthy (n = 15) and arthritic (n = 18) rats. Successive lesions of the dorsal columns (DC) of one dorsolateral funiculus (DLF) then of half the spinal cord were performed at the level of prepared laminectomies of C2, C4 and C6 vertebrae. Histological controls of the lesions were systematically done.

In the two experimental conditions, DC and DLF lesions did not modify the receptive fields of the VB neurons. In contrast, hemisection - i.e. essentially the addition of the lesion of one anterolateral quadrant (ALQ) - produced differential effects :

. in healthy animals, it eliminated the receptive field located on the hindlimb contralateral to the lesion. Noxious inputs are therefore conveyed, in healthy rats, by and ascending pathway located in the ALQ and completely crossed, most probably the direct spinothalamic tract.

. in arthritic rats, the effects of the ALQ lesion was more complex ; indeed, a lesion on the side of the thalamic recording failed most often to modify the receptive field of the VB neurons; in contrast the lesion of the ALQ opposite to the recording site did eliminate the responses elicited from the hindlimb ipsilateral to the recorded neuron. This shows that in this experimental model of chronic pain, afferent inputs involved in some VB neuronal responses are subserved by a pathway located in the ALQ, with both a crossed and a direct components. This pathway is probably a part of the spinoreticular system.

These observations are discussed with reference to the results of therapeutic unilateral cordotomies, in particular to some paradoxical results with elimination of sensori-discriminative aspects of acute pain and failure of a consistant effect upon chronic pain.

56.9 CLUSTER ANALYSIS OF SOMATOSENSORY NEURONS. J.M. Chung, D.J. Surmeier, K.H. Lee*, L.S. Sorkin, C.N. Honda, Y. Tsong* and W.D. Willis. Marine Biomedical Institute, Departments of Anatomy, Physiol. & Biophys. and Office of Academic Computing & Biostatistics, Univ. Texas Med. Branch, Galveston, TX 77550.

An attempt is made to classify primate spinothalamic tract (STT) cells and neurons of the caudal part of the ventral posterior lateral nucleus (VPLc) of the thalamus using a cluster analysis of the responses of these cells to graded mechanical stimulation of the skin.

Data were collected from a total of 75 young monkeys (1.8-3.0 kg; Macaca fascicularis). Animals were anesthetized with an initial dose of α-chloralose (60 mg/kg), and then sodium pentobarbital (4 mg/kg/hr) was infused throughout the experiment to maintain a constant level of anesthesia. Single unit activity of STT cells antidromically activated from the contralateral VPLc thalamus were recorded with a microelectrode in the lumbosacral enlargement of the spinal cord. For thalamic unit recording, a microelectrode was driven slowly into the VPLc nucleus while applying electrical search stimuli to the sciatic nerve. A k-means clustering analysis, a non-hierarchical cluster technique, was done based on the relative responsiveness of the cells to graded mechanical stimuli applied to their receptive fields.

For 128 STT cells, a classification scheme with 3 clusters was found statistically to be the best. This yielded groups of 22, 57 and 49 cells in clusters 1, 2 and 3, respectively. Response characteristics of cells in each cluster showed that cluster 1 cells are activated best by low threshold mechanical stimuli whereas cluster 3 cells are activated mostly by nociceptive stimuli. Cluster 2 cells had intermediate characteristics. When the classification scheme based on the cluster analysis was compared with the conventional classification of low threshold (LT), wide dynamic range (WDR) and high threshold (HT) cells, cluster 1 cells were divided into LT and WDR cells whereas cluster 2 and 3 cells included WDR and HT cells. For 110 thalamic neurons, a classification scheme with 5 clusters was found statistically to be the best. Clusters 1-5 contained 25, 34, 17, 10 and 24 cells, respectively. Response characteristics of cells in each group indicated a gradual change in sensitivity to higher threshold peripheral input from cluster 1 to 5. When this classification scheme was compared with the conventional classification, cluster 1 cells belonged to LT group, clusters 2 and 3 split into LT and WDR cells, and clusters 4 and 5 included WDR and HT cells.

Classification schemes based on a cluster analysis of the responses of cells may provide an objective and functionally meaningful categorization for somatosensory neurons. (Supported by NIH grants NS09743, NS11255, NS18830, NS21266 and a grant from the Moody Foundation. Also supported by NIH postdoctoral fellowships NS07216 (D.J.S.), NS07185 (L.S.S.) and NS07574 (C.N.H.).)

56.10 CLUSTER ANALYSIS OF THE RESPONSES OF PRIMATE SPINOTHALAMIC NEURONS TO NOXIOUS THERMAL STIMULATION. D.J.Surmeier, C.N.Honda and W.D. Willis. Marine Biomed. Inst., Univ. Texas Med. Br., Galveston, TX 77550.

A k-means cluster analysis was performed on the responses of spinothalamic (STT) neurons to graded mechanical and thermal stimulation in an attempt to ascertain whether functional subdivisions within the STT population could be resolved.

Macaque monkeys (M. fascicularis) were initially anesthetized with α-chloralose (70 mg/kg) and maintained by continuous intravenous infusion of sodium pentobarbital (5 mg/kg/hr). Recordings from antidromically-identified STT neurons in the L7-S1 segments of the spinal cord were made with glass microelectrodes. The majority of cells were isolated within laminae IV-VI. Thermal stimulation was performed with a servo-controlled Peltier stimulator. Thirty second thermal pulses were delivered to the skin in ascending, two degree steps from 43 to 55°C.

The responses to a standard series of mechanical stimuli were used to classify neurons according to the scheme proposed by Chung et al., 1985. A cluster analysis was also performed on the responses evoked by noxious thermal stimulation. Four well-defined clusters of STT neurons were found when the analysis was based upon the alteration in discharge rate in the stimulation and post-stimulation periods. No clustering was evident based upon only the alteration in rate during stimulation. Members of the 'A' class (35/56) had relatively poor thermal responses and were either suppressed following stimulation or exhibited no change in rate. Members of the 'B' class (8/56) had relatively large thermal responses and were suppressed following stimulation. Members of the 'C' class (2/56) possessed the largest responses to thermal stimuli and had mixed post-response activity patterns. Members of the 'D' class (11/56) had intermediate thermal responses and a post-response afterdischarge. The clustering was independent of the type of skin stimulated. The mechanical and thermal responsivity of the four classes strongly covaried.

The STT population's pattern of discharge to thermal stimuli was also examined with multidimensional scaling (MDS) techniques. The across-neuron pattern of discharge has been correlated with the quality of sensation in other sensory systems (Smith et al., J. Neurophysiol., 50: 541-558, 1983). The MDS analysis revealed that STT neurons are capable of qualitatively ordering stimuli from 45-55°C in an intuitively reasonable manner. In addition, the across-neuron pattern derived from hairy skin was found to undergo a marked change between 47-49°C, whereas that from glabrous skin underwent a similar change near 51°C. An MDS analysis of the mechanical type 2 and 3 subgroups suggest that although their coding capacities differ, each is essential to the coding of thermal quality in the 45-55°C range.

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56.11 CORTICAL NOCICEPTIVE RESPONSES AND BEHAVIORAL CORRELATES TO TOOTH PULP STIMULATION IN THE MONKEY. E.H. Chudler*, W.K. Dong and Y. Kawakami*. Departments of Anesthesiology and Psychology and Multidisciplinary Pain Center, University of Washington, Seattle, Washington 98195.

The location and function of cortical mechanisms that subserve trigeminal nociception are uncertain. To investigate such mechanisms, we characterized the distribution of tooth pulp-evoked potentials (TPEPs) in the sensory and motor cortices of anesthetized and unanesthetized monkeys. In lightly anesthetized monkeys (n = 5), bipolar electrical tooth stimulation elicited components P23 and N44 over the primary somatosensory cortex (SI), P26 and N72 over the primary motor cortex (MI) and P72, N161, P280, N420, P561 and N662 over the secondary somatosensory cortex (SII). Anesthetics attenuated TPEPs in SII more than TPEPs in SI and MI. TPEPs were not contaminated by extradental input and muscle artifacts because pulpectomy of the stimulated tooth eliminated all TPEPs and neuromuscular blocking agents had no effect on TPEPs, respectively. Compound action potential recordings from the trigeminal ganglion indicated that the shorter latency TPEPs recorded from SI and MI required the activation of only A-beta nerve fibers while the longer latency TPEPs recorded from SII required the additional recruitment of A-delta nerve fibers. Intracortical recordings revealed polarity reversals of P23 and N44 in cytoarchitectonic area 3b, P26 and N72 in area 4 and P72 to N662 in the superior bank of the lateral sulcus (SII/r).

The relationships between nociceptive cortical events and behavioral indices of pain were determined in monkeys trained on a simple escape task or an appetitive-tolerance escape task. An array of epidural or subdural electrodes to record TPEPs was placed over SI, MI and SII in four monkeys. Long latency components were found exclusively in SII when TPEPs were recorded from the pial surface. These late TPEPs recorded from SII required the recruitment of A-delta nerve fibers and were correlated with high rates of escape and short mean escape latencies. At low stimulus intensities, few escapes occurred and only short latency TPEPs were observed. Administration of morphine (2-4 mg/kg, s.c.) significantly increased escape threshold and latency and selectively reduced the amplitude of only the longer latency potentials recorded over SII.

These results support the assumption that a large population of nociceptive neurons exists in SII. The noninvasive measurement of nociceptive evoked potentials over the SII region may provide a useful tool in assessing the efficacy of pharmacological, electrical, and surgical manipulations that modulate nociception.

This research was supported by National Institute of Dental Research Grant DE 05130.

56.12 DO ELECTRICALLY INDUCED PAIN AND THERMALLY INDUCED PAIN SUM? A. C. Brown, A. C. Kloka*, R. W. Fields*, W. J. Beeler*, and A. P. Hainisch*. Physiol. & Biophysics SD, Oregon Health Sci. U. and VA Md. Center, Portland, OR 97201.

The purpose of these experiments was to ascertain whether tooth pain and detection (pre-pain) thresholds determined from electrical stimulation were altered by the simultaneous application of heat. Stimuli were applied to maxillary central incisors of normal human volunteers. All teeth used were free of restorations or had at most one small composite restoration, were free of disease, and gave normal responses to standard clinical tests (percussion, heat, cold, vitalometer). Stimuli were applied through a custom-molded acrylic splint applied to the maxillary anterior teeth. A 2.5 mm hole through the splint on the lingual surface was filled with conducting paste for delivery of electrical pulses (0-100 uamp, 1 msec duration, repeated at 2 sec intervals). Heated air was circulated through a chamber on the labial surface and tooth surface temperature monitored with a thermocouple. The subject indicated sensation intensity (pre-pain, brief pain, continuous pain) by pressing a pushbutton. Stimulus amplitude was determined by a computer-controlled stimulator which was used to implement staircase and threshold tracking algorithms. The experimental design was to use electrical stimuli to determine pain and detection thresholds, starting at a tooth surface temperature of 35C, and then raising surface temperature in 5C increments until the subject reported continuous pain. Each temperature was maintained for a two-minute "epoch" while electrical thresholds were tested; every few epochs, temperature was returned to the 35 C baseline and electrical stimulation withdrawn for two minutes to reduce the possibility of adaptation or sensitization. We found that increasing temperature produced no significant effect on either detection or pain electrical thresholds, even when the temperature was raised to a level which caused thermally induced continuous pain (50-55 C in most subjects). From this lack of interaction, we conclude the following: First, pain due to electrical stimulation and pain due to heat stimulation are mediated by separate neural pathways. Possibly electrical stimulation activates tooth pulp A-beta and A-delta axons, while heat activates C-fibers. Second, there is no significant convergence or summation of the two pathways in relation to sensation. Supported by NIDR grant DE-05895.

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57.1 DISTRIBUTION OF NCAM IN THE CHICK HINDLIMB DURING AXON OUTGROWTH AND THE ESTABLISHMENT OF MATURE NERVE-MUSCLE RELATIONSHIPS. K. Tosney, M. Watanabe, L. Landmesser, U. Rutishauser. Division of Biology, Univ. of Michigen, Ann Arbor, MI 48109, Anatomy & Developmental Genetics, Case Western Reserve Univ., Cleveland, OH, 44106, Depts. of Physiology and Neuroscience, Univ. of Connecticut, Storrs, CT 06268, Anatomy & Developmental Genetics, Case Western Reserve Univ., Cleveland, OH, 44106.

Motoneurons in the chick lumbosacral spinal cord have been shown to interact with the developing limb bud to form a stereotyped gross anatomical nerve pattern and a precise pattern of specific connections with muscles. Adhesive interactions between the neuronal growth cone and limb components would be expected to play an important role in these processes. We therefore determined the distribution of NCAM (a molecule known to mediate neural adhesion) in this system from the initiation of axon outgrowth through hatching using both immunocytochemistry and SDS-Page gel immunoblots of microdissected limb components.

NCAM staining was intense on neurites throughout development. At early stages, weak to moderate NCAM staining was also always found on mesenchyme cells in advance of growing nerves, suggesting that it may play at least a supportive role in axon elongation. However, no differences were detected in NCAM levels between regions where axons grow and adjacent regions (i.e., the anterior vs. posterior half of each somite; nerve pathway vs. surrounding mesenchyme) suggesting that the pathways these axons follow are not delineated by NCAM.

However, axon invasion of developing muscles was temporally correlated with the onset of intense NCAM staining of developing myotubes. Axons enter muscle at st 28, but do not ramify within muscle until st 31-32 (Tosney & Landmesser, J. Neurosci., in press). Between st 28-32, the intensity of NCAM staining increased in all thigh muscles. The onset of NCAM staining in muscle was not triggered by nerves or nerve-evoked activity, since it also occurred in aneural limbs.

While NCAM increased in amount during st 28-32, it did not alter in form. NCAM in nerves (plexus and sciatic nerve) and muscle was similar with an apparent molecular weight ranging smoothly between 140-250Kd at each stage.

Muscle NCAM remained high between st 34 and 40, when large numbers of myotubes are added and synapses formed, but declined sharply around hatching, as mature contacts become stabilized and polyneuronal innervation is removed. These results suggest that high levels of NCAM may facilitate formation and rearrangement of synapses. Since NCAM staining declined earlier in fast than in slow muscles, nerve-mediated activity patterns, which differ between fast and slow muscles, may play a role in reducing NCAM expression.

In summary, the distribution of NCAM is not correlated with pathfinding in this system. However, dynamic changes in the levels of NCAM seem to be associated with the subsequent interaction of these neurons with their targets.

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57.2 RELATIVE RECEPTOR INDUCING ACTIVITY ALONG NEURITE. J.M. Dubinsky, M. Morgan*, G.D. Fischbach. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Mo.

Accumulation of acetylcholine receptors occurs on cultured myotubes soon after contact by a cholinergic growth cone. Several neurites issue directly from neuron cell bodies and each of them is capable of inducing several receptor patches within a few hours of coculture. We have searched for a gradient of receptor inducing activity along neurites by examining receptor patches as a function of distance from the cell body. Acetylcholine receptor patches were identified by labelling all receptors in cocultures of chick neurons and myotubes with Rhodamine-conjugated alpha-Bungarotoxin (R-BTX). Neurites were visualized by injection of Lucifer Yellow or 5,6 Carboxyfluorescein into individual ciliary ganglion neurons or spinal cord motorneurons. Images of neurites and individual neurite-associated AChR patches (NARPs) were digitized using a Dage SITCAM connected to a Grinnell Image processor and PDP 11/44 computer. The entire neuritic arbor was traced and the locations of muscle contact and NARPs were noted. For each NARP the maximum receptor density within a cluster was judged by fluorescence intensity of the cluster relative to the background intensity of the adjacent muscle membrane. Total area of the NARP was measured by calculating the number of pixels above a threshold value. We also made note of variations in fluorescence intensity within individual NARPs. These values were compared for all NARPS along a given process. A total of 33 neurons were studied, between 4 hours and ten days after plating. 360 NARPs were analyzed along 80 neurites contacting 22,656 micrometers of muscle surface. The mean incidence of NARP occurrence was 1.6 per 100 um of muscle contact (cf. Role et al Nsci Abst. 9:1179, 1983). While several neurites had only one or two NARPs, some neurites induced up to 30 NARPs along their length with the avarage range being 8-15 per neurite. Most NARPs along a given neurite fell within a relatively narrow intensity range with the exception of one or two NARPs of up to ten-fold greater intensity. These bright NARPs were usually located at an intermediate position along the course of the neurite rather than at the extreme proximal or distal positions. The area of individual NARPs varied significantly (from 2 to 20 μm^2) with one or two NARPs up to 10 times larger than others along the same neurite. As observed with the most intense NARP, the largest ones were usually found midway along the neurite, although the largest NARPs were not necessarily the brightest. Based on these parameters, no gradient of receptor induction by the neurites was apparent. We are currently using time-lapse techniques to study the formation, organization, and stability of NARPs at different times after the initial neurite-muscle contact is formed. Supported by USPHS 5 T32 NS07057-07.

57.3 **NEURITE-PROMOTING ACTIVITY OF A LAMININ-RELATED POLYPEPTIDE FROM MUSCLE-CONDITIONED MEDIUM IS INCREASED BY DENERVATION.** T.N. Stitt*, (Spon.: M. Gurney). Committee on Neurobiology, Univ. of Chicago, 947 E. 58th St., Chicago, IL 60637.

The composition of the substrate to which neuronal growth cones adhere in large part determines the extent of outgrowth of neuronal processes. It may be postulated that postsynaptic tissues supply substrate-bound factors which influence neuron-target interactions. This report is a preliminary characterization of a polypeptide secreted into muscle-conditioned medium which promotes neurite outgrowth from embryonic spinal cord cells.

Rat hemidiaphragm, denervated 3 days in vivo, was organ cultured as a source of serum-free conditioned medium (Ddia CM). When bound to polyornithine-coated tissue culture plastic, a component of the Ddia CM promoted neurite outgrowth from 25% of cells from dissociated 5-day embryonic chick neural tube. A 190 gm/L ammonium sulfate-precipitable fraction of the Ddia CM (190 gm AS) was 10-fold more active (half max. activity = 10 µg/ml using 0.3 ml to coat 16 mm wells) than the Ddia CM and the percentage of cells responding increased to 50%. In comparison, laminin, an extracellular matrix glycoprotein purified from mouse EHS sarcoma, had a half-maximal activity of 2 µg/ml in the assay. The effects of denervation on neurite-promoting activity was examined by comparing the activity of Ddia CM with medium conditioned by muscle placed into culture without prior in vivo denervation (Ndia CM). Dose-response curves of 190 gm AS fractions from 24-hour cultures of Ndia CM and Ddia CM indicated that 3-day prior denervation increased neurite-promoting activity 2-fold.

The active component of the Ddia CM may be laminin or a laminin-related polypeptide. The activity of the Ddia CM was removed by precipitation with antisera to laminin. Laminin antisera blocked the neurite-promoting activity of laminin but not that of the Ddia CM material. On Western blots of Ddia CM proteins electrophoresed on reduced, SDS-polyacrylamide gels, laminin antisera reacted with a doublet of approx. 210 kilodaltons and a faint band at 400 kd, corresponding to the molecular weights of the subunits of laminin. These results suggest three non-exclusive possibilities: 1) the neurite-promoting functionality of the muscle-derived material is in some way masked such that it is inaccessable to antibody, 2) the laminin-related polypeptide acts as a carrier for the neurite-promoting activity, or 3) the Ddia CM polypeptide is antigenically related to laminin but contains regions which differ from laminin in function and primary structure. Currently, work is being done to map both the laminin-related polypeptide from muscle and authentic laminin using proteolytic enzymes.

Supported by the Searle Scholars Program and the Sloane Foundation.

57.4 PATTERN AND GROWTH RATES OF NEURITES IN PERIPHERAL NEURONAL CULTURES ARE MOST PRONOUNCED ON EXTRACELLULAR MATRIX PREPARATIONS OBTAINED FROM SCHWANN CELL-NEURON CULTURES. Carson J. Cornbrooks Dept. of Anatomy & Neurobiology, University of Vermont Burlington, Vermont 05405

Neuronal regeneration within the peripheral nervous system (PNS) is promoted when the proximal and distal portions of the injured nerve are in close apposition. This indicates that the cellular components distal to the site of injury are required for the promotion and continuation of the initial sprouting response. To date, the cellular source and the molecular nature of neurite promoting factors remain unclear although adequate evidence suggests that extracellular matrix (ECM) components, Schwann cell membrane molecules and molecules secreted by nonneuronal cells may facilitate PNS neuronal regeneration.

In order to characterize neurite promoting factors in peripheral nerve tissue, pure populations of neurons from embryonic superior cervical ganglia (SCG) and dorsal root ganglia (DRG) were prepared in culture. Neurons from embryonic day (E) 21 SCG and E15 and E18 DRG were grown in vitro, subjected to axotomy and transplanted to a second dish with modified substrata. Substrates consisted of untreated collagen (control), detergent extracted collagen, pure populations of Schwann cells, and ECM prepared by detergent extraction of mature Schwann cell-neuron cultures. Growth rates of the transplanted neurons were constant for at least 5 days after the axotomy and varied with respect to age and neuron type (E21 SCG > E15 DRG > E18 DRG). Neurite growth on untreated collagen (E21 SCG 13um/hr; E15 DRG 10um/hr; E18 DRG 5um/hr) was approximately equal to the growth rates on detergent treated collagen. Surprisingly, neurons transplanted onto Schwann cells extended highly fasciculated neurites at the same approximate rate as neurons grown on collagen (E15 DRG 9um/hr;E18 DRG 4um/hr). In contrast neurons placed on preparations of ECM maintained growth rates which were consistently 2 to 3 fold higher than those calculated with collagen substrata (E21 SCG 34um/hr;E15 DRG 21um/hr;E18 DRG 14um/hr). Neurites regenerating on ECM substrata grew without fasciculation and faithfully oriented within the matrix channels. Neurons grown on Schwann cell cultures for 1 week increased their growth rates at least 3 fold when transferred to ECM cultures, but neurite growth decreased to control rates when the paradigm was reversed.

These observations indicate that components of the ECM produced in mature Schwann cell-neuron cultures contain neurite promoting factors which facilitate regeneration of PNS neurons.

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57.5 COMPARISON OF LAMININ AND NEURONECTIN (A CONDITIONED MEDIUM-DERIVED NEURITE EXTENSION FACTOR) USING RADIATION INACTIVATION ANALYSIS. M.D. Coughlin, A.K. Grover* and C.Y. Jung*+. Department of Neurosciences, McMaster University, Hamilton, Ontario, L8N 3Z5, Canada, and +Biophysics Lab., Veterans Administration Medical Center, SUNY, Buffalo, New York, 14215.

Neurite outgrowth from a wide variety of peripheral neurons is stimulated and may be directed in culture by a substrate-binding factor derived from medium conditioned over numerous types of cells. We shall refer to this factor as neuronectin by reason of its ability to serve as an adhesion molecule for neurons. The extracellular matrix molecule laminin (M_r-900 kDa) possesses a similar capacity for eliciting neurite extension (Baron van Evercooren et al., J. Neurosci. Res. 8: 179, 1982), and some studies suggest that laminin and neuronectin may be identical (Lander et al., Soc. Neurosci., 10: 40, 1984).

To obtain an estimate of the molecular weight of neuronectin and to compare it with laminin, both laminin and the partially purified neuronectin were subjected to target size analysis using radiation inactiviation. This method makes use of the finding that biologically active proteins exposed to high energy radiation are rendered inactive by direct "hits" occurring within the volume of the molecule possessing activity. The degree of inactivation at any radiation dose is proportional to the size of the target (i.e., its M_r), regardless of the state of purification or non-covalent association.

Partially purified neuronectin was frozen in thin layers in aluminum trays, as similarly laminin and enzyme standards, and exposed to a graded series of radiation dosages from 0.2 to 8 Mrads. The activity remaining in each sample of neuronectin or laminin was determined by the standard dissociated cell bioassay using dose-response curves of dilutions either preincubated in culture dishes or added directly to the culture medium.

Comparison of the activity decay slope of neuronectin with those of enzyme standards indicates that the M_r of neuronectin is 352 ± 21 kDa. By the same method, the size of laminin as assayed by its neurite-promoting properties has been confirmed to be ~900 kDa. Moreover, unlike neuronectin, laminin gives rise to toxic or inhibitory products when exposed to high energy radiation. These results indicate clear differences between laminin and neuronectin and suggest that the neurite-promoting activity from conditioned medium is distinct from laminin. The existence of multiple neurite-promoting factors suggests a mechanism for the formation of specific axonal pathways during embryogenesis.

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57.6 INDUCTION OF PROCESS OUTGROWTH BY A 2-ACETYLPYRIDINE THIOSEMICARBAZONE IN INSECT AND RAT CLONAL CELL LINES. G.M. Carrow and R.G. Van Buskirk*. Marine Biological Laboratory, Woods Hole, MA 02543 and Dana-Farber Cancer Institute, Boston, MA 02115.

Cells of the embryonic line Kc of Drosophila melanogaster respond to the insect molting hormone, 20-OH-ecdysone, by ceasing proliferation and elaborating processes (Cherbas et al., 1977, Science 197:275). Similarly, cells of the rat pheochromocytoma line PC12 elaborate processes in response to nerve growth factor (NGF) and Bt_2cAMP (Greene and Tischler, 1976, PNAS 73:2424). Induction of process outgrowth in both neuron-like cell lines is highly specific. We have now found that a 2-acetylpyridine thiosemicarbazone, one of a class of antineoplastic agents, blocks proliferation and induces process outgrowth in Kc and PC12 cells.

The [1-(2-pyridinyl)ethylidene]hydrazide of 1-pyrrolidinecarbothioic acid was dissolved in dimethyl sulfoxide and added to cultures of Kc or PC12 cells; the cells were incubated for several days and scored for the fraction of cells showing processes. The thiosemicarbazone at 10^{-6} M to 10^{-5} M blocked Kc cell proliferation and induced process elaboration equivalent to about 75% of the response to 10^{-6} M 20-OH-ecdysone. Notably, cells of a mutant subclone of the Kc line that was selected for lack of a morphological response to 20-OH-ecdysone did elaborate processes in response to the thiosemicarbazone. Finally, the compound did not induce process outgrowth in cells of an unrelated embryonic cell line, Schneider's 2, that do not extend processes when treated with 20-OH-ecdysone. The 2-pyridyl moiety was found to be essential for the process promoting activity of the thiosemicarbazone.

PC12 cells exposed to 10^{-6} M to 10^{-5} M of the thiosemicarbazone elaborated processes if they adhered to the culture dish. The response was similar to that evoked by 1 mM Bt_2cAMP. Remarkably, cells of a mutant subclone of PC12 that do not elaborate processes in response to either NGF or Bt_2cAMP did grow processes in response to the thiosemicarbazone.

These data suggest that the 2-acetylpyridine thiosemicarbazone initiates differentiation of neuron-like cells derived from diverse species. Its mode of action may be downstream or distinct from that of the factors that have been previously shown to regulate process outgrowth in these cell lines.

We thank K. Wing for discussions and providing the thiosemicarbazones and P. Cherbas for providing the Drosophila cell lines. Supported by a Grass Foundation Fellowship to G.M.C., NIH Fellowship AM0722 to R.G.V.B., and NSF grant PCM-8215638.

57.7 CONTACT MEDIATED AVOIDANCE BETWEEN SPECIFIC PAIRS OF GROWTH CONES AND NEURITES IN CULTURE. J.A. Raper, J.P. Kapfhammer*, and E.B. Grunewald*. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, West Germany.

Experiments in invertebrates suggest that growth cones distinguish between and crawl upon reproducible sequences of particular axons, implying that axons possess specific labels on their surfaces. Bray, Wood, and Bunge (Exp.Cell.Res.130:241,1980) describe a tissue culture system in which embryonic rat SCG neurites grown on collagen will not mix with retinal neurites, while SCG neurites mix with SCG neurites, and retinal neurites mix with retinal neurites. We have replicated their results using embryonic chick sympathetic and retinal tissues grown on laminin. We have also made a time lapse study of the behavior of growth cones as they approach and touch like and unlike neurites.

Retinal tissue was taken from 6 day embryos, sympathetic and dorsal root (DRG) ganglia from the lumbar region of 8 day embryos. Non-neuronal cells spread poorly on laminin, and were suppressed by the addition of anti-mitotics to the culture medium. Consequently, we were able to observe neuron-neuron interactions without their interference.

After 5-7 days in culture, the neurites from adjacent sympathetic and retinal explants form two nearly exclusive interdigitating territories, separated by a mostly axon free zone. In contrast we see no signs of exclusive territoriality when the following pairs are explanted together: retina-retina, sympathetic-sympathetic, DRG-DRG, or DRG-sympathetic. Ambiguous results are obtained when DRGs confront retinae.

Time lapse examination of these cultures 1-2 days after explantation show that, upon contact, sympathetic growth cones retract from retinal axons (23 retractions/24 encounters), but less often from sympathetic axons (8/29). Retinal growth cones retract from sympathetic axons (30/38), but not from retinal axons (1/44). The probabilities that retinal or sympathetic growth cones are incapable of distinguishing between retinal and sympathetic neurites are less than .001 . Similar data collected for DRG-sympathetic confrontations yielded no evidence that either DRG or sympathetic growth cones distinguish between DRG or sympathetic neurites in this assay.

These results demonstrate that the growth cones of vertebrate neurons can distinguish between particular neurites in a cell specific manner. Since contact appears to be required, tissue specific cell surface labels may be involved. Finally, our results highlight the role active avoidance might play in growth cone navigation.

57.8 USE OF VEC-DIC MICROSCOPY AND HVEM-IMMUNOLOCALIZATION TO STUDY ADHESIVE INTERACTIONS BETWEEN NEURONAL FILOPODIA H-C. T. Tsui, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201

Observation of cultured chick retina neurons with VEC-DIC microscopy showed that the motile filopodia of growth cones were extremely adhesive. Motile filopodia that contacted each other often became attached at their tips, remaining attached despite drastic movements of the filopodia. Attached filopodia were seen to push or pull on each other, move laterally together, or stretch tightly and undergo small vibratory movements. Attached filopodia separated slightly at times, but within seconds re-attachment occurred at the original position. If one filopodium moved away, another from the same cell often became attached to the same region of the stable filopodium. These observations are consistent with the hypothesis that adhesive molecules are specifically localized within filopodial membranes. We therefore studied the distribution of adheron molecules (J. Cell Biol. 96:990-999) on neuron surfaces using immunogold labeling of whole mounts of cultured neurons. Treated cells were then critical-point-dried and examined with the high voltage electron microscope (HVEM). Antibodies were located primarily on membranes at the edges of neurites, growth cones, and filopodia, regions where the cells were attached to the substratum. In addition, particularly high concentrations of antibodies were present at areas where filopodia contacted each other. The HVEM immuno-labeling data are consistent with the possibility that adherons were responsible for the localized adhesive interactions of filopodia observed with VEC-DIC microscopy.

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Antibodies kindly supplied by David Schubert of the Salk Institute)

57.9 GROWTH CONE FORM CHANGES WITH AGE AND POSITION ALONG THE EMBRYONIC MOUSE RETINAL AXON PATHWAY. P. Bovolenta and C.A. Mason. Department of Pharmacology, New York University School of Medicine, New York, NY 10016.

In vivo studies of the mammalian CNS have never asked whether growth cone form varies with growth cone behavior, or with features of the environment. It is also not known how these two factors in turn relate to differential adhesion to the surfaces on which axons grow. To address these questions, we have been studying the morphological changes which growth cones undergo along the mouse retinal axon pathway. This system offers the advantage of a relatively homogenous population of axons which pass through microenvironments with different cellular organizations (optic nerve or neuroepithelium, chiasm, tract and target tissue). Retinal axon growth cones were visualized along the entire pathway by inserting HRP-coated micropipettes into mouse retina at embryonic day (E)11 to E15 and into the chiasm at E14-E19; injections were made in whole embryos or fresh isolated brains kept *in vitro* for several hours. Vibratome sections were reacted with DAB both for light and electron microscopy. Between E11 and E15, growth cones in the optic nerve consist of flattened irregular expansions up to 50 μm long, often having wing-like extensions but rarely bearing filopodia. In general the growth cones that are found further along the optic nerve are larger and more expanded than the followers. Moreover, in older embryos growth cones along the nerve are simpler and more fusiform. Between E13-E16 growth cones reach the chiasm, where they are shorter (20 μm), more broad and three-dimensionally complex; most of them bear filopodia. As in the nerve, the shapes vary somewhat and simpler forms are more common in older embryos. Finally, growth cones enter the optic tract between E14-E19, where they are much smaller and more sleek. They do not have expansions or filopodia, but give off small spines along their length. When near their first target (lateral geniculate nucleus), the main axon extends branches that end in growth cones with filopodia and shapes similar to the ones in the chiasm.

These results suggest that simple growth cone forms are prominent when axons follow a well defined pathway, and that more elaborated forms appear at "choice points". Since growth cones are actively involved in the guidance of the axons, it is conceivable that the complexity of growth cones correlates with a more active role in recognition. EM analysis is in progress to determine the interactions between various growth cone forms and axonal and glial processes in their microenvironments.

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57.10 Ca^{2+} MEASUREMENTS USING THE FLUORESCENT INDICATORS QUIN 2 AND FURA 2 COMBINED WITH DIGITAL IMAGING IN MAMMALLIAN CNS CELLS. John A. Connor, Dept. Mol. Biophys., AT&T Bell Laboratories, Murray Hill, NJ 07974.

Cells in two types of culture were examined, trypsin dispersed cells from embryonic rat diencephalon (E17,18), and cerebellar explants (P3,8). In the explants, granule cells were the predominant type studied. Cells were loaded with indicator by bathing them (30 min., T=36°C) in defined media containing either 60 μM quin 2 or 8 μM fura 2. After loading and a 2 hr. post-load incubation, cells growing on #1 thickness coverslips were mounted on the stage of an inverted microscope and viewed through a 40X objective (1.3 N.A., glycerine). U.V. illumination was provided by a 100 w, short-arc Hg lamp. A charge coupled device (CCD) photometer was mounted at the 35 mm camera port of the microscope in the image focal plane. The CCD had 320 x 512 pixels, though generally, pixel binning was employed to reduce thermal noise variance (operating temperature -40 °C). Experimental procedure was to obtain sequential images (340 and 360 nm excitation for quin, 340 and 380 nm for fura) and then to form the ratio of the two images. The value of this ratio gives a measure of spatially resolved Ca^{2+} concentration independent of indicator concentration and cell geometry to a first approximation. Exposure times of .15 to .25 s were employed for each image and a temporal separation of about 1 sec was required to change the excitation filter. In dispersed cultures 5 to 15 cells could be included in a given field, while for small, densely packed granule cells from explants, the number was often greater than 20.

In the dispersed cultures there were large differences in the ratio images of the various cells in a given field (>100%), while there was uniformity to within a few percent in granule cell populations. Exposure to high K saline (25 mM) produced measurable increases in Ca^{2+} within 30 sec to 1 min. Only a fraction of the cells in diencephalon cultures responded to high K. Addition of TTX (.3 μM) to high K saline caused a rapid decrease in Ca^{2+} indicating that the increase was due in some cases to enhanced action potential firing. Nitrendipine and nifedipine (10 μM) caused a decrease in Ca^{2+} in subpopulations of the diencephalon cells, but the cell types affected have not been worked out at this time. Cells new in culture (growing) showed higher levels of Ca^{2+} than cells of more mature cultures. Examples of growth cones with extended filapodia have been observed. In these cases, Ca^{2+} in the cone was generally higher than in the soma. Where filapodia were not evident the ratio image was nearly uniform throughout the cell.

57.11 PRESENCE OF REGENERATING PROCESSES IS ASSOCIATED WITH PROLONGED BURSTING OF CALCIUM-ACTIVATED NONSELECTIVE CATION CHANNELS. Stuart A. Lipton. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

Calcium-activated channels that are permeable to multiple cations were first described in heart muscle and neuroblastoma. In the present study, conductances with similar permeation selectivity have been found in cultured neurons from the mammalian CNS, and the degree of channel activity was different in cells regenerating processes from those without processes.

Patch-electrodes were sealed to the soma of 65 rat retinal ganglion cells that had been cultured and identified as described previously (Leifer, Lipton, Barnstable, and Masland, Science 1984;224:303-6). In culture, ganglion cells were found as solitary cells or within small clusters of other retinal cells, and many of the solitary ganglion cells regenerated long processes within 1 to 3 days (ibid). Each cell-attached patch exhibited bursts of inward currents at rest (about -60 mV). Judged from continuous records lasting 5-30 minutes, solitary ganglion cells with processes displayed longer bursts of channel openings than cells without processes. In patches with comparable numbers of channels the mean burst duration was 1.3 sec (± S.D. 0.6 sec, n= 25 patches) for cells lacking processes vs 1.2 min (± S.D. 0.45 min, n=12) for cells with one or more processes. Because of the long duration of the bursts in cells with processes, the mean interburst interval could not be accurately determined in these recording sessions. For cells both with and without processes at least 4 conductance levels were encountered (30, 85, 165, and 300 pS at rest, 36°C). Inward channels of similar amplitudes were observed in the same patch of membrane after excision from the cell to the inside-out configuration. The conductance levels were little affected by varying monovalent cations, including Na, K and Cs, or by substituting the relatively impermeant anion MES for Cl. These findings support the notion that the current is carried by cations rather than by anions. For inside-out patches the current-voltage relation of the smallest conductance exhibited some degree of inward rectification. In these patches, channel activation was only slightly affected by varying the membrane potential from -60 to +60 mV. However, raising Ca^{2+} on the internal side of the membrane patch from 10 nM to 18 micromolar increased the activity of the conductances. Thus, the bursting activity of these channels may reflect variations in the level of an internal messenger such as Ca^{2+}, especially in cells that are regenerating processes, but rapid measurement of intracellular Ca^{2+} will be necessary to test this hypothesis.

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57.12 **GENERATION OF ACTION POTENTIALS SUPPRESSES NEURITE ELONGATION IN ISOLATED IDENTIFIED NEURONS.** C.S. Cohan and S.B. Kater. Department of Biology, University of Iowa, Iowa City, Ia 52242.

The dynamic actions of the neuronal growth cone are well documented but little understood. Growth cones are thought to be intimately involved in the process of neurite outgrowth and may play an important role in determining final neuronal morphology. Elucidation of the cellular events which regulate growth cone properties may provide a better understanding of the organizing principles underlying architecture and connectivity in the nervous system.

We recently suggested (J. Neurosci. Res. 13:285 1985) that voltage sensitive ion channels may influence growth cone motility. The growth cone membrane of isolated *Helisoma* neurons possesses a 70 pS ion channel. In excised patches in isotonic saline this channel mediates an inward current at negative membrane potentials, which is not blocked by TTX (100 μM) and shows little inactivation during maintained depolarizations. Our ability to distinguish morphologically between growth cones that are actively elongating and those that have ceased elongating has enabled us to demonstrate further that the activity of this ion channel is correlated with growth state. Growing growth cones contain active ion channels whereas nongrowing growth cones contain masked channels which are in a nonactivatible state. This physiological difference suggests that ion channel activity may play a role in growth cone movement and neurite elongation.

In the present series of experiments we show that the generation of action potentials suppresses neurite elongation. Gigohm seals were obtained with patch pipettes on the somata of isolated *Helisoma* neurons 5 and 19 which were in an active state of outgrowth. After setting a holding potential of -50 mv, the membrane patch was ruptured to gain access to the intracellular compartment as in whole cell clamps. Photographs were taken at 15 minute intervals to monitor neurite outgrowth. Neurite elongation was analyzed after the experiment by measuring the advance of each growth cone from the photographed neuron at each 15 min. time point. During periods in which the patch pipette was at the holding potential, neurite elongation proceeded at a constant rate (15.5 μm/h, n=14). Similarly, during hyperpolarizing pulses, neurite elongation was unchanged. However, when depolarizing pulses which produced overshooting action potentials were used, neurite elongation stopped. This occurred within 15 min. of the onset of the stimulus pulses and was accompanied by a change in growth cone morphology. Growth cones which had ceased elongating showed some additional outgrowth over the following 12 h. (nonstimulated) after the patch pipette was removed. These experiments demonstrate that the generation of action potentials within an actively outgrowing neuron can inhibit neurite elongation. Furthermore, they suggest that electrical activity may be one mechanism for regulating neuronal morphology as neurons grow, interconnect, and form spontaneously active circuitry.

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FEEDING AND DRINKING III

58.1 SALT APPETITE EVOKED BY SODIUM DEPLETION IS ABOLISHED BY CONCURRENT INTERFERENCE WITH ENDOGENOUS ANGIOTENSIN II AND ALDOSTERONE R.R. Sakai*, A.N. Epstein & S. Nicolaidis*, University of Pennsylvania, and Collège de France.

During sodium deficiency, angiotensin II (ANG II) and aldosterone (ALDO) are produced and cause renal sodium conservation. Epstein (Peptides, 3, 493, 1982) has suggested that the behavioral counterpart to sodium conservation, sodium appetite, is aroused by the same two hormones in the sodium deplete rat. Moe et al (AJP. 247, R356, 1984) have demonstrated that blockade of endogenous ANG II formation with captopril, an angiotensin converting enzyme antagonist, suppresses but does not abolish sodium appetite induced by sodium depletion

The role of ALDO in the expression of sodium appetite was studied by giving RU 28318 (Roussel-UCLAF), an anti-mineralocorticoid, either systemically or directly into the brain ventricles of rats that were sodium depleted by combining a natriuretic drug, furosemide (10 mg/rat, SC), with removal of ambient sodium (home cage washed, removal of 3% NaCl, and food replaced with a sodium deficeint diet). At least 18 hours after the beginning of the depletion 3% NaCl was returned for 2 hours and the rats expressed a sodium appetite.

Peripheral ALDO receptors were blocked in sodium deficient rats (N=6) by giving RU 28318 in their drinking water (1 mg/ml, PO) and by SC injection (5 mg/kg, BW) 15 minutes before furosemide and at 2 hours and at 30 minutes before 3% NaCl access the following day. There was no suppression of the volume of 3% NaCl drunk during the 2 hour access period (veh 6.8 ± 0.6 ml, RU 7.5 ± 0.5 ml).

Central ALDO receptors were blocked in other rats (N=5/group) receiving RU 28318 (0.5, 5 and 25 μg/hr) by continuous intracerebroventricular (cICV) infusion during the depletion through an indwelling cannula. Their sodium appetite was suppressed in a dose related fashion by RU 28318 (veh 8.2 ± 0.6 ml, 0.5 μg/hr 5.6 ± 0.8 ml, 5 μg/hr 4.2 ± 0.9 ml, 25 μg/hr 2.2 ± 0.7 ml).

In a final group, rats (N=6) were treated with both cICV RU 28318 (25 μg/hr) and captopril (CAP) (SC, 50 mg/kg/BW, and PO, 0.1 mg/ml), during the depletion. By interfering with the actions of both ANG II and ALDO, sodium appetite was abolished. In all experiments, the drug treatments did not reduce water intake and did not affect sodium excretion during the depletion (veh 1.6 ± 0.09 mEq, RU + CAP 1.67 ± 0.24 mEq). These data are strong support for the idea that ANG II and ALDO are essential for both the arousal and expression of sodium appetite.

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58.2 THE ENDOCRINE CONSEQUENCES OF SODIUM DEFICIENCY PREPARE THE BRAIN FOR SALT APPETITE A.N. Epstein, R.R. Sakai*, and W. Fine*, (SPON. J. Sprague). University of Pennsylvania.

A salt appetite is aroused in the sodium replete rat by endogenous aldosterone (ALDO) and angiotensin II (ANG II) acting in synergy (Neurosci. Abstracts, 10, 1984, 1010) and the appetite of the sodium deplete rat can be abolished by preventing the actions of endogenous ALDO and ANG II (Neurosci. Abstracts, 11, 1985).

We now report 1) that a single sodium depletion enhances the salt appetite that is expressed after a second and subsequent sodium depletions, 2) that this enhancement does not depend on the drinking of salt after the first depletion, and 3) that it can be produced by the actions of ALDO and ANG II on the brain in the absence of sodium depletion.

1) Rats (N=5) living in their home cages with water, 3% NaCl, and Purina pellets freely available were depleted of sodium by combining pharmacological natriuresis (furosemide, 10 mg/rat, SC) with removal of sodium (Na deficient diet, removal of the 3% NaCl, and thoroughly washed cage). Eighteen to 20 hours later they expressed a reliable salt appetite (latency of 10 seconds and 6.8 ± 0.5 ml volume drunk) when the 3% NaCl was returned for 2 hours of access. When depleted a 2nd time 1 week later, they drank more rapidly (0 latency) and nearly twice as much (10.5 ± 1.3 ml). Total sodium loss was the same after the 1st and 2nd depletions. There was no further enhancement. Latencies and volumes drunk after a 3rd and 4th depletion were the same as after the 2nd.

2) Other rats (N=17) were depleted overnight but were not permitted to drink 3% NaCl the next morning. Their appetite after a 2nd depletion was enhanced and was indistinguishable from that of rats that drank after both their 1st and 2nd depletions. The same result was obtained whether or not the animals were repleted of sodium (by SC injection) after the first depletion.

3) A third group of rats (N=11) were treated while sodium replete with systemic ALDO (40 μg/day) for 5 days and with a pulse intracerebroventricular injection of ANG II on day 5 which, as we have shown (Neurosci. Abstracts, 10, 1984, 1010), arouses a robust sodium appetite. But these animals were not offered salt. One week later they were depleted and allowed to drink salt. Their appetite was indistinguishable in latency and volume drunk from the enhanced appetite of animals that were depleted twice and were expressing an appetite for the 2nd time.

When the levels of hormones of sodium deficiency, angiotensin II and aldosterone, are raised by a rat's first sodium depletion they not only cause a salt appetite, but they also leave a long-lasting trace in the animal's brain that prepares it for more rapid and more vigorous expression of the appetite in response to future depletions. Supported by NS 03469-24 and T32 MH 17168-01.

58.3 HEPATIC-PORTAL NaCl INFUSION ATTENUATES SALT APPETITE. M.G. Tordoff, J. Schulkin* and M.I. Friedman*. Monell Chemical Senses Ctr., and Dept. Anatomy, Univ. PA, Philadelphia, PA 19104.

We investigated the possibility that sodium-sensitive elements in the liver or hepatic-portal vein can mediate the satiation of salt appetite in sodium-depleted rats. All experiments used male Sprague-Dawley rats (300-350 g) given hepatic-portal or jugular catheters. NaCl consumption was measured in 30-min two-bottle tests (0.5 NaCl vs. water). A sodium deficit was induced by providing a low-sodium diet 48 hr before, and injecting furosemide (5 mg, sc) 24 hr before tests were conducted. Infusions were of 1 ml volume given over 30 min, starting 15 min before access to the two bottles. Infusions were given in counterbalanced order with at least 6 days between tests. Nine rats with hepatic-portal catheters received 0, 0.15, 0.6 and 1.2 M NaCl. All three doses of NaCl significantly suppressed NaCl intake relative to the 0 condition (mean ± SEM [ml/30 min] 0 = 7.1 ± 0.71, 0.15 M = 5.5 ± 0.69, 0.6 M = 5.7 ± 0.49, 1.2 M = 4.4 ± 0.83). Note that the suppression of NaCl intake was probably not due to an osmotic effect of the infusate because 0.15 M (isotonic) NaCl significantly suppressed NaCl intake. Water intakes during the same period were not significantly altered, although there was a trend for the 0.6 and 1.2 M infusions to increase water consumption (0 = 1.3 ± 0.48, 0.15 M = 0.6 ± 0.22, 0.6 M = 2.7 ± 0.73, 1.2 M = 3.4 ± 1.35). On the other hand, 10 rats given jugular infusions of 1.2 M NaCl did not decrease NaCl intake (0 = 6.0 ± 1.10, 1.2 M = 5.2 ± 1.05) but did significantly increase water intake relative to the 0 condition (0 = 1.9 ± 0.41, 1.2 M = 5.0 ± 0.83).

The reduction of salt appetite produced by hepatic-portal NaCl infusion is apparently mediated by the vagus nerve: NaCl solution consumption was measured in seven rats before and after selective hepatic vagotomy. Before vagotomy, hepatic-portal infusion of 1.2 M NaCl significantly reduced NaCl intake relative to intake in response to distilled water infusion. However, after vagotomy the suppressive effect of 1.2 M NaCl was abolished.

Thus, the results support the existence of a vagally mediated porto-hepatic mechanism that can modulate NaCl appetite. This may be a function of brainstem components of the visceral-taste pathway, which have been shown by electrophysiological and anatomical methods to integrate afferent information from the liver and oral cavity.

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58.4 THE "VASCULAR HYPOTHESIS" FOR INITIATION OF THIRST AT THE SUBFORNICAL ORGAN. R.G. Blasberg*, P.M. Gross and J.D. Fenstermacher, Department of Neurological Surgery, SUNY at Stony Brook, NY 11794 and Department of Nuclear Medicine, NIH, Bethesda, MD 20205.

The subfornical organ (SFO) is a midline tubercle in the third ventricle having a dense, highly permeable capillary bed that makes this structure a primary target for circulating hormones. One hypothesis (1) for stimulation of thirst and hormonal responses during dehydration is that vasoactive hormones, such as angiotensin II and vasopressin (whose plasma concentrations are elevated during dehydration), cause contraction of the blood volume and reduced blood flow to circumventricular organs, including the SFO; such vascular effects then lead to behavioral, cardiovascular and endocrine responses to restore fluid balance ("vascular hypothesis" for thirst). We comprehensively evaluated this hypothesis in the SFOs of conscious rats that were normally hydrated or severely dehydrated (5 days of water deprivation). Using quantitative autoradiographic methods and computerized image processing, we determined in the SFO local blood flow (14C-iodoantipyrine), microvascular plasma volume (125I-albumin), microvascular erythrocyte volume (51Cr-erythrocytes), and tissue glucose utilization (14C-deoxyglucose) (Reference 2).

	Control	Dehydration
Blood flow (ul/g/min)	1010±160(5)	1520±190(6)
Plasma volume (ul/g)	42± 10(5)	44± 7(6)
Erythrocyte volume (ul/g)	12± 1(5)	8± 1(4)
Glucose utilization (umoles/g/min)	0.47±.05(8)	0.81±.04*(8)

Means ± SE; Number of animals in parenthesis; *p<0.05.

The results do not support the "vascular hypothesis" for initiation of thirst through microvascular adjustments in the SFO. Capillary blood flow and total vascular volume (plasma + erythrocyte volumes) in the SFO were not altered by severe dehydration. Glucose utilization, however, was substantially increased, both by the conditions of chronic dehydration and by direct intravenous administration of angiotensin II (3). The findings suggest that the vasodilatatory effects of increased metabolism, both from angiotensin II itself and from metabolic products such as H+, effectively oppose the actions of any circulating substances that may cause vasoconstriction. There appears to be no significant net effect of these antagonistic stimuli on the SFO microcirculation.

1. Fitzsimons, J.T. The Physiology of Thirst and Sodium Appetite, Cambridge: Cambridge University Press, 1979, pp. 374-379.
2. Gross, P.M. et al., Brain Res.330: 329-336, 1985.
3. Gross, P.M. et al., Peptides 6: Suppl. 1, 1985.

58.5 MONOSODIUM L-GLUTAMATE LESIONS: EFFECTS ON NOREPINEPHRINE STIMULATED FEEDING AND INSULIN SECRETION IN THE RAT. J. Steves Sims and J. F. Lorden, Dept. Psych., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Neonatal treatment with monosodium L-glutamate (MSG) produces lesions of the medial basal hypothalamus in rodents. Relatively little is known about the cause of the resulting obesity syndrome. MSG obesity is distinguished from other hypothalamic obesities by the absence of hyperphagia but may be associated with altered carbohydrate metabolism. Therefore, we examined the feeding response of MSG-treated rats to norepinephrine (NE) stimulation and studied the dependence of the MSG obesity on vagal function.

Litters of 8-10 rats were injected on postnatal days 2 and 4 with 4 mg/g of MSG or isotonic saline. In adulthood, 10 saline and 10 MSG-treated rats were implanted with bilateral cannulae aimed at the paraventricular nucleus of the hypothalamus (PVN). After recovery, the rats were given sweetened condensed milk for 1 h each day. Intake was measured and a fresh supply given for a second hour. After 10 days training, rats received bilateral injections of either saline (.5 ul) or NE (20 nM) through the PVN cannulae immediately prior to the second hour. For rats treated neonatally with saline, milk intake was 18.4 ml ± 4.0 (M ± SD) during the first hour. Following a saline injection, milk intake was 4.4 ml ± 2.2 and following NE, 10.6 ± 2.7. In the MSG group, rats drank 13.7 ± 3.1 ml prior to the injections. Consumption following saline injections was 3.2 ml ± 1.3 and following NE, 4.2 ± .9. Thus, the MSG-treated rats consumed less milk overall and showed a significant attenuation of NE-induced feeding. It is possible that the reduced responsiveness of the MSG rats to stimulating effects of NE contributes to their hypophagia.

In the second study, adult female MSG and saline treated rats from the same litters used in the first study were given either complete subdiaphragmatic vagotomies (Vx) or sham surgery (SVx). Animals were monitored for 180 days. At that time, all rats were fasted overnight, anesthetized and implanted with venous catheters. A fasting blood sample was drawn, .3 cc of 2 M glucose injected and additional blood samples drawn 5, 10 and 15 min later. Blood glucose levels were similar in all groups. SVx MSG-treated rats were obese and showed a significant fasting hyperinsulinemia in comparison with SVx Saline-treated rats. Vagotomy produced a complete reversal of both the obesity and hyperinsulinemia of the MSG group, suggesting that MSG obesity depends on vagal function. (Supported by NINCDS grant 14755.)

58.6 CHOLINERGIC AFFERENTS TO THE SUBFORNICAL ORGAN IN RATS. David A. Wheeler and John B. Simpson. Department of Psychology, University of Washington, Seattle, WA 98195.

Acetylcholine is involved in the central regulation of body fluid balance. Intraventricular injection of cholinergic agonists stimulates water ingestion, raises blood pressure, increases vasopressin secretion, induces natriuresis, and suppresses sodium appetite. Some of these effects may be mediated by the subfornical organ (SFO)— an area of the brain involved in angiotensin II body fluid regulation. Direct application of cholinergic agonists into the SFO in quantities smaller than are required by intraventricular injection produce water ingestion, elevated blood pressure, and increased vasopressin secretion.

There is also anatomical evidence which suggests that the SFO receives cholinergic afferents. Acetylcholine, the synthetic enzyme, choline acetyltransferase (ChAT), and the degradation enzyme, acetylcholinesterase (AChE) have been found there. Electron microscopy reveals vessicles in nerve terminals which appear to contain acetylcholine. Lewis and Shute (Brain, 90:521, 1967) suggested that the SFO receives cholinesterase containing axons originating from the area of the dorsal fornix but the specific neurons were not identified. The study presented here uses modern neuroanatomical tract tracing techniques to identify the nuclei which send afferents to the SFO.

The retrogradely transported molecules wheat germ aggultinin, cholera toxin subunit-B, or succinylated concanavalin-A were injected directly into the SFO in rats through stereotaxically implanted cannulas and they were visualized using the Vectastain ABC immunohistochemical method. Initially, possible cholinergic neurons were identified using AChE histochemistry in diisopropylflourophosphate treated rats. Once possible cholinergic afferents were identified, adjacent sections were stained using a monoclonal antibody against ChAT (Immunonuclear). Neurons which contained both the retrogradely transported tracers and choline acetyltransferase were considered to be likely cholinergic afferents to the SFO.

To confirm that these nuclei projected to the SFO and not to adjacent tissue, they were injected with the anterogradely transported phaseolus vulgaris leucoagglutinin (PHA-L). Nuclei were considered to be the source of cholinergic afferents to the SFO only when the PHA-L provided confirmation of the projection to the SFO.

Preliminary results suggest that the medial septum is the source of cholinergic afferents to the SFO.

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58.7 HISTAMINERGIC AND VAGAL CONTROL OF GASTRIC ACID SECRETION BUT NOT DRINKING IN ANTICIPATION OF EATING IN THE RAT. F.S. Kraly, S.M. Specht*, L.A. Coogan*, J.E. McOsker* and S. Geasey*. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

Male Sprague-Dawley rats (n = 10), surgically equipped with stainless-steel gastric cannulas, were tested in a paradigm similar to that used by Weingarten and Powley (Physiol. Behav., 27: 217, 1981) to achieve conditioning of cephalic phase gastric acid secretion in the rat. After 24-hr food deprivation, each rat was prepared for testing by having its fistula opened, its stomach washed with .9% NaCl, and a collecting tube attached to the cannula. On Test 1 (Baseline), no food was offered, but water intake and gastric acid secretion (through the fistula) were measured for 60 min. For Tests 2-6, rats were offered sweetened milk to sham feed with open fistula for 60 min. On Test 7 rats were not offered milk (though they should have expected it), and water intake and gastric acid secretion were again measured. Subsequent tests evaluated the ability of atropine methyl nitrate (AMN; 1 mg/kg i.p.) or combined antagonism of H_1 and H_2 receptors using dexbrompheniramine (DXB; 1 mg/kg i.p.) and cimetidine (C; 16 mg/kg i.p.) to affect water intake and acid secretion when rats expected to eat but were not offered food.

		Anticipation of eating		
	Test 1	Test 7		
	Baseline	.9% NaCl	DXB + C	AMN
Water intake (ml)	0.6 ± 0.4	6.9 ± 2.5	5.6 ± 1.4	5.7 ± 2.0
Gastric acid (μEq H^+)	61 ± 20	155 ± 38	28 ± 10	12 ± 7

(Scores are means ± SE for 60-min tests)

Rats appeared to have been successfully conditioned to anticipate eating in the experimental paradigm; gastric acid secretion ($p < .02$) and water intake ($p < .05$) on Test 7 were greater than baseline scores. Combined antagonism (DXB + C) of peripheral H_1 and H_2 receptors inhibited ($p < .01$) cephalic phase gastric acid secretion, such that it was not different ($p > .10$) from baseline, but failed to affect water intake ($p > .20$). Blockade (AMN) of peripheral cholinergic vagal efferents inhibited cephalic phase gastric acid secretion ($p < .01$), bringing it below baseline ($p < .05$), but failed to affect water intake ($p > .20$). This dissociation between drinking and acid secretion in anticipation of eating was further supported by the failure to find correlations (all $ps > .05$) between 60-min water intake and acid secretion for each experimental treatment.

These results suggest no vagal histaminergic mediation of drinking elicited by the anticipation of eating, in contrast to evidence for histaminergic control of drinking elicited by pregastric food-contingent stimulation in the rat (Behav. Neurosci., 98: 349, 1984).

58.8 FUNCTIONAL COUPLING BETWEEN TRANSIENTS IN BLOOD GLUCOSE AND FEEDING BEHAVIOR:. TEMPORAL RELATIONSHIPS. F.J. Smith* and L.A. Campfield (SPON: C. Enroth-Cugell) Depts. of Physiology and Engr. Sci., Northwestern University, Chicago and Evanston, Il. 60611 and 60201.

A premeal decline in blood glucose (BG) has been recently shown to be causally related to meal initiation in free-feeding rats (Brandon, Smith, Campfield, Soc. Neurosci. Abstr. 10, 534,1984). In order to assess the degree and time course of the functional coupling between declines in BG and meal initiation, access to food was prevented by covering the food cup prior to and throughout declines in BG, followed by restoration of access to food 5 minutes after BG returned to baseline. The latency to feeding and the next decline in BG were measured. 200 gm female Wistar rats implanted which chronic cardiac cannulas were allowed to recover for 7 days prior to experiments. Powdered chow and water were available, except when the food cup was covered. Continuous blood withdrawal (25μl/min) for BG monitoring was initiated 45 minutes after the IV administration of 200 U heparin. The food cup was then covered and the rat was returned to its home cage and remained undisturbed throughout the rest of the experiment. In five experiments across the light/dark transition, declines in BG, similar to those described previously under free- feeding conditions, were observed. Food seeking behavior (moving to food cup, sniffing, try to remove cover) occurred in each experiment with an average latency of 10 ± 1.4 minutes after the beginning of the fall in BG. (This compares with the average latency to meal initiation of 12.1 ± 1.7 minutes after the beginning of the decline in the free-feeding condition.) After the food cup was uncovered, no food seeking behavior and no feeding was observed until after another similar decline in BG occurred. Meal initiation following the second decline occurred an average of 88.5 ± 5.2 minutes after the food seeking behavior following the first decline in BG.

In preliminary studies, a novel food (orange slice, cookies) was introduced 30 minutes after the food cup was uncovered. No significant decrease in BG occurred following food presentation. The food was eaten with a very short latency but without a prior decline in BG.

Therefore, we conclude that transient declines in BG strongly signal food seeking and meal initiation but this functional coupling between BG and feeding behavior is of short duration. The transient nature of this coupling requires a second decline in BG to initiate feeding if the rat is unable to eat within a narrow temporal window. In addition, transient declines in BG appear to be an endogenous cue for food seeking and meal initiation that is not strongly influenced by sensory cues.

58.9 SELECTIVE ALTERATIONS IN OPIATE-INDUCED FEEDING FOLLOWING NALOXONAZINE PRETREATMENT. P.E. Mann, R.J. Bodnar, M.T. Romero*, L.S. Truesdell and G.W. Pasternak. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367 and George C. Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Ctr. and Depts. of Neurology and Pharmacology, Cornell Univ. Medical Ctr., New York, NY 10021.

Naloxonazine, a selective and irreversible antagonist of mu-1 opioid binding sites and naloxone, a short-acting antagonist which interacts with a number of opiate receptor sub-types, inhibit food intake in freely feeding and food-deprived rats. Yet, 2-deoxy-D-glucose hyperphagia is reduced by naloxone and potentiated by naloxonazine. The present study evaluated the effects of long-term (24 h) pretreatment with naloxonazine and short-term (5 min) pretreatment with naloxone upon feeding induced by the mu receptor agonist, morphine (MOR) and the kappa receptor agonists, ethylketocyclazocine (EKC) and dynorphin (DYN). First, food intake of 14 mildly-deprived (5 h) rats was assessed 4 h following MOR (5 mg/kg, SC) alone or following naloxonazine (10 mg/kg, IV) or naloxone (10 mg/kg, SC) pretreatment. MOR hyperphagia was completely blocked by naloxone, yet was significantly potentiated by naloxonazine. Second, MOR (1-10 mg/kg) hyperphagia was absent in 15 rats neonatally treated with monosodium glutamate which destroys medial-basal hypothalamic cells which contain beta-endorphin and met-enkephalin. Third, food intake of freely-feeding rats was assessed 4 h following EKC at doses of 5 mg/kg (n=19, SC) or 2 mg/kg (n=13, SC). While neither naloxone nor naloxonazine blocked EKC (5 mg/kg) hyperphagia, naloxone, but not naloxonazine blocked EKC hyperphagia at the 2.5 mg/kg dose. Fourth, food intake of 12 freely-feeding rats was assessed 1 h following DYN (10 ug, ICV). Again, naloxone, but not naloxonazine blocked DYN hyperphagia. These data indicate that both mu and kappa receptor subtypes are involved in the short-term opioid-induced increases in feeding behavior. Further, the mu-1 binding site appears to differentially modulate various forms of feeding paradigms. (Supported by NIDA Grant DA002615 and PSC/CUNY Grant 6-64187.)

58.10 (^{14}C) 2-DEOXYGLUCOSE UPTAKE IN THE NUCLEUS TRACTUS SOLITARIUS INCREASES IN RESPONSE TO GASTRIC DISTENTION. M. F. Gonzalez, F. R. Sharp and J. A. Deutsch*. V. A. Medical Center, Department of Neurology, San Francisco, CA 94121 and University of California, San Diego, Department of Psychology, La Jolla, CA 92093.

The presence of fibers in the vagus nerve that respond to gastric distention has been extensively documented. However the areas where this information projects in the brain are still not well established. The present work mapped these areas using the (^{14}C) 2-deoxyglucose (2DG) method.

Under urethane anesthesia (1.5g/kg body weight) the left cervical vagus was severed and intragastric balloons were implanted in six, food and water deprived, Sprague-Dawley rats. An injection of 2DG (167μCi/kg body weight dissolved in 0.3 ml sterile saline) was injected as a bolus into each rat's tail vein. Immediately following this injection, the intragastric balloons of three subjects were inflated with 20 ml of warm water to produce stomach distention. In the other three subjects, a nondistending volume of 2 ml was injected into their balloons. 45 min after the 2DG injection the rats were sacrificed, and their brains removed, frozen, sectioned, and autoradiographed as previously described (Sharp and Evans, J. comp. Neurol., 208:255, 1982).

Gastric distention induced increases of 2DG uptake in the right nucleus tractus solitarius (NTS), contralateral to the vagotomized side. This effect was confined to the medial aspects of the NTS and extended from the most caudal, commissural end of the nucleus to the level of appearance of the fourth ventricle. The locus of maximal activation was at the level of the area postrema. A nonparametric Mann Whitney U test comparing the optical densities of the activated sites in the right NTS to those of the contralateral, non-activated NTS was significant at the $p<0.05$ level. A significant left versus right difference in 2DG uptake was not found in control subjects or in other brain stem structures. The present results confirm the notion that gastric distention information travels to the brain, at least partially, through the vagus nerve. They are also compatible with our knowledge of neural connections between the stomach and the brain.

58.11 ESTROGENIC CONTROL OF FEEDING BEHAVIOR AND BODY WEIGHT REGULATION IN RATS WITH KAINIC ACID LESIONS OF THE LATERAL SEPTAL AREA. T.R. KING* AND D.M. NANCE. Department of Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7 (SPON: D.A. Hopkins).

Extrahypothalamic control of feeding behavior and body weight (BWt) regulation is well documented. We have suggested that the lateral septal area (LS) may have a primary role in the hormonal control of energy balance (Nance P.B.&B., 18:605, 1983). In support of this, we have shown that rats with kainic acid (KA) LS lesions exhibit a systematic increase in BWt and an attenuation in the anorexic effects of estrogen on energy balance. However, it is not known whether these changes are correlated to alterations in feeding behavior. We examined the short term effects of KALS lesions on food intake (FI) and BWt regulation in female rats. In addition, the effects of ovariectomy and exogenous estrogen treatment on these parameters were determined. Using a microsyringe, KA [2µg/µl] was infused bilaterally (total volume 1.0 µl) into the LS at a rate of 0.1 µl/minute and the needle left in place for an additional 10 minutes. In all animals KA lesions of the LS produced major cell loss in the LS, however the extent of damage to the LS was variable. Associated with KALS lesions was the concurrent loss of CA3-CA4 cell groups in the hippocampus which was comparable for all lesioned animals. The extent of septal damage was quantified morphometicaly using a Zeiss IBASS Image Analyzer and correlated with changes in FI and BWt. The significant effects of the KALS lesions, relative to the controls were: 1) an increase in percent BWt gain from baseline which was significant 22 days following brain surgery. 2) an increase in percent food intake which was significant by day 6 post surgery, 3) an attenuation in the anorexic effects of estrogen on FI and BWt, and 4) a decrease in the percent days of vaginal estrus. Moreover, the anorexic effects of estrogen were inversely correlated with the extent of LS damage, but not the amount of hippocampal damage. The present study has confirmed that there is an increase in BWt following KALS lesions and further indicates that a sustained period of increased FI precedes the increase in BWt. These results verify that cells in the LS are involved in the estrogenic control of energy balance and further suggest that the increase in FI and BWt following KA lesions of the LS is attributable to the concurrent reduction in the anorexic effects of estrogen. Finally, the morphometic analysis of the brain lesions further suggest the ventral and/or intermediate subdivisions of the LS as the location of the cell bodies mediating the estrogenic control of feeding behavior. Supported by MRC of Canda.

58.12 CONCENTRATION DEPENDENT SUCROSE INTAKE IN CHRONIC DECEREBRATE RATS. F.W. Flynn, G. Acevedo* and H.J. Grill. Dept. Psychol. and Inst. Neurol. Sci. University of Pennsylvania, Philadelphia.

In neurologically intact rats, the volume of sucrose solution ingested increases with concentration up to a maximum. Further increases in concentration are accompanied by decreases in intake. These data are interpreted as evidence that taste and post-ingestional feedback interact in determining the amount ingested. Previously, Grill and Norgren provided evidence that chronic decerebrate rats show normal discriminative oromotor responses to taste stimuli. The volume of the taste stimulus infused into the rats' mouths (50µl) did not allow the quantification of the interaction of taste and postingestional feedback. In the present study, the interaction of taste and postingestional feedback in determining the amount of sucrose solution ingested was measured.

Brains of Sprague-Dawley rats were transected in the supra-collicular plane using a 2-stage procedure. Decerebrate (N=6) and pair-fed control rats (N=8) were implanted with intraoral cannulae and were maintained by intragastric feedings (4, 8ml meals/day). Intraoral intake tests began 10 days postsurgery. One hour following the morning meal, a taste stimulus-filled tubing was attached to the intraoral cannula and the rat was placed in a clear plastic chamber. A syringe mounted on an infusion pump maintained a constant rate of intraoral stimulus delivery (0.8ml/min). Intraoral infusions continued until the stimulus was expelled from the rat's mouth. The amount consumed was then computed by multiplying the rate of infusion x time spent ingesting. On alernate days, rats were given the following order of stimuli: water, 0.03M, 0.1M, 0.3M, 1.0M, 1.3M, 2.0M sucrose.

The volumes of the stimuli consumed showed the same concentration dependent relationship for control and decerebrate rats. For both groups, the amount of water and 0.03M sucrose consumed were not significantly different. The amounts consumed by control and decerebrate rats significantly increased when sucrose concentration was increased to 0.1M, and again when increased to 0.3M ($p<.05$). Intake of 0.3M, 1.0M, and 1.3M was similar. Both groups significantly decreased their intake when sucrose concentration was increased to 2.0M. Overall, control rats ingested more of all the stimuli than did decerebrate rats. The concentration dependent function in decerebrate rats shows that caudal brainstem mechanisms are sufficient for the detection of taste and postingestional feedback, and their integration to control the ingestion of orally-delivered sucrose solutions.

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58.13 MEDULLARY AND CEREBELLAR DEGENERATION AND CYTOLOGIC CHANGE FOLLOWING THERMAL ABLATION OF THE AREA POSTREMA. Jon N. Kott, Nancy J. Kenney, and L. E. Westrum. Departments of Psychology and Neurological Surgery, University of Washington, Seattle, WA 98195.

The area postrema and underlying caudal-medial portion of the nucleus of the solitary tract (AP/cmNTS) have received a great deal of attention from researchers in the areas of food-intake and body-weight regulation, fluid and electrolyte balance, blood-pressure regulation and food aversion. Much of the research dealing with the function of the AP/cmNTS has utilized a thermal method for ablation of this region. Considering the rapid rate at which these studies are accumulating, it seems appropriate to examine the extent to which nearby structures may be damaged by the ablation procedure. We are undertaking a limited series of studies utilizing a modified Fink-Heimer degeneration technique, as well as, critical cytological staining to examine any ancillary damage resulting from thermal ablation of the AP/cmNTS.

Preliminary examination of material stained for degneration shows little advanced cell-body degeneration beyond the area that is carbonized and removed by the cautery tip. Cells within the dorsal motor nucleus of the vagus and the hypoglossal nucleus appear normal using this technique. However, critical cytologic examination may still reveal subtle changes.

Degenerating fibers and terminals appear more widespread; both are often seen in the gracile, cuneate and external cuneate nuclei. Lobule X of the cerebellum shows degeneration that may be both axonic and dendritic. As the Xth cerebellar lobule lies immediately dorsal to the AP, damage to this structure might be attributable to heat rising from the cautery tip at the time of lesioning. However, it seems more likely that both the dorsal column and the cerebellar damage may be attributable to the mechanical manipulation necessary to dry the medullary surface and to elevate the cerebellum or simply from exposure of these surfaces to the air.

Thus, while cell-body degeneration is quite limited around the lesion site, degenerating fibers, terminals and, possibly, dendrites, appear more widespread than might be predicted from the connectivity of the area.

59.1 EVIDENCE FOR DYNAMIC ELECTROTONIC COUPLING IN MAMMALIAN INFERIOR OLIVE IN VIVO. K. Sasaki* and R. Llinás (SPON: J. A. Gruner). Dept. of Physiology & Biophysics, New York Univ. Med. Ctr., New York, NY 10016.

Anatomical and physiological studies have demonstrated that the inferior olive (I.O.) neurons are electrotonically coupled by dendro-dendritic gap junctions at the I.O. glomerulus (Llinás et al. & Sotelo et al.: J. Neurophysiol. 37, 1974; Llinás & Yarom: J. Physiol. 315, 1981). The possibility that the functional organization of this nucleus may be modulated by the dynamic decoupling of neuronal groups was suggested some years ago (Llinás: Physiologist 17, 1974). In order to investigate this question, we recorded complex spikes from 32 Purkinje cells simultaneously using extracellular micropipettes in the cerebellar cortex of the rat (Crus IIA) as a variance of a technique developed in this laboratory (Bower & Llinás: Soc. Neurosci. Abst., 1982, 1983). Rats were anesthetized with Nembutal or Ketamine. Recording micropipettes were individually placed 250 μm from each other and were arranged in a 4x8 matrix in a rostrocaudal and mediolateral direction, respectively. The degree of electrotonic coupling between the I.O. neurons (the origin of the climbing fibers) was determined by the normalized degree of cross-correlation of Purkinje cell complex spikes. This spontaneous oscillatory firing of complex spikes occurred at a frequency of 8-12 Hz. The synchronous firing was observed in rostrocaudal bands which would cover most of the rostrocaudal span of a folium but which had a mediolateral span of 250-500 μm. I.O. reflexes (Eccles et al.: J. Physiol. 182, 1966) evoked from direct white matter stimulation also demonstrated the rostrocaudal organization of complex spikes, indicating that such arrangement is not dependent on co-activation of I.O. cells by common afferent inputs. Physiological stimulation of the trigeminal nerve produced responses in three rostrocaudal bands separated by two corridors of nonresponse bands. A similar spatial organization of cross-correlation was observed after injection of harmaline which increased the level of cross-correlation but does not modify the spatial organization of the climbing fiber input. Blockage of GABA inhibition by systemic application of picrotoxin induced clear cross-correlation throughout Crus IIA. Similarly following this pharmacological treatment, previously well defined trigeminal responses became widespread. These results suggest that the global organization of the I.O. can be changed through modulation of electrotonic coupling between neurons mediated by the activation of GABAergic inhibitory terminals on the I.O. glomerulus. [Supported by USPHS grant NS13742 from NINCDS]

59.2 NON-ISOCHRONOUS GENERATION OF PURKINJE CELLS DESTINED FOR SINGLE CEREBELLAR CORTICAL ZONES: A TRITIATED THYMIDINE AUTORADIOGRAPHIC STUDY IN THE CAT. B.L. Brown. Department of Anatomy, Emory University School of Medicine, Atlanta, Georgia 30322.

Considerable evidence (Voogd, 1969, Neurobiol. of Cerebellar Evol. and Devel.) suggests that the cerebellar cortex can be subdivided into a series of roughly longitudinally oriented strips, or zones, which constitute fundamental units of cerebellar cortical organization. Purkinje cells in each zone receive climbing fiber input from a single subdivision of the inferior olivary complex and send their output to a single deep cerebellar nucleus. The question arises as to how this precise pattern of connectivity is achieved during prenatal development. A simple hypothesis states that differences in connectivity between zones are related to differences in time of cell generation in neurons destined for those zones. Furthermore, this hypothesis requires that there is an orderly temporal sequence of neuron generation in cerebellar cortical zones and the deep nuclei and inferior olivary subdivisions to which they are related, coupled with an orderly sequence of axon outgrowth and synapse formation. Accordingly, the earliest-generated inferior olivary subdivision would form connections with Purkinje cells in the earliest-generated cerebellar cortical zone. To test this hypothesis, cat fetuses were exposed to tritiated thymidine (^{3}H-TdR) in utero at ages ranging from embryonic day 22 (E22) to E51 and sacrificed postnatally. Following tissue processing by standard autoradiographic techniques, the distribution of heavily labeled Purkinje cells was plotted on drawings or photographs of the cerebellum. Heavily labeled Purkinje cells were present in cerebella which had been exposed to ^{3}H-TdR on embryonic days 22 through 29, with peak Purkinje cell generation occurring around embryonic days 25-26. In cats injected with ^{3}H-TdR between E22 and E29, heavily labeled Purkinje cells are scattered across the cerebellar cortex, rather than being confined to a single cerebellar cortical zone. Since recent studies (Hickey et al., 1983, J. Neurosci. Meth. 8, 139-147) show that ^{3}H-TdR is available for approximately 4h after administration in cat fetuses, this scatter cannot be attributed to limitations of the method as applied to cats. These data therefore refute the simple hypothesis stated above by showing that individual cerebellar cortical zones are not made up of a single population of isochronously generated Purkinje cells.

The author wishes to thank Drs. Terry Hickey, Carla Shatz, and Ed Polley, who provided much of the tritiated thymidine autoradiographic material. Supported by the Sloan Foundation, the Emory University Research Fund, and NIH RR5364.

59.3 AUTORADIOGRAPHIC STUDY OF THE OLIVOCEREBELLAR PROJECTION IN ADULT NORMAL AND WEAVER MUTANT MICE. G.J. Blatt and L.M. Eisenman. Daniel Baugh Institute, Jefferson Medical College, Philadelphia, PA 19107.

The organization and topography of the olivocerebellar projection was studied in normal mice (+/+) and in weaver (wv/wv; wv/+) mutant mice. The weaver cerebellum is characterized by reduced numbers and ectopia of Purkinje cells (PCs); the primary target for incoming climbing fiber (CF) afferents. This study was designed to address the following questions: (1) will the organization of olivocerebellar afferents be altered in weaver due to the PC abnormalities? and (2) in the normal animal, does the olivocerebellar projection terminate in a series of parasagittal zones alternating with zones devoid of CF input or do CFs supply all regions of the molecular layer (ML)? To investigate these questions, single injections of ^{3}H-L-leucine were made into the inferior olivary complex (IO) in normal and weaver mice. In addition, some normal mice received either multiple injections of ^{3}H-leucine directly in the IO or multiple injections around, but not in, one side of the IO to label all or most IO cells by diffusion. The results from nine cases in +/+ mice demonstrate that olivocerebellar afferents end in terminal bands oriented orthogonal to the long axis of the folia. Many labeled bands extend across all cerebellar lobules but in some areas the label was restricted to one or a few lobules leaving other lobules devoid of label. More extensive CF terminal labeling was seen in cases where the injections were placed around the IO. A comparison of similar sections from different cases (of multiple injections) indicates that in one or another case there is label present in all parts of the ML. Unlabeled areas in individual cases appear to be the result of either cell death at the injection site or the insufficient labeling of some IO cells.

Tritiated leucine injections in wv/+ mice yielded similar results to +/+ mice. In wv/wv, single injections into the IO resulted in many band-like areas of label. The topographic organization of CF terminals was similar in all three types of mice: the caudal medial accessory olive projects to the contralateral vermis; the dorsal accessory olive projects to the contralateral paravermis; and the rostral medial accessory olive and principal olive send a projection to the opposite hemisphere and paraflocculus.

These findings suggest that (1) CFs project to PCs in all regions of the cerebellum, and (2) in the weaver mutant, exact target cell position and number do not appear to be important factors in determining the organization and topography of CF terminals.

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59.4 DISTRIBUTION PATTERN OF INFERIOR OLIVE AXON COLLATERALS WITHIN CAT CEREBELLAR STRIPS. A.Rosina* and L.Provini* (SPON: European Neuroscience Association), C.N.R., Ist. Fisiol. Centri Nervosi , Via Mario Bianco, 9, 20131 Milano, Italy.

While it is now generally accepted that the olivocerebellar axons distribute over the cerebellar cortex along parasagittally oriented strips, it is still largely obscure how the collaterals (10 on the average in the cat) of single inferior olive(IO)axons distribute within the strips.

One way to address this problem is to ascertain whether climbing fiber (CF) collaterals sustain any relation between somatotopically homologous areas of cerebellar cortex. Along this line it was shown (Rosina and Provini,Brain Res.,289:45-63,1983) that single IO neurons supply CF collaterals to pairs of face-forelimb (FL) related areas in the pars intermedia of the anterior lobe (PIAL), lobulus simplex, crus II and paramedian lobule (PML), within strips C_1 to D_2.While this mosaic type of interlobar branching involves the majority of IO neurons in each pertinent olivary subdivision, it only accounts for 2 of the 10 estimated collaterals. The residual branches could therefore distribute within a single folium and/or adjacent folia within each somatotopically homogeneous cerebellar area.The questions we then addressed were: a)do CFs branch over contiguous folia?, and b)how do these interfolial branches relate to the interlobar collaterals? To this end two spectrally different fluorescent tracers, Fast blue (2%,w/v) and Diamidino yellow (1%, w/v), were microinjected into single adjacent folia (V_b and $V_{c,d}$) of PIAL. In the same animal a third fluorescent tracer (Evans blue, 10%, w/v) was microinjected into the FL folia of PML.

The results showed that a number of IO neurons do give off branches directed to contiguous folia of V-PIAL as well as to the FL folia of PML. These data suggest that in the FL areas of strips C_1 to D_2,IO neurons give two interlobar collaterals which further split into branches directed to adjacent folia; finally a few (two to three) branches would be given within each folium. CF input from single IO neurons might thus sustain the replication of the same peripheral receptive field according to this distribution pattern.

59.5 THE GRACILE NUCLEUS PROJECTION TO THE INFERIOR OLIVE: AN ELECTRON MICROSCOPIC STUDY. H.H. Molinari. Department of Anatomy, Albany Medical College, Albany, N.Y. 12208.

The gracile nucleus is one of several sources for the inferior olive of cutaneous feedback from the hindlimb. The present study defined the mode of termination of gracile axons in the dorsal accessory portion of the cat inferior olive. The gracile terminals were labeled by anterograde transport of wheat germ agglutinin-horseradish peroxidase and visualized, after a 24 hr survival time, with tetramethylbenzidine (TMB). The tissue was prepared for electron microscopy following Carson and Mesulam (1982). Ultrathin and 0.25 µm thick sections were examined with either a JEOL 100CX or High Voltage (N.Y. State Department of Health) electron microscope. The labeling patterns were analyzed by scanning selected sections, photographing all labeled elements within those sections, and classifying the labeled elements using published ultrastructural descriptions of the inferior olive.

The TMB reaction product was largely confined to myelinated axons and synaptic terminals. Virtually all of the labeled terminals measured less than 2 µm in diameter, contained round synaptic vesicles, and formed asymmetric contacts with postsynaptic elements. Thus, in keeping with physiological data, the gracile terminals had the properties of excitatory synapses.

The majority of labeled gracile terminals contacted distal dendritic shafts. They did not participate in synaptic glomeruli. Despite this, only a third of the terminals formed isolated contacts. The majority terminated within loosely organized synaptic complexes termed dendritic thickets by Sotelo et al. (1974). The dendritic thickets were composed of three or four serially connected, distal dendritic shafts that received input from three or four different presynaptic elements. Only one presynaptic element per thicket contained TMB reaction product. The unlabeled terminals within the thickets resembled the gracile terminals in that they were also small, contained round synaptic vesicles, and formed asymmetric contacts with the dendritic shafts.

Dendritic thickets provide a means by which postsynaptic elements within the inferior olive might simultaneously receive input from a number of presynaptic elements and through which interactions between postsynaptic elements might occur. It is suggested that the convergence of somatosensory inputs in the inferior olive, demonstrated with physiological techniques by Oscarsson and Sjolund (1977), occurs within dendritic thickets.

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59.6 ANATOMY AND PHYSIOLOGY OF DORSAL COLUMN AFFERENTS TO FORELIMB DORSAL ACCESSORY OLIVE. C. Weiss, M.L. McCurdy, J.C. Houk and A.R. Gibson. Neuroscience Program and Department of Physiology. Northwestern University, Chicago, Ill. 60611

The origin of forelimb afferents from the dorsal column nuclei to the dorsal accessory olive (DAO) has been elusive. Using WGA-HRP we have identified these cells as forming a narrow column, 0.6 mm wide, which extends from the dorsal caudal cuneate nucleus (CCN) to the rostro-ventral CCN. This region provides dense input to the DAO.

Using bipolar stimulation (.1ms pulses) of the forelimb DAO we located antidromically activated cells within the CCN. Only cells showing low threshold activation and collision were studied. Thus far we have data on 22 cells that have been histologically verified as being within the column of cells that project to DAO. They had a mean antidromic threshold of 17.8uA (SD=11.6uA) and a mean antidromic latency of 1.67ms (SD=.94ms). A majority of these cells(N=13) had zero spontaneous activity or the low irregular firing frequency characteristic of olivary neurons.

These cells were activated by stimulation of hairs(N=9) and by deep rapidly adapting(N=3) and slowly adapting(N=2) receptors, or they were inhibited(N=2). Six cells could not be driven. The majority of receptive fields(N=12) were on the distal paw, mostly the dorsal surface. The others were on the proximal forelimb, shoulder or back. The majority of cells had simple responses, a few had more complex properties such as prolonged excitation, post excitatory inhibition and surround inhibition.

We have also found a reciprocal connection between this narrow column of cells in the CCN and the forelimb area of the magnocellular red nucleus(RNm). To determine if input from the RNm mediates inhibition of olivary activity, which occurs during movement (Gellman et al., Soc. Neuro. Abs. 9:607,'83), we stimulated either the RNm or rubrospinal tract and looked at the effects on DAO unit activity. We found that 50-200ms trains of electrical stimuli (200-400Hz, .2ms), to either the nucleus or tract (within the brainstem), inhibited the DAO response to a peripheral stimulus delivered approximately 30ms later. All 16 rostral DAO cells were inhibited by this conditioning stimulation ($\bar{X}$=37.0uA, SD=24.8uA).

We conclude that the input from the dorsal column nuclei to the forelimb DAO arises from a restricted region of the caudal cuneate nucleus, and that the cells of this region demonstrate receptive field properties similar to DAO cells (Gellman et al., JCN 215: 228-243,'83). Input from the rubrospinal tract (probably via a collateral) to the vicinity of the DAO projecting cells, may provide the anatomical basis for electrophysiological as well as behavioral suppression of DAO responses.

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59.7 MAPPING THE CLIMBING FIBER PROJECTION FROM THE BETA NUCLEUS TO THE UVULA-NODULUS OF THE CEREBELLUM OF THE RABBIT. N. H. Barmack and Y. Sato*. Neurological Sciences Institute, Department of Ophthalmology, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

The beta nucleus comprises the caudal-medial aspect of the medial accessory olive; lying ventromedially to the dorsal cap of Kooy, the major source of visual climbing fiber projections to the cerebellar flocculus. The beta nucleus is of functional interest for two reasons: 1) It receives information of otolithic origin from the vestibular nuclei, and 2) It is the recipient of a major GABAergic projection, the origin of which is at present undetermined. Previous investigations have demonstrated a projection of the beta nucleus onto the nodulus and uvula of rat, cat and monkey. As a first step in understanding the significance of olivo-cerebellar circuitry mediating otolithic information, using retrograde tracers, we have studied the pattern of olivary projections in the rabbit onto the uvulo-nodulus; with the particular aim of describing the projections from the beta nucleus.

Small and large (40nl-120nl) pressure injections of HRP or fluorescent retrograde tracers (fast blue or 2,4-diamidino nuclear yellow) were injected into one or several folia of the uvula and nodulus in 20 rabbits at different medio-lateral locations. The topographic patterns of contralateral climbing fiber projections onto the cerebellar surface reveal longitudinally oriented, transfolial strips.

The caudal beta nucleus projects onto the most medial aspect of the uvula-nodulus. The rostral part of the beta nucleus projects more laterally. Together these strips comprise a longitudinal band which is approximately 1.3-1.4mm wide. Further laterally on the cerebellar surface is another longitudinal strip projection from the caudal-lateral medial accessory olive, an olivary area from which vestibularly-evoked activity has also been evoked. Still more laterally, about 2mm from the midline is a projection from the dorsomedial cell column. The ventral lamellae of the principal olive projects to a strip which is about 3.5mm from the midline, and the rostrolateral MAO projects to the most lateral region of the uvula-nodulus. These findings indicate the medial 1.5mm of the posterior cerebellar vermis may be involved in processing vestibular as well as other information mediated by climbing fibers.

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59.8 LOSS OF GABAERGIC NERVE TERMINALS IN THE INFERIOR OLIVE OF CEREBELLECTOMIZED RATS. B. Nelson and E. Mugnaini. Lab. of Neuromorphology, Univ. of Conn., Storrs, CT 06268.

Nerve terminals immunoreactive for the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), are densely distributed throughout all subnuclei of the mammalian inferior olive (IO). In rat, small GABAergic nerve terminals (0.5-1.0 um) are present in principal olive (PO), dorsal accessory olive (DAO), and medial accessory olive (MAO). Large, densely stained GABAergic terminals (up to 2.5 um) are distributed in the ß nucleus, while dorso-medial cell column and caudal portion of DAO contain GABAergic terminals of intermediate size and density. One source of olivary GABAergic terminals are small neurons situated in the cerebellar nuclei (Nelson et al., 1984). The present study is an attempt to ascertain the extent of the GABAergic cerebello-olivary projection by the loss of GAD-immunoreactive nerve terminals induced by cerebellectomy. Sprague-Dawley rats (150-450 g b.w.) were divided into four groups: 1) unoperated rats; 2) totally cerebellectomized rats; 3) hemicerebellectomized rats; and 4) rats subjected to large cerebellar cortical ablations without damage to the cerebellar nuclei. Ablations were made without apparent damage to vestibular nuclei. After 4, 10, and 21 days survival, rats were perfused transcadially with a low pH zinc-aldehyde fixative. Serial sections of the brainstem were obtained on a freezing microtome. Sections were processed for GAD-immunoreactivity with the PAP method using sheep antiserum S3 of Oertel et al., 1981. Within 10 days, total cerebellectomy results in conspicuous bilateral reduction of GAD-immunoreactive nerve terminals in areas known to receive the cerebello-olivary projection, i.e., PO, DAO, and rostral MAO. This terminal loss is contralateral to the aspirated side in unilaterally cerebellectomized rats. A small reduction of GAD immunoreactive nerve terminals perhaps occurs in caudal MAO ipsilaterally. After 21 days the GAD immunoreactivity is partially recovered. The observed loss of GABAergic nerve terminals in the IO may be the result of the removal of the source nuclei of the GABAergic cerebello-olivary projection and/or of a retrograde olivary cell response to severance of the climbing fibers. Removal of large areas of cerebellar cortex without damage to the cerebellar nuclei fails to elicit loss of GAD immunoreactivity in IO, but climbing fiber collaterals projecting to the undamaged cerebellar and vestibular nuclei may prevent a retrograde effect. Complete bilateral cerebellectomy does not produce the same effect throughout IO. Beta nucleus, dorsal cap, and medial DAO remain unchanged in spite of an expectable retrograde effect. Experiments are in progress to further elucidate these observations, and to investigate the nature of the recovery of GAD-immunoreactivity observed 21 days after cerebellectomy.

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59.9 GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN THE DEEP CEREBELLAR NUCLEI FOLLOWING CLIMBING FIBER LESIONS OR IN DYSTONIA. G.A. Oltmans, J.F. Lorden and M. Beales*. Dept. of Pharmacology, Chicago Med. Sch., N. Chicago, IL 60064 and Dept. of Psychology, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The inferior olive(IO)-climbing fiber(CF) input to the cerebellar cortex constitutes one of the two major excitatory inputs to the cerebellar Purkinje cell(PC). Activation of the IO-CF system by the drug harmaline produces increased complex spike activity in the PCs, an increase in cerebellar cGMP, and behavioral tremor. Removal of the CF input to the PCs by destruction of the IO with the toxin 3-acetylpyridine (3AP) results in an absence of complex spikes, a decrease in cerebellar cGMP, and a block of the behavioral responses to harmaline. The 3AP lesion also produces multiple motor dysfunctions.

Motor abnormalities similar in part to those found following 3AP lesion are seen in the genetically dystonic rat (genotype dt). This mutant also has decreased cerebellar cGMP levels and is refractory to the tremorogenic effects of harmaline (Lorden et. al., 1985, J. Neurosci.), suggesting a similarity to animals with CF lesions. The dt rat also has increased levels of glutamic acid decarboxylase (GAD) activity in the projection area of the Purkinje cells, the deep cerebellar nuclei (Oltmans et. al., 1984, Exp. Neur.,85:216). The purpose of the present study was to determine if lesions of the CF system in normal animals would produce a change in GAD activity in the deep cerebellar nuclei. In addition, GAD activity in specific cerebellar nuclei of the dt rat was measured to determine if this neurochemical change was localized to a specific site.

To produce CF destruction, normal 16-day old rats were injected wtih 65 mg/kg of 3AP. The animals were then killed 4 hr., 24 hr., 14 days, or 28 days later, and GAD activity determined in the cerebellar nuclei and vermis. There was a significant increase (115% of control) in GAD activity in the deep nuclei at 24 hr. post-treatment. This increase became larger with time, reaching 134% of control at day 28. A significant increase in GAD activity in the vermis was found only on day 28, and this increase was smaller (114% of control) than that found in the deep nuclei.

Analysis of GAD activity in specific cerebellar nuclei of the 16 day old dystonic rat was accomplished using a modification of the "punch" technique of Palkovits (Br. Res., 59:449, 1973). A significant increase in GAD activity was found only in the nucleus interpositus(125% of control). No significant change was found in either the lateral or medial cerebellar nuclei.

The results indicate the presence of increased GAD activity in the cerebellar nuclei of both dystonic rats and rats with CF lesions. Differences in their motor syndromes may be due to the specific sites of neurochemical change. (Supported by NS 18062).

59.10 ULTRASTRUCTURAL CHANGES OF THE NERVE TERMINALS IN THE CEREBELLAR NUCLEI FOLLOWING PURKINJE CELL DEPRIVATION OF THEIR CLIMBING FIBER INPUT. P. Strata, F. Rossi and D. Cantino (SPON: European Neuroscience Association). Inst. of Human Physiol. and Inst. of Human Anatomy, University of Turin, 10125 Turin, Italy.

It has been reported that following inferior olive lesion, Purkinje cell inhibitory function is reduced. This effect may be the consequence of a loss of a throphic factor carried along the climbing fiber input (Ito et al., Nature 277: 568-569, 1979) or the consequence of an increased Purkinje cell activity (Montarolo et al., J. Physiol. 332: 187-202, 1982; see Strata P. In: Cerebellar Function, Bloedel et al. Eds. Springer, Heidelberg, 1984). We examined the intracerebellar nuclei following inferior olive lesion, in order to recognize possible morphological changes in the Purkinje cell axon terminals.

The inferior olive of adult female Wistar rats was lesioned by means of 3-acetylpyridine and the cerebellum was processed for electron microscopy 3 days to 6 months after lesion. A quantitative analysis of the axon terminals exhibiting a series of ultrastructural changes was made in the dentate nucleus. For each animal, we counted the number of altered terminals in 10 squares, each measuring 90x90 μm, randomly chosen according to the method described by Weibel (Stereological methods, Academic Press, London, 1979).

We have counted the axon terminals characterized by the presence of i) clusters of vesicles, ii) large vacuoles, iii) elongated cysternae of the smooth endoplasmic reticulum, iv) whorled bodies. The number of altered axons for each class was remarkably higher than in control material and depended on the time interval from the lesion. Axon terminals of class i), ii) and iii) increased in number up to day 15, and decreased until 6 months. In contrast, those characterized by whorled bodies showed an increase up to 6 months.

These results will be discussed in relation to the functional modifications described above.

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59.11 EVIDENCE FOR ENKEPHALINERGIC CLIMBING FIBERS IN THE OPOSSUM'S CEREBELLUM J.S. King, R.H. Ho and G.A. Bishop. Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210.

Previous investigations suggest that climbing fibers contain an amino acid transmitter (likely aspartic acid) and arise from the inferior olivary complex. In the present study, adult opossums were perfused with Zamboni's fixative, and their brains serially sectioned on a freezing microtome in the transverse and sagittal planes. The sections were processed for the localization of enkephalin immunoreactivity using the indirect antibody peroxidase-antiperoxidase (PAP) technique. Enkephalin-like immunoreactivity is present in the cerebellum within axons that have the anatomical characteristics of climbing fibers. The parent fiber branches in the lower third of the molecular layer giving rise to fine beaded axons that either course at right angles or in parallel to the parent axon. Enkephalingeric climbing fibers are not present throughout the cerebellum, but are limited to restricted areas of cortex in vermal lobules II-VIII and X, the medial paramedian lobule, the paraflocculus and the flocculus. Brains of other animals were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde, sectioned on a vibratome at 60 um and processed for electron microscopic immunohistochemistry. Serial thin sections (90 nm) reveal enkephalinergic profiles that approximate Purkinje cell dendrites from the level of the Purkinje cell bodies to the area just beneath the pial surface. The labeled terminals contain clear synaptic vesicles and make multiple synaptic contacts on the thorns of Purkinje cells. In order to localize the cell bodies of origin for enkephalin positive axons, horseradish peroxidase (HRP) was injected into the cerebellum. After a survival time of 24-36 hours the animals received intraventricular injections of colchicine. Twelve to twenty-four hours later, the animals were perfused with 3.5% paraformaldehyde and their brains processed for HRP histochemistry followed by enkephalin immunohistochemistry. Neurons retrogradely labeled with HRP are located in all precerebellar nuclei as well as in several reticular and raphe nuclei. Enkephalinergic neurons are present primarily in the reticular formation rostral to the inferior olive, (gigantocellular and the parvicellular reticular nuclei), several raphe nuclei and the periaqueductal gray. In addition, a population of enkephalinergic neurons is located immediately lateral to the rostral dorsal accessory olivary nucleus, adjacent to the ventral brain surface. Neurons that are both retrogradely labeled with HRP and demonstrate enkephalin immunostaining have only been observed in this latter region and the nucleus reticularis gigantocellularis. To date, no enkephalinergic or double-labeled neurons have been seen in the inferior olivary complex although a dense plexus of enkephalinergic varicosities is present. These data in the opossum suggest that: 1) climbing fibers are not uniform in their (putative) transmitter content and 2) enkephalin which is inhibitory in other brain areas is present in cerebellar axon terminals which are well-characterized physiologically as excitatory. (Supported by NS-08798).

60.1 SELECTIVE EFFECT OF AMIFLAMINE, A REVERSIBLE MONOAMINE OXIDASE INHIBITOR, ON SEROTONERGIC NEUROTRANSMISSION. P. Blier, C. de Montigny and A.J. Azzaro. Université de Montréal, Canada and University of West Virginia, Morgantown, USA.

Amiflamine, a reversible type A monoamine oxidase inhibitor (MAOI), has been reported to exert a preferential effect on serotonin (5-HT)-containing neurons (Ask et al., Neuropharmacology 21: 299, 1982). Pilot observations suggest that amiflamine might be effective in major depression.

Male Sprague-Dawley rats received amiflamine (2 mg/kg, i.p.) twice daily for 2, 7 or 21 days. Biochemical determinations and electrophysiological experiments were carried out 2-6 h after the last dose of amiflamine. Whole brain concentrations of 5-HT and norepinephrine (NE) were increased respectively by 62% and 29% after 2 days, by 52% and 40% after 7 days, and by 89% and 40% after 21 days of treatment.

Unitary extracellular recordings of 5-HT and NE neurons were obtained from the raphe dorsalis and the locus coeruleus under chloral hydrate anesthesia (400 mg/kg, i.p.). The two-day amiflamine treatment markedly reduced the firing activity of 5-HT neurons. However, there was a partial recovery of their activity, after 7 days of treatment, and the recovery was complete after 21 days of treatment. This recovery was attributable to a desensitization of the 5-HT autoreceptor indicated by a decreased responsiveness of 5-HT neurons to LSD, a 5-HT autoreceptor agonist. In contrast, the firing activity of NE neurons was normal after 2 days of treatment but decreased progressively with the prolongation of the treatment. The responsiveness of NE neurons to the α_2 agonist clonidine was unchanged after 21 days of treatment, indicating that the NE autoreceptor had not desensitized.

The responsiveness of hippocampal pyramidal neurons to microiontophoretic applications of 5-HT and NE and the effect on the activity of these neurons of the electrical activation of the ventromedial 5-HT pathway and of the dorsal NE bundle were assessed in control rats and in rats treated with amiflamine for 21 days. The responsiveness of pyramidal neurons to exogenous 5-HT and NE was unchanged by the treatment. However, the efficacy of the stimulation of the ascending 5-HT pathway was increased by amiflamine, whereas that of the stimulation of the NE pathway was not modified.

These data confirm the preferential effect of amiflamine on 5-HT neurons resulting in a selective enhancement of 5-HT neurotransmission without any modification of the postsynaptic neuron responsiveness. Hence, should the antidepressant activity of amiflamine in major depression be confirmed, this would provide novel evidence supporting the hypothesis that an increase of 5-HT neurotransmission might contribute to the therapeutic activity of antidepressant treatments.

60.2 EFFECTS OF TRAZODONE ON ALPHA-2 ADRENOCEPTOR FUNCTION IN DEPRESSION. L.H. Price, D.S.Charney*, G.R.Heninger, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT., 06508.

Based on preclinical findings, it has been hypothesized that antidepressant drugs may exert their therapeutic effects by reducing the sensitivity of alpha-2 adrenoceptors during long-term treatment. Clinical studies have demonstrated this property for the tricyclic antidepressants desipramine and amitriptyline. Mianserin, an atypical tetracyclic drug, lacked this effect. In the present investigation alpha-2 adrenoceptor function was measured before and during treatment with the atypical triazolopyridine antidepressant trazodone.

METHODS: 11 patients with DSM III major depression gave voluntary informed consent to participate. Alpha-2 adrenoceptor function was assessed by measuring the ability of the alpha-2 agonist clonidine to decrease plasma 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG) and blood pressure, and to increase sedation and plasma growth hormone. After a minimum 2-week placebo period (at least 3 weeks psychotropic drug-free), patients underwent 2 double-blind test days. Placebo was given the first day and clonidine 5 ug/kg p.o. was given the second day. The test procedures were repeated after 4 weeks of active trazodone treatment. Clinical change was assessed with the use of rating scales. A gas chromatography mass spectrography method was used to measure MHPG and an RIA method to measure growth hormone.

RESULTS: Clonidine-induced changes in MHPG, blood pressure, sedation, and growth hormone were not significantly affected by trazodone. Trazadone treatment was associated with a small (.2 ng/ml) nonsignificant decrease in baseline plasma MHPG. There was no clear difference between the patients whose symptoms improved during trazodone treatment and the nonimproved patients on any of the measured variables or the effects of clonidine before or after trazodone treatment.

CONCLUSION: Long-term clinical treatment with trazodone has no clear effect on pre- and postsynaptic measures of alpha-2 adrenoceptor function. Since some patients improved during trazodone treatment this study suggests that trazodone, like some other atypical antidepressants, does not exert its therapeutic effects via changes in alpha-2 adrenoceptor sensitivity. Trazodone acts as an antagonist at serotonin receptors and one of its metabolities (m-chlorophenylpiperazine) acts as a serotonin agonist. The therapeutic mechanism of action of trazodone may therefore predominantly involve the serotonin system and not the noradrenergic system.

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60.3 THE EFFECTS OF ANTIDEPRESSANTS, WHICH ATTENUATE CLINICAL ANXIETY ASSOCIATED WITH DEPRESSION, IN TWO ANTIANXIETY PROCEDURES IN RATS, D. Luttinger and P. A. Mason.* Dept. of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144

Several antidepressants have been reported to alleviate anxiety associated with depression. In the present studies we examined the effect of five antidepressants, with purported antianxiety properties, in two operant procedures used to assess antianxiety activity in water deprived male Sprague-Dawley rats. One procedure was a metrazol drug discrimination procedure (Shearman and Lal, Neuropharmacol, 1980). In this paradigm rats are trained to discriminate between saline and metrazol (15mg/kg, i.p.). Antianxiety compounds such as chlordiazepoxide routinely inhibit metrazol discrimination in a dose related fashion. Mianserin (1-10 mg/kg), trazodone (0.1-30 mg/kg), amoxapine (3-30 mg/kg), maprotiline (1-10 mg/kg), and doxepin (0.1-10 mg/kg) injected i.p. did not affect metrazol discrimination, suggesting that these compounds do not produce demonstrable antianxiety activity in this procedure. This may be due, in part, to the observation that when the antidepressant was administered at the highest dose tested, with saline instead of metrazol, 25-50% of the rats choose the drug lever; suggesting that the antidepressants possess some metrazol like discriminable properties. The second procedure was a punishment procedure (Cook and Davidson, The Benzodiazepines, 1973) with water used as the positive reinforcer and electric footshock (0.6 mA) as the punishing stimuli. Mianserin (3-30 mg/kg), trazodone (0.1-30 mg/kg), amoxapine (0.3-10 mg/kg), maprotiline (3-30 mg/kg) and doxepin (0.1-10 mg/kg) were injected i. p. sixty minutes before testing. Trazodone, amoxapine, maprotiline and doxepin did not demonstrate any antianxiety activity in this procedure. Mianserin (3 mg/kg) tended to increase punished responding, suggesting antianxiety activity, though the magnitude of this effect was not as large as that observed with chlordiazepoxide.

In summary, in two procedures that detect antianxiety activity in rats, antidepressants with purported antianxiety activity were not active. Three possible reasons for the lack of effect of the antidepressants are a) anxiety reduced by these antidepressants may be associated with depression and distinct from the anxiety routinely treated with the benzodiazepines, b) these antidepressants may work by a unique mechanism for the alleviation of anxiety which is not detected by metrazol discrimination or punishment procedures, or c) these antidepressants may only work at reducing anxiety when administered on a chronic basis.

60.4 5-HYDROXYTRYPTOPHAN ALTERATION OF ENDOGENOUS OPIOIDS: IS IT RELATED TO THE CLINICAL EFFECTS OF THE DRUG ? E. Parati, G. Bussone*, A. Groppetti*, M. Parenti*. Inst. Neurol. "C. Besta", and Dept. of Pharmacol., Univ. of Milan, Milano, Italy.

Drugs affecting serotonergic neuronal activity are often used in the clinical treatment of migraine.

In our hands the administration of 5-hydroxytryptophan (5-HTP) to patients affected by common migraine ameliorated the clinical symptoms.

In the attempt to potentiate the therapeutic efficacy of 5-HTP, the peripheral catabolism of the drug was decreased by the concomitant administration of the inhibitor of L-aromatic aminoacid decarboxylase carbidopa (CAR). In fact, while only a modest raise of plasma 5-HTP levels was observed when the drug was given orally at the dose of 100 mg to normal or migraineous subjects, a dramatic increase of plasma drug concentrations (about 10 fold) occurred after treatment with 5-HTP plus CAR (10 mg). However the association induced a marked nausea and vomiting in all the subjects shortly after the treatment. The appearance of these side effects was significantly delayed by the slow intravenous infusion of naloxone (16 mg in an hour) but was not affected by the administration of the dopamine antagonist domperidone (20 mg in an hour) suggesting that endogenous opioids could be affected by 5-HTP. Indeed interactions between serotonergic and opioid neuronal systems have been already reported.

In this context we have found that, in rats, the i.p. administration of 5-HTP, in combination with CAR (25 mg/kg 5-HTP; 2.5 mg/kg CAR) strongly enhanced serotonin (5-HT) metabolism in different brain areas such as hypothalamus (see Table), striatum and brainstem. On the contrary met-enkephalin-like immunoreactive material (ME.IR) concentrations were decreased in all these regions.

Further investigations are needed to establish whether the clinical effects of 5-HTP are related to endogenous opioid modifications.

	HYPOTHALAMIC CONTENT		
	5-HT	5-HIAA	ME.IR
	(ug/g$\pm$SE)	(ug/g$\pm$SE)	(ug/g$\pm$SE)
CONTROLS	0.85 ± 0.05	0.58 ± 0.04	0.94 ± 0.04
5-HTP+CAR	1.64 ± 0.11*	4.86 ± 0.39*	0.78 ± 0.03*

* $p < 0.01$ vs control

60.5 WY-45,030, A NEW ANTIDEPRESSANT, AND ZIMELIDINE DECREASE 5-HIAA LEVELS IN WHOLE RAT BRAIN. E.A. Muth and K.M. Cilmi*. Wyeth Laboratories, Division of Experimental Therapeutics, Philadelphia, PA 19101.

The novel antidepressant compound, Wy-45,030, possesses monoamine uptake inhibitory properties. The compound potentiates the behavioral actions of L-DOPA in rats, antagonizes reserpine in mice, is active in vitro vs. the rat brain synaptosomal uptake of norepinephrine (NE; IC50=0.64 μM), serotonin (5-HT; IC50=0.21 μM), and dopamine (DA; IC50=2.8 μM), but lacks affinity for alpha-1/alpha-2/beta adrenergic, muscarinic cholinergic, histaminic, or opiate receptors (Muth et al. and Moyer et al., Neuroscience Abstracts 10:261, 1984). In addition, the compound caused a rapid (24 h) beta noradrenergic subsensitivity in the pineal model (Moyer et al., ibid.)

In the present follow-up study, we compared the in vivo biochemical effects of Wy-45,030 to those of desipramine (DMI), imipramine (IMI), and zimelidine (ZIM). Each compound was administered at 10 mg/kg i.p. either acutely or once a day for 5 days (also b.i.d. for 18 days for Wy-45,030). Two hours after the last injection, rats were decapitated and monoamines and their major metabolites were measured by reverse-phase HPLC with electrochemical detection. In whole rat brain minus the cerebellum, neither IMI nor DMI, after acute or chronic dosing, caused any significant changes in the concentration of any of the neurochemicals monitored, although IMI had a tendency to decrease the concentration of the serotonin metabolite, 5-hydroxyindole acetic acid (5-HIAA). In contrast, both ZIM and Wy-45,030 decreased 5-HIAA levels by 31% after acute treatment and by 43% and 35%, respectively, after 5-day dosing. Wy-45,030 also decreased 5-HIAA content by 21% after the 18-day b.i.d. regimen. These results suggest that Wy-45,030 shares with ZIM the ability to slow the turnover of 5-HT without affecting NE metabolism, despite the fact that ZIM, but not Wy-45,030, is a specific 5-HT uptake inhibitor.

60.6 PR 903-574:BIOLOGICAL PROFILE OF A NOVEL NON-TRICYCLIC AS A POTENTIAL ANTIDEPRESSANT. S.McCreedy*, J.Blosser, J.M. Ordy, J. Frankenheim*, R.Griffith*, and B. Watkins* Depts. of Pharmacol., Chem., Pennwalt Corporation, Pharmaceutical Division, Rochester, NY 14623.

PR 903-574A, a halogenated isoquinoline derivative modeled after mianserin (Griffith et al., J. Med. Chem. 27:995, 1984), exhibited CNS and biochemical properties predictive of anti-depressant activity. Like most clinically active antidepressants, PR 903-574A antagonized tetrabenazine (TBZ) induced ptosis in mice; it exhibited a minimal effective dose (2 mg/kg) intermediate between that of mianserin (10 mg/kg) and imipramine (<0.5 mg/kg). The effect of PR 903-574A on adrenergic receptor function in rat frontal cortex following chronic (14 days) but not acute treatment was also indicative of antidepressant activity. PR 903-574A (30 mg/kg) as well as mianserin (30 mg/kg) and imipramine (15 mg/kg) decreased norepinephrine (NE) sensitive adenylate cyclase (AC) activity by 40-50%. Similar chronic treatment had little effect on β-adrenergic receptor number in rat whole cortex. PR 903-574A produced small (10%) decreases in receptor number as did mianserin (12%). This contrasted with imipramine treatment which produced marked reductions of 25-30%. There were no changes in receptor affinity. PR 930-574A demonstrated an affinity for histamine-2 receptor sites that was comparable to that of other tri- and tetracyclic agents including mianserin. EEG power spectra of PR 903-574A was consistant with that of other antidepressants. A 60 min analysis of EEG power (picowatts) time curves with respect to pretreatment baselines following 1 mg/kg iv dose in beagle dogs showed that imipramine increased total power maximally at 15 minutes while mianserin produced a lesser effect which was maintained over the entire period. Total power spectra of PR 903-574A exhibited elements of both reference agents; that is, a dose response characteristic of mianserin and a time response characteristic of imipramine. Certain aspects of the biochemical profile of PR 903-574A were dissimilar from other classes of anti-depressants. PR 930-574A did not inhibit total monoamine oxidase (MAO) or MAO-A activity in vitro or in vivo following acute or subacute treatment. Unlike many tricyclic agents, PR 903-574A weakly inhibited NE and dopamine uptake and had no effect on serotonin (5HT) uptake in vitro. Despite structural similarities with mianserin, PR 903-574A demonstrated very low affinity for α_2 adrenergic and 5HT-2 binding sites in vitro. Based on the antagonism of TBZ induced ptosis, the inhibition of NE sensitive AC following chronic treatment, and characteristic EEG spectra PR 903-574A appears to have potential as a new antidepressant agent. The lack of effect on amine transport and low affinity for α_2 adrenergic and 5HT-2 receptors suggest that its mechanism of action differs from that of tricyclic antidepressants and mianserin.

60.7 A NOVEL COMPOUND, AMPEROZIDE (FG 5606), ACTIVATES LOCUS COERULEUS NEURONS AND INHIBITS DORSAL RAPHE NEURONS IN VIVO. J.T. Haskins, D.E. Jones*, G.J. Zingaro* and E.A. Muth. Wyeth Laboratories, Inc., Dept. of Experimental Therapeutics, Philadelphia, PA 19101.

A novel compound, amperozide (Ferrosan FG 5606; 4-[4,4-bis(4-flurophenyl)butyl]-N-ethyl-1-piperazine carboximide, hydrochloride) has in vivo and in vitro activities indicating a diverse preclinical profile. Amperozide (AMPZ) has atypical neuroleptic activity including blockade of spontaneous exploratory behavior in mice and inhibition of conditioned avoidance response in rats, but no cataleptogenic activity and no effect on amphetamine-induced stereotypies.

AMPZ is also reported to be an antidepressant compound as a result of its inhibition of rat muricidal behavior, reduction of immobility in behavioral despair and moderate activity in blocking serotonin-dependent behavior in mice. The anxiolytic activity of AMPZ is reflected in its suppression of anxiety induced by an approach avoidance conflict test in rats and inhibition of aggression in isolated male mice.

Biochemically, we found AMPZ to be a ligand of only moderate affinity at limbic dopamine-2 receptors (Ki=190 nM). The compound was weaker in binding to alpha-1, serotonin-1 and alpha-2 receptors, with Ki's of 0.3, 2.7, and 3.5 μM, respectively. Interestingly, AMPZ was most potent vs. serotonin-2 binding, with a Ki of 43 nM. AMPZ is also a monoamine uptake inhibitor, with IC50's of 2.1 μM vs. norepinephrine and 0.7 μM vs. serotonin.

Considering the diverse biochemical and behavioral actions of AMPZ, it was of interest to determine the acute electrophysiological effects of AMPZ on noradrenergic and serotonergic neuronal activity. Neurons were recorded with a single-barreled electrode and observed during intravenous (lateral tail vein) or intraperitoneal administration of increasing doses of AMPZ. Intravenous administration of low doses of AMPZ (0.5 mg base/ml saline) resulted in a rapid acceleration of locus coeruleus (LC) neuronal activity. Cumulative doses of AMPZ up to 0.6 mg/kg produced increases in activity from 218 to 313% of predrug activity in all cells tested. Additional doses of AMPZ, up to 1.4 mg/kg, caused only minimal increases (approximately 20%) in LC firing rate. Intraperitoneal administration of AMPZ (10 mg/kg) caused a rapid reduction in serotonergic neuronal activity in the dorsal raphe nucleus. Neuronal firing rate was reduced to about 30% of baseline activity 8-9 minutes post-injection. By 25 minutes after the i.p. injection neuronal activity had usually dropped to less than 10% of baseline activity. In view of the behavioral, biochemical and electrophysiological actions of AMPZ, it seems likely that interactions with serotonergic and noradrenergic systems play an important role in its mechanism of action.

60.8 BUPROPION (WELLBUTRIN®) INHIBITS THE FIRING RATES OF NORADRENERGIC BUT NOT SEROTONERGIC NEURONS. V.K. Shea* and C.M. Wang* (SPON: J. L. Howard), Dept. of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Bupropion is a clinically effective antidepressant with novel neurochemical and neuropharmacological properties. While active in animal antidepressant models (ED_{50} ≈ 10 mg/kg i.p.), bupropion fails to inhibit monoamine oxidase and, compared to tricyclic antidepressants, is a weak inhibitor of norepinephrine (NE) uptake and a very weak inhibitor of serotonin (5-HT) uptake in vitro. Bupropion also weakly inhibits the uptake of dopamine (DA) in vitro and also in vivo at high doses (ID_{50} ≈ 45 mg/kg i.p.). Consistent with this weak inhibition of DA uptake, bupropion was found to suppress the firing rates of DA neurons at high doses (ID_{50} ≈ 45 mg/kg i.p.)(Shea and Wang, Neurosci. Abst., 1984).

Since tricyclic antidepressants and MAO inhibitors typically suppress the firing rates of 5-HT and/or NE neurons, we examined the effects of bupropion on 5-HT neurons in the dorsal raphe nucleus and on NE neurons in the locus coeruleus in the chloral hydrate anesthetized rat by conventional extracellular recording techniques.

Injection of bupropion did not alter the firing rates of 5-HT neurons up to a total cumulative dose of 12 mg/kg i.v., or when administered i.p. at a dose of 25 mg/kg. However, the firing rates of NE neurons were consistently suppressed by bupropion with an ID_{50} of about 4 mg/kg by the i.v. route. By the i.p. route, bupropion suppressed the firing rates of NE neurons in a dose-dependent manner with an ID_{50} of approximately 14 mg/kg. Suppression of NE neuronal firing rates by bupropion was rapidly reversed by yohimbine (0.25 - 1.0 mg/kg i.v.).

It is unlikely that bupropion's ability to suppress NE neuronal firing rates is secondary to inhibition of NE uptake because bupropion only weakly inhibits NE uptake in vitro and only slightly inhibits NE uptake at doses of 100 mg/kg i.p. in vivo (Ferris et al., J. Clin. Psychiat. 44:74-78, 1983). While bupropion's ability to suppress NE neuronal firing rates cannot be attributed to inhibition of NE uptake or MAO inhibition, the fact that suppression of NE firing rate occurred with a potency by the i.p. route comparable to bupropion's antidepressant potency suggests an unusual noradrenergic link in its antidepressant activity.

60.9 DISSOCIATION OF BEHAVIORAL AND BIOCHEMICAL INDICES OF CORTICAL $5HT_2$ RECEPTOR SENSITIVITY AFTER CONTINUOUS GEPIRONE (BMY 13805) TREATMENT. A.S. Eison and F.D. Yocca. Preclinical CNS Research, Pharmaceutical Research and Development Division of the Bristol-Myers Company, Evansville, IN, 47721.

Gepirone, known chemically as 4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedione hydrochloride (BMY 13805) is a novel nonbenzodiazepine compound with activity suggestive of anxiolytic potential. Recent behavioral and electrophysiological studies have demonstrated that, acutely, gepirone interacts with brain serotonin systems. Gepirone induces a behavioral serotonin syndrome in rats and the drug's anticonflict activity is significantly attenuated in rats depleted of serotonin by the neurotoxin 5,7-dihydroxytryptamine (Eison et al., Soc. Neurosci. Abs. 9:436, 1983). Systemic administration of gepirone potently inhibits the activity of serotonin-containing dorsal raphe neurons (Eison et al., Soc. Neurosci. Abs. 10:259, 1984).

In addition to acute effects consistent with an anxiolytic preclinical profile, gepirone administered chronically induces behavioral and neurochemical alterations common to clinically-effective antidepressants. Both chronic (oral) and continuous (subcutaneous) administration of gepirone for 28 days decreased rat cortical $5HT_2$ receptors (Bmax) and reduced the frequency of head shakes induced by the serotonin agonist, quipazine (Eison et al., 1984; Eison and Yocca, Eur. J. Pharmacol., 1985, in press). Chronic administration of antidepressant drugs, which reduce the number of cortical $5HT_2$ receptors, also reduce the head shake response associated with $5HT_2$ receptors (Lucki et al., Soc. Neurosci. Abs. 10: 1067, 1984).

In the present study, the time course for gepirone's effects upon behavioral and biochemical reflections of cortical $5HT_2$ receptor sensitivity were examined following continuous treatment in the rat. Gepirone was administered via continuous pumping systems (Alzet minipumps) implanted subcutaneously to deliver a daily dose of 40 mg/kg. Minipumps were removed 24 hr. prior to behavioral testing with quipazine. Animals were sacrificed at the completion of behavioral testing and binding parameters were determined in cortical homogenates using [3H]-spiperone as ligand.

Following 3, 7 and 14 days of continuous administration, gepirone significantly decreased $5HT_2$ receptor density by 17%, 18% and 23%, respectively. In contrast, an enhanced behavioral response (head shakes) was observed after 3 and 7 days treatment (161% and 200% of control, respectively) with a return to control levels at 14 and 21 days. The time course of gepirone's actions will be compared to classical antidepressant agents (tricyclics and monoamine oxidase inhibitors).

F.D. Yocca is a Bristol-Myers Postdoctoral Fellow.

60.10 THE NONBENZODIAZEPINE ANTIDEPRESSANT-ANXIOLYTIC CANDIDATE, GEPIRONE, INHIBITS SEROTONERGIC DORSAL RAPHE NEURONS IN THE RAT BRAIN SLICE. G. Gehlbach and C. P. VanderMaelen, Preclinical CNS Research, Bristol-Myers Company, Evansville, IN 47721.

Preclinical testing of the nonbenzodiazepine compound, gepirone (MJ 13805, BMY 13805), indicates this compound may be potentially useful as an antidepressant-anxiolytic agent. Gepirone interacts with brain serotonin (5-HT) systems as exemplified by its ability to produce a behavioral 5-HT syndrome in rats. Its anticonflict action is reduced in animals depleted of brain 5-HT by 5,7-dihydroxytryptamine (Eison, et al., Soc. Neurosci. Abstr., 1983,9: 436). Chronic treatment of animals with gepirone down-regulates $5HT_2$ receptors (Eison and Yocca, Eur. J. Pharmacol., in press). Gepirone binds to $5HT_1$ receptors in the hippocampus with an IC_{50} of 471 nM (D.P.Taylor, personal communication). Systemic administration of gepirone inhibits the firing of 5-HT containing neurons in the dorsal raphe (DR) nucleus (Eison et al., Soc. Neurosci. Abstr., 1984, 10:259). The present study examined the effects of gepirone on the activity of these neurons in the rat brain slice.

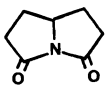
Adult male albino Sprague-Dawley rats were anesthetized with chloral hydrate and allowed to breathe 100% O_2 for at least 5 min. Frontal sections 400 μm in thickness were cut through the DR nucleus and placed in a chamber through which 95% O_2, 5% CO_2 flowed. The slices were bathed in continuously flowing artificial CSF containing in mM: NaCl, 130; KCl, 5.0; $CaCl_2$, 1.25; $MgSO_4$, 1.25; $NaHCO_3$, 24; NaH_2PO_4, 1.25; d-glucose, 10.0. Phenylephrine (PE, 10 μm) was present to provide increased neuronal activity. Gepirone in concentrations of 50, 75, 100, 125, 200 or 400 nM was added to the bath and its effect on the firing of DR neurons observed using extracellular single unit recording techniques.

Gepirone inhibited the firing of DR neurons in a manner similar to that of 5-HT and 5-methoxy-N,N-dimethyl tryptamine. This inhibition was concentration-dependent with a calculated IC_{50} of 93.5 nM. The anxiolytic, buspirone, which is structurally related to gepirone, was 5 times more potent (IC_{50}=18.9nM) in inhibiting DR cells under similar, but not identical experimental conditions. Interestingly, buspirone is also 5 times more potent than gepirone in binding to $5HT_1$ receptors in the hippocampus (IC_{50}=95nM; Riblet et al., Psychopathology, 1984, Suppl. 3, 17:69). The fact that gepirone does not bind to α receptors combined with the observation that it inhibited spontaneously active DR neurons indicates that its inhibition of PE-activated DR cells was not simply due to antagonism of α receptors.

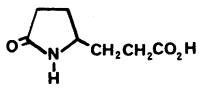
This study, combined with previous studies, suggests that the inhibitory effect of the antidepressant-anxiolytic candidate, gepirone, on DR neurons is due to a direct effect of this agent on these cells, and may involve an interaction with 5-HT receptors.

60.11 SYNTHESIS AND AMNESIA-REVERSAL ACTIVITY OF 1-AROYL-5-OXO-2-PYRROLIDINEPROPANOIC ACID DERIVATIVES: IDENTIFICATION OF CI-933 AS A POTENTIAL COGNITION-ACTIVATOR. D.E. Butler*, J.D. Leonard*, B.C. Caprathe*, R.E. Davis, J.G. Marriott, and F.M. Hershenson (Spon: R. Marriott). Depts. of Chemistry and Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

A number of simple pyrrolidone derivatives, including piracetam, aniracetam, and pramiracetam, have been reported to reverse ECS-induced amnesia in rodents, as well as exhibiting cognition-activating properties in other animal models. CI-911, 1, a pyrrolizinedione, was also recently identified to possess these biological features. It has also been discovered that CI-911 undergoes a rapid conversion in vivo to 5-oxo-2-pyrrolidinepropanoic acid, 2. As a result of this finding, the preparation of additional analogs of 2 was undertaken. Three structural variations were examined (see 3): (1) changes in the substituent, R, of the aroyl group on the pyrrolidine ring nitrogen, (2) alteration of the length (h) of the alkyl side-chain bearing the carboxylic acid, and (3) derivatization of the carboxylic acid moiety. These compounds were tested for their ability to reverse ECS-induced amnesia in mice and rats trained on a one-trial, inhibitory avoidance task. From these series, CI-933, 4, was found to exhibit the best profile of amnesia-reversal activity in mice and rats. On the basis of these results, CI-933 was selected for additional preclinical studies, and is currently in Phase 1 safety and tolerance trials in humans as a potential treatment of cognitive disorders.



1, CI-911



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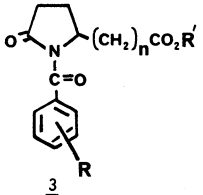

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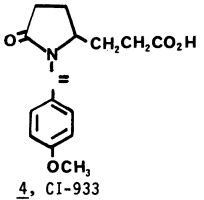

4, CI-933

60.12 ENHANCEMENT OF DELAYED-ALTERNATION PERFORMANCE IN RAT AND DELAYED-RESPONSE PERFORMANCE IN RHESUS MONKEY BY CI-933. J.G. Marriott, R.E. Davis, R.E. Voigtman*, and T. Tew*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

CI-933, (1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid), was initially found to have potential cognition-activating properties in tests measuring reversal of ECS-induced amnesia in mice and rats. CI-933 administered over a broad dose range reversed the transient amnesia seen in this model of impaired memory. Additional studies were undertaken to determine if CI-933 would also improve performance in animal models of normal and impaired short-term memory (STM).

CI-933 was first tested for effects upon STM using an operant, delayed-alternation task in well-trained, drug-sophisticated Long-Evans rats. These discrete trial, correctional procedures required the animals to remember from one trial to the next which of two levers was correct. The correct response was always the opposite (or alternate) of that chosen on the previous trial. Groups of rats were tested twice a week under either drug or control conditions. CI-933 (0.1 to 32 mg/kg PO) was administered 30 min prior to the start of a 100 min test session.

Additional tests for effects upon STM were conducted using an automated delayed response task in young and old rhesus monkeys. In these procedures, recall of the spatial position of a stimulus within a 3 x 3 matrix is tested following retention intervals of various lengths. Animals were tested twice a week, once under control conditions and once 30 min following drug administration (0.32 to 10 mg/kg PO).

In the rat delayed-alternation task, CI-933 produced small, statistically significant improvements in performance at doses above 0.32 mg/kg in several replications of this test. This improvement was not related to the length of the retention interval. Similarly, CI-933 improved performance on the delay-response of both aged rhesus monkeys and poor performing adult monkeys. This improvement was most evident when no delay was interposed between the presentation of the correct stimulus and the opportunity to respond. On trials with retention intervals interposed, CI-933 produced only minimal improvement over control levels of performance.

Taken together, these results indicate that CI-933 has performance-enhancing effects in these tasks that do not appear to be due to direct effects upon short-term memory. Based on these findings, CI-933 may be useful in the treatment of human cognitive declines related to attentional, motivational, and/or learning disorders.

60.13 CI-933 REVERSES DIAZEPAM-INDUCED MEMORY IMPAIRMENTS IN YOUNG AND AGEND MONKEYS. R.E. Davis, T.F. Tew*, and J.G. Marriott, Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105.

Besides their well known anxiolytic actions, certain benzodiazepines have been found to impair human memory. We have reported previously that diazepam produces a reliable short-term memory deficit in aged-rhesus monkeys performing a delayed response task. This memory impairment is similar to that produced in humans by this agent, suggesting that this may be an excellent animal model of human impaired cognition. In light of this and the finding that CI-933, 1-(4-methoxybenzyl)-5-oxo-2-pyrrolidine-propanoic acid, improves performance of monkeys in a delayed response task, CI-933 was studied for its ability to reverse diazepam-induced delayed response deficits in young and aged monkeys.

Performance on a delayed response task was measured in aged (>18 yrs old) and adult (<15 yrs old) rhesus monkeys using an automated testing apparatus. Monkeys were presented a stimulus in one of nine spatial locations in a 3 x 3 array on a CRT. Recall of the spatial position of the stimulus was tested following various retention intervals (0-30 secs). Diazepam (2.0 mg/kg, IM) or vehicle was administered 45 minutes prior to testing. Fifteen mins later CI-933 (0.32-10.0 mg/kg, PO) was administered. Monkeys were tested twice each week, once under drug and once as a control day.

Diazepam significantly decreased performance across all intervals tested in young and aged monkeys. CI-933 did not reverse the diazepam-induced deficits on nondelay trials. However, CI-933 significantly reversed this deficit on all delay trials in both age groups.

These data demonstrate that CI-933 antagonizes the memory impairing effects of diazepam. This occurs despite the fact that CI-933 does not interact with any known receptor including benzodiazepine receptors. Thus, CI-933 appears to antagonize the effects of diazepam through a novel mechanism. To the extent that diazepam induced memory impairments serve as a model of human cognitive dysfunction, these data suggest that CI-933 may be useful in treating congitive dysfunction in humans.

60.14 CI-933 REVERSES THE ANTINEOPHOBIC ACTIVITY BUT NOT THE SURVIVAL TIME ENHANCING PROPERTIES OF DIAZEPAM. R.E. Voigtman*, M. Smith*, R.E. Davis and J.G. Marriott (Spon: E. Gamzu). Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI. 48105.

CI-933, 1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidine propanoic acid, possesses a broad spectrum of activity in animal models of cognition. Among its many behavioral properties, CI-933 antagonizes the ability of diazepam to impair short-term memory. It is not known whether this effect of CI-933 is selective for the memory-impairing effects of diazepam or is reflective of a general ability to antagonize most actions of benzodiazepines. To examine this question, CI-933 was tested for its ability to block the antineophobic and survival time increasing actions of diazepam.

CI-933 (0.32 - 100.0 mg/kg, PO) was tested in rats alone or in combination with diazepam (3.2 mg/kg, IP) or vehicle for effects on consumption of a novel milk solution in a novel environment. Diazepam, vehicle or CI-933 was administered 30 mins prior to testing and 15 mins before CI-933 or vehicle. Milk consumption was measured at 1/2 hr intervals for the next 2 hrs. Diazepam increases milk consumption in this model.

For survival time testing mice received diazepam (3.2 mg/kg, PO) 45 mins prior to testing. Fifteen mins later CI-933 (0.32-100.0 mg/kg, PO) or vehicle were given. At the time of testing, core body temperature was measured and the animals were immediately placed into a glass chamber containing 4.1% oxygen and 95.9% nitrogen. Time to respiratory arrest was measured for each animal. Diazepam increases survival time under these conditions.

CI-933 antagonized the ability of diazepam to increase milk consumption in a novel environment while having no effect when given alone. In contrast, CI-933 did not antagonize diazepam-induced body temperature decreases or survival time increases.

Insofar as antineophobic activity of diazepam in animals is related to its anxiolytic activity in man, these data suggest that CI-933 may block the therapeutic effects of benzodiazepines. Taken together with previous findings, these data indicate that CI-933 may possess a generalized ability to antagonize most effects of diazepam. The exception to this is that CI-933 did not block diazepam induced changes in survival time or body temperature. However, it should be noted that benzodiazepine antagonists such as BCEE and Ro 15-1788 also do not block these effects of diazepam.

60.15 THE EFFECTS OF CI-933 UPON ACQUISITION OF A WATER MAZE TASK IN INBRED MICE. J.P. Symons*, R.E. Davis, J. Kalanik*, and J.G. Marriott (Spon: F. Gullotta). Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105.

CI-933 (1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid) has been shown to reverse ECS-induced amnesia in rats and mice, enhance delayed response performance in young and aged monkeys and reverse diazepam-induced memory deficits in young and aged monkeys. The mechanisms responsible for these actions of CI-933 are not known. However, these studies suggested CI-933 influenced performance by altering non-mnemonic processes such as attention or motivation. If this is the case, then performance on a task requiring animals to attend to distal spatial cues might also be enhanced by administration of CI-933. This hypothesis was tested using a swim maze, a task in which an animal is required to locate the position of a hidden platform using spatial cues.

Inbred C57BL/10 mice, a strain deficient in the number of pyramidal cells of the hippocampus (Wimer, et al, 1980) and impaired in swim maze acquisition (Symons, et al, 1985), were trained and tested over two consecutive days. CI-933 (0.0, 0.1, 0.32, 1.0, 3.2, 10.0, 100.0 mg/kg, PO) was administered 30 mins prior to testing. Mice were given four trials in the maze each day. A trial consisted of placing the animal in one of the four corners of the pool and recording latency to locate the submerged platform (maximum of 2 min per trial). Results indicated that low doses of CI-933 enhanced acquisition and/or retention of this task. With doses levels less than 3.2 mg/kg there were significantly more trials in which the platform was located within the first 15 sec of each trial when compared to the higher dose levels and vehicle control.

CI-933 improved performance in hippocampally deficient mice on the spatial swim maze task. Taken together with previous results, these data support the hypothesis that CI-933 enhances performance by altering non-mnemonic processes such as attention or motivation.

60.16 ELECTROPHYSIOLOGICAL EFFECTS OF CI-933 IN THE AGED RAT. J. French, J.J. Kinsora*, M.E. Smith*, and J.G. Marriott, Warner-Lambert/ Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

CI-933, 1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidine-propanoic acid, has been shown to enhance performance of learned behaviors in many animal models of cognition. The EEG changes produced by CI-933 in aged rats were studied to identify changes in the brain which might (1) correlate with the bioavailability of CI-933 and/or (2) suggest possible mechanisms underlying the performance-enhancing properties of CI-933.

Bipolar electrodes were implanted in the cortex and hippocampus of aged Fisher-344 rats (>24 months) since increased arousal or attention is often correlated with changes in electrical activity in these areas of brain. A computerized system for conducting power spectral density analyses of the EEG below a Nyquist frequency of 40 Hz was used to evaluate 6 rats over a 2 hr period. Rats were tested twice a week using a crossover design to test each dose of CI-933 and vehicle control once in every animal. CI-933 was administered 5 min prior to beginning the test session.

Oral doses of CI-933 increased the power seen in hippocampal 4-6 Hz theta and 16-40 Hz beta activities. Increases in beta activity (centered at 19 Hz) occurred at several doses, beginning immediately after dosing and becoming maximal after 60 minutes (68% increase over control levels at the most effective dose). A second peak in the 16-40 Hz activity occurred 95 minutes after dosing. Cortical changes consisted of increases in the 6-8 Hz band.

The most prominent electrophysiological effect of CI-933 was to increase hippocampal beta activity during the first two hours after dosing. Similar increases in hippocampal beta activity can be elicited by direct electrical stimulation of the brainstem reticular activating system. Taken together, these results suggest CI-933 may produce its effects on hippocampal beta by activation of brainstem mechanisms. Further, the unique performance-enhancing effects of CI-933 may be due to direct effects of this compound upon the brainstem reticular formation, which in turn have diffuse effects upon the hippocampus and cortex. The unique behavioral and EEG profiles of CI-933 in animal models and the absence of any side effects over a wide range of doses, support its use in clinical populations with arousal-related performance deficits.

60.17 **CI-933 INCREASES BRAIN FUNCTIONAL ACTIVITY IN DISCRETE BRAIN REGIONS.** H.E. Gompers*, R.E. Davis and J.G. Marriott (Spon: R. Richardson). Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI. 48105.

CI-933, 1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid, has been shown to broadly affect cognitive performance in animals. This agent reverses ECS-induced amnesia in rodents, improves delayed response performance and reverses diazepam-induced memory impairments in monkeys. As part of a series of experiments to determine possible sites of action and mechanisms mediating these cognition enhancing effects of CI-933, we studied the ability of this agent to modulate functional activity of discrete brain regions as estimated by 2-deoxyglucose utilization. This technique was chosen since glucose utilization in the brain is sensitive to procedures and drugs which modulate cognitive performance in man and other animals.

Male albino rats were food deprived 24 hrs prior to initiation of the experiment. CI-933 (0.0, 1.0, 3.2 and 10 mg/kg PO) was administered 15 minutes before tail vein injection of [3H]- or [14C]-2-deoxyglucose (200 uCi/kg). Forty-five mins later these animals were decapitated and their brains were frozen in dry ice and prepared for apposition to LKB-ultrafilm or Kodak x-ray film. Brains were analyzed using videocapture microdensitometry.

CI-933 globally increased functional activity at all doses tested. In addition there were dose related increases in functional activity in most cortical areas and in regions receiving reticular formation input including most thalamic structures, the inferior colliculi and the dorsal but not ventral cochlear nuclei.

These data demonstrate that at behaviorally active doses, CI-933 increases functional activity in brain regions thought to be involved in cognitive performance. Among the most interesting are those seen in the inferior colliculi and the dorsal cochlear nuclei. Changes similar to these in magnitude and pattern are seen after strong electrical stimulation of dorsal reticular sites which also produces intense arousal or orienting reactions. This similarity suggests that CI-933 may influence behavior by driving reticular structures. This interpretation is consistent with studies indicating that CI-933 drives hippocampal EEG activity in the beta bandwidth and increases behavioral arousal or attention.

60.18 NEUROCHEMICAL ASPECTS OF CI-933: A NOVEL PERFORMANCE ENHANCING AGENT. R. D. Schwarz, T. A. Pugsley*, L. L. Coughenour* and S. F. Stewart*. Warner-Lambert/Parke-Davis Pharm. Research, Ann Arbor, MI 48105.

Alzheimer's disease, and other age-related disorders which show marked declines in cognitive function, are being diagnosed with greater frequency as the age of the general population increases. At present, no therapy exists which effectively treats this decline in mental ability. As part of an ongoing program to develop new therapeutic agents to treat cognitive dysfunction, a behavioral testing strategy has identified 1- 4-methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid CI-933) as an agent which enhances performance in certain animal models of cognition.

CI-933 was studied in a variety of biochemical assays in order to gain an understanding of its mechanism of action. There was no in vitro binding of CI-933 to any of the various receptors examined: adenosine A1 and A2, alpha and beta adrenergic, benzodiazepine, Ca^{++}, dopamine, glutamate, muscarinic or serotonin receptors. Brain monoamine functions have been shown to be involved in learning and memory. In examining monoamine metabolism CI-933 10 and 30 mg/kg) elevated DOPAC levels in rat mesolimbic, but not striatal areas. Serotonin metabolism was unaffected. Since a profound loss of short-term memory has been associated with a cholinergic deficit, CI-933 was tested for effects on cholinergic function. It failed to affect either spontaneous or K^+-stimulated release of ^{3}H-ACh from rat brain slices, did not alter in vitro Na^+-dependent high affinity choline uptake into hippocampal synaptosomes following in vivo administration, or alter choline acetyltransferase in vitro. A third neuronal system believed to impact short-term memory involves glutamate/aspartate asp) neurotransmission. CI-933 was found to decrease the Ca^{++}-dependent, K^+-stimulated release of ^{3}H-D-asp from rat hippocampal slices. This effect is similar to that of muscarinic agonists and certain other agents which positively improve cognitive function in rodent models. Since CI-933 appeared to antagonize some of the behavioral effects of diazepam, a biochemical correlate to this activity was examined. Like the known benzodiazepine antagonist, RO15-1788, CI-933 was able to inhibit the diazepam blockade of the chlorpromazine-induced rise in HVA and serum prolactin levels. Thus, CI-933 blocks certain effects of diazepam, while exhibiting no in vitro or in vivo affinity for rat brain benzodiazepine receptors.

In summary, CI-933 appears to be a novel agent which may show behavioral activity by one or more mechanisms. In addition to effects on neurotransmission mediated by monoamines and excitatory amino acids, it represents a unique compound in its ability to reverse the action of diazepam while not binding to the benzodiazepine receptor site.

60.19 PHARMACOKINETICS AND BIOAVAILABILITY OF CI-933, A NEW COGNITION ACTIVATOR, IN LABORATORY ANIMALS. A. Black and T. Chang (SPON: D. Lobstein). Warner-Lambert/Parke-Davis, Ann Arbor, MI. 48105.

CI-933 (I), 1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid, is a new cognition activator currently undergoing phase I clinical evaluation. The pharmacokinetics and bioavailability of I were determined in beagle dogs and Wistar rats following 10 mg/kg intravenous and oral doses. A rapid and selective HPLC assay was develop for quantitation in plasma in which I and the internal standard (PD-116,312) are extracted from acidified plasma with methylene chloride. Separation was achieved on a Lichrosorb RP-8 column (5 micron; 250 x 4 mm) with a mobile phase of 0.0087M tetrabutylammonium sulfate in 0.05M phosphate buffer (pH 7.1):acetonitrile (75:25) at flow rate of 1 ml/min. Calibration curves are linear from 0.1 to 5 mcg/ml of plasma. Assay precision ranges from 0.7-5.6% (within-day) to 1.1-5.2% (between-day). Accuracy was determined by replicate analysis of spiked control plasma at low, medium, and high concentrations. The measured values differed from theoretical by 2.5% or less.

Following intravenous dosing, plasma concentrations of I declined monoexponentially. The kinetic parameters were: $t_{\frac{1}{2}}$, 0.45 and 0.35 hr, total plasma clearance, 9.7 and 8.5 ml/min/kg, and volume of distribution, 0.36 and 0.26 1/kg for dogs and rats, respectively.

Oral absorption of I was rapid with peak plasma concentrations being attained in 0.25-1 hr after dosing. The absolute systemic oral bioavailability was 82% in dogs and 72% in rats. Repeated oral administration (10 mg/kg/day) for 5 days resulted in no drug accumulation or evidence of autoinduction.

60.20 NEURAL ACTIVITY AND MYO-INOSITOL-1-PHOSPHATE LEVELS IN HIPPOCAMPAL SLICES EXPOSED TO LITHIUM AND A CHOLINESTERASE INHIBITOR. Rinaldi, P.C., Fairchild, M.D.*, Jenden, D.J. & Wasterlain, C.G.*, Neurosurgery Div., UCIrvine, 92717; Neurology Dept., V.A.Med.Ctr., Sulpulveda & Pharmacology Dept.,UCLA.

Lithium (Li) has been shown to stimulate cholinergic activity and alter D-myo-inositol-1-phosphate (MIP) levels in the CNS. This interaction in brain with inositol levels and associated modulation of receptor sensitivity may be part of the mechanism through which Li exerts its therapeutic effects. In addition, MIP levels were increased, behavioral seizures produced and limbic, cortical and thalamic morphological changes observed when Li treated rats were challenged with a cholinesterase inhibitor. We have begun investigations to determine whether these results can be studied in the in vitro hippocampal preparation.

In the present work extracellular field potentials and MIP levels have been studied in perfused hippocampal slices in response to LiCl substituted for NaCl in perfusion media and acetylcholinesterase inhibition following addition of edrophonium HCl to the media. Studies were conducted in the CA3 region which has been shown to be susceptible to epileptiform activity. A fast-slow (FS) potential reflecting both antidromic discharge and orthodromic synaptic activation of pyramidal cells was recorded in s.pyramidale in CA3 following fimbria stimulation. A portion of the FS complex is expected to represent activation of cholinergic elements since the septal-hippocampal pathway projects via the fimbria and innervates CA3.

Initially we determined that combined Li/edrophonium perfusion produced sustained paroxysmal bursting in CA3. Li alone (10 mM) would only occasionally produce such activity. Experiments have now been conducted in 51 hippocampal slices. After stabilization in normal media, slices were perfused for 30 min. in media with 10 mM LiCl or 150 μM edrophonium HCl or both. In several experiments atropine methylbromide (10 μM) was added to the Li/edrophonium combination. The FS potential was recorded during perfusion. At the end of perfusion, slices were collected for extraction and MIP levels for each slice were analyzed by GC-MS techniques.

The results (in μM/mg protein) indicated that neither Li ($\bar{x}$=0.116 ±0.015) nor edrophonium ($\bar{x}$=0.145 ±0.019) had a significant effect on MIP levels over control values ($\bar{x}$=0.102 ±0.017) while the combined perfusion of these two agents resulted in a substantial increase ($\bar{x}$=0.464 ±0.077). Neither Li nor edrophonium perfusion reliably produced abnormal neural activity. However, during combined administration spontaneous epileptiform discharges were observed and fimbria stimulation produced a sustained and recurring afterdischarge. The addition of atropine to the Li/edrophonium combination modified MIP levels ($\bar{x}$=0.311 ±0.035) but did not totally block the increase.

60.21 SULFONYL FLUORIDES: A STRUCTURE/ACTIVITY STUDY AS CNS SELECTIVE CHOLINESTERASE INHIBITORS. L.A. Rodriguez*, D.E. Moss and M.L. Camarena*. Psychobiochemistry Laboratory, Department of Psychology, University of Texas at El Paso, El Paso, Texas 79968.

A decline in CNS cholinergic function appears to be correlated with dementia in several disorders including senile dementia of the Alzheimer type (e.g., Whitehouse et al., Science 15, 1237-1239, 1982), Parkinson's disease (Nakano and Hirano, Ann. Neurol. 15, 415-418, 1984), and parkinsonism-dementia complex of Guam (Nakano and Hirano, Ann. Neurol. 13, 87-91, 1983). Because of this, there has been interest in enhancing CNS cholinergic function but these attempts have been limited by the peripheral toxicity of drugs that are not CNS selective. As one possible solution to this problem, a device for intracranial infusion of cholinergic drugs was tested in Alzheimer's disease (Harbaugh et al., Neurosurgery 15, 514-518, 1984). A more satisfactory approach to the problem of enhancing CNS cholinergic function without unacceptable levels of peripheral toxicity is to develop CNS selective drugs. Moss et al. (In: Senile Dementia of the Alzheimer Type, J.T. Hutton and A.D. Kenny (Eds.), Alan Liss, New York, 1985, in press) reported that certain sulfonyl fluorides show a high degree of selectivity as CNS cholinesterase inhibitors as compared to enzyme from several peripheral tissues. In fact, under certain circumstances, up to 90% inhibition of CNS cholinesterase could be inhibited with 30% or less inhibition of peripheral enzyme. In addition, some representatives of these compounds were relatively free from effects on extrapyramidal motor behaviors as compared with physostigmine (Moss, Rodriguez and McMaster, Pharm. Biochem. Behav. 22, 479-482, 1985).

In view of the potential therapeutic applications for CNS selective cholinesterase inhibitors, an additional 33 sulfonyl fluorides of widely varying structure were screened for reactivity against CNS and peripheral cholinesterases in vivo in rats. The tissues assayed included the forebrain, ileum, skeletal muscle and heart. The results showed that all of the larger molecular weight compounds and those with two or more rings were without effect at the standard test dose of 0.575 mmole/kg). Of all of the compounds tested, only 5 have shown significant activity against CNS enzyme as compared to peripheral enzyme. There was no clear structure/activity relationship observed by comparison of the various compounds. The mechanism responsible for inducing CNS selectivity is currently obscure.

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CARDIOVASCULAR REGULATION

61.1 AUTORADIOGRAPHIC LOCALIZATION OF ATRIAL NATRIURETRIC FACTOR BINDING SITES IN THE PERIPHERY AND CENTRAL NERVOUS SYSTEM OF THE RAT AND GUINEA PIG. C.R. Mantyh*, N.C. Brecha, L. Kruger, and P.W. Mantyh Center for Ulcer Research and Education, VA Wadsworth, Bldg. 115, Room 217, L.A., CA., 90073 and Brain Research Institute, UCLA, L.A., CA., 90024

Specific high affinity atrial natriuretic factor (ANF) binding sites are localized and quantified in 20um cryostat sections of the nervous system and periphery of rats and guinea pigs using autoradiographic techniques and the radioligand ^{125}I-ANF$_{1-28}$ (Amersham). Specific binding is defined as that binding that was displaced in the presence of 1uM of ANF$_{1-28}$, but not by unrelated peptides, and is greater than 75% of the total binding.

In the periphery, the highest concentration of specific ANF binding sites is observed in the glomerular apparatus, collecting tubules, and small arteries of the kidney, the zona glomerulosa of the adrenal cortex, the smooth muscle of the aorta and gall bladder, the epithelium of the lung, the posterior lobe of the pituitary and the ciliary body of the eye.

ANF receptor binding sites in the brain are present in several neuroendocrine and circumventricular structures including the pituitary stalk and caudal neurohypophysis, subfornical organ, and in the subcommisural organ, pineal, medial habenular nucleus and the preoptic region of the organum vasculosum of the lamina terminalis. Within the brain one of the highest concentrations of receptors is present in the dendritic field of the olfactory bulb mitral cells extending along the entire axonal extent of the olfactory tracts but fails to penetrate the neuropil of the primary olfactory tract. The fasciculus retroflexus and its diencephalic terminations also reveal a moderate concentration of binding sites. A high concentration of binding sites is also present in the chorioid plexus epithelium, whereas the ependymal lining of the ventricles has a negligible concentration of binding sites. The leptomeningeal margins and expanded subarachnoid spaces also reveal a high concentration of binding sites.

Species differences are significant only in the presence of extensive ANF binding sites in the neuropil of the cerebellar cortex of guinea pigs but not in rat. The distribution of specific ^{125}I-ANF$_{1-28}$ binding sites is consistent with earlier physiological and pharmacological studies which suggest that ANF plays a functional role in the regulation of extracellular fluid volume, electrolyte concentration and blood pressure.

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61.2 SUBDURAL INJECTION OF A SUBSTANCE P ANTIBODY ATTENUATES THE REFLEX PRESSOR RESPONSE TO STATIC MUSCULAR CONTRACTION. K.J. Rybicki*, G.P. Kozlowski*, and M.P. Kaufman* (Spon: G.A. Iwamoto). Dept. of Physiol., Univ. of Texas Health Science Center, Dallas, Texas 75235.

Static contraction of hindlimb muscles has been firmly established to reflexly increase mean arterial pressure, an effect initiated by the stimulation of group III and IV afferents. Although the specific neurotransmitters or neuromodulators released by these muscle afferents are not known, substance P is one candidate. Therefore, in chloralose anesthetized cats, we have examined the effect of injecting subdurally a substance P antibody on the reflex pressor responses to static contraction of the triceps surae muscles. This antibody was found not to cross-react (less than 0.01%) with neurotensin, met-enkephalinamide, or somatostatin. Before injection of the antibody, static contraction, induced by stimulating the tibial nerve (40 Hz; 2 times motor threshold), increased mean arterial pressure by 28±2 mmHg (n=6 cats). After injection of the substance P antibody, via a cannula whose tip was located at the entry point into the spinal cord of the L_7 and S_1 dorsal roots, contraction increased arterial pressure by 11±2 mmHg. Injections of equal volumes of normal rabbit serum, the vehicle containing the antibody, had no effect on the pressor response to contraction. In 2 cats, the pressor response to electrical stimulation of the posterior hypothalamic area was not decreased by injection of the substance P antibody, suggesting that the antibody was not directly inhibiting sympathetic outflow. Last, after waiting about 60 minutes or opening the dura and washing out the cerebral spinal fluid, the pressor response to contraction returned towards its pre-injection level (17±3 mmHg). Our results are consistent with the hypothesis that substance P functions as a neuromodulator in mediating the reflex cardiovascular responses to static muscular contraction. (Supported by HL06296 and HL30710.)

61.3 LHRH ANALOGUE ACTS AS SUBSTANCE P ANTAGONIST BY INHIBITING SPINAL CORD VASOMOTOR RESPONSES. Y. Takano*, W.B. Sawyer*, N.L. Sanders* and A.D. Loewy. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Intrathecal injections of the LHRH analogue - D-pGlu^1, D-Phe^2, D-Trp^{3,6} LHRH caused dose-dependent decreases in mean arterial blood pressure in anesthetized rats similar to those seen with the substance P analogue D-Pro^4, D-Trp^{7,9}-substance P(4-11). Similarly, intrathecal injections of either peptide reversed the hypertension elicited by application of kainic acid on the ventral surface of the medulla oblongata. Both analogues had similar I.C.$_{50}$ values ($\sim 10^{-5}$M) for inhibition of specific [^{3}H]-substance P binding in tissue sections through the region of the intermediolateral cell column. LHRH had no effect on [^{3}H]-substance P binding in sections of rat spinal cord. Furthermore, no specific [^{3}H]-LHRH binding sites could be demonstrated in the spinal cord.

The LHRH analogue failed to block substance P-induced contractions of the isolated guinea pig ileum and did not affect tail-flick withdrawal time in a thermal nociceptive test, whereas the substance P analogue acted as an antagonist in both of these bioassays.

In conclusion, these results suggest that D-pGlu^1, D-Phe^2, D-Trp^{3,6} LHRH may act as an antagonist which can inhibit the sympathetic vasomotor outflow and potentially by a relatively specific substance P-physalaemin (SP-P) receptor antagonist in the central nervous system but without effect on peripheral SP-P receptors.

61.4 SINGLE NEURONS IN NUCLEUS TRACTUS SOLITARIUS PROCESS CONVERGENT AFFERENT INPUT FROM CARDIAC VAGUS, CAROTID SINUS AND RENAL NERVES. R.B. Felder, Department of Internal Medicine and Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242.

Afferent nerves carrying cardiovascular sensory information from cardiac, aortic and carotid sinus receptors terminate centrally in the nucleus of the tractus solitarius (NTS). Renal afferent nerves have also been shown to influence neurons in the vicinity of NTS. Stimulation of sensory receptors in each of these regions can reflexly modify arterial pressure and heart rate, and a number of central interactions among these receptor groups have been described. Recent single unit recording studies have suggested that neurons in the NTS may integrate multiple sensory inputs rather than simply transmitting unaltered sensory signals to other centers involved in cardiovascular reflex regulation. In the present experiments, the influences of the cardiac branch of the right vagus nerve (CBVN), the right carotid sinus nerve (CSN) and the right renal nerve (RN) on NTS neurons were examined. In 5 alpha-chloralose anesthetized, paralyzed and artificially ventillated cats, microelectrode recordings were obtained from 11 single units in NTS excited by stimulation of CBVN. Peak response latencies to electrical stimulation of CBVN ranged from 9-64 msec. Seven of these units were also evoked by electrical stimulation of CSN at peak response latencies ranging from 5-51 msec. Two units evoked by both CBVN and CSN were also excited by stimulation of RN at peak response latencies of 33 and 39 msec. Recording sites marked by iontophoresis of Pontamine Blue were recovered histologically for 6 units, 3 of which had combined CBVN and CSN input. All were medial to tractus solitarius with 5 in dorsomedial or commissural subnuclei and 1 ventromedial to tractus. These results suggest that single neurons in medial NTS may integrate afferent signals from cardiopulmonary, carotid sinus and renal sensory receptors.

61.5 CENTRAL EFFECTS OF ADENOSINE AND ITS ANALOGS ON BLOOD PRESSURE AND HEART RATE IN THE RAT. E.P. Schoener, W. Richard Campbell*, R.A. Barraco and D.R. Marcantonio*. Departments of Physiology and Pharmacology, Wayne State University Medical School, Detroit, Michigan 48201.

Adenosine (ADO), its precursors and the enzymes involved in both its synthesis and degradation are ubiquitous to neural and other tissues. In the CNS, ADO and its analogs exert potent depressant effects on neural firing at many levels of the neural axis. ADO is released from neurons and synaptosomes following electrical stimulation and the notion that ADO acts as a synaptic modulator in the CNS is supported by demonstrations of its inhibitory effects on the presynaptic release of a number of neurotransmitters. Perhaps the best characterized effect of ADO at the biochemical level is its capacity to modulate adenylate cyclase activity through two separate purinergic receptors, A_1 and A_2. Male Sprague-Dawley rats with chronic indwelling cannulae were injected in the lateral cerebral ventricle with two adenosine analogs and their effects on blood pressure and heart rate were examined. NECA (5'-N-ethyl-carboxamidoadenosine) and L-PIA(L-phenyl-isopropyladenosine) produced dose-related reductions in blood pressure and heart rate. NECA exerted slighly more potent hypotensive action while L-PIA was more potent in depressing heart rate. These effects were antagonized by intraperitoneal injections of caffeine. In other experiments using the technique of push-pull perfusion, the nucleus tractus solitarius and adjacent brainstem regions were unilaterally perfused with adenosine or NECA. ADO produced diphasic effects in blood pressure, producing increases at nanomolar concentrations and decreases in micromolar concentrations. NECA, and A_2 specific agonist, produced mostly decreases in blood pressure. These studies on the central effects of ADO analogs on blood pressure and heart rate and their antagonism by caffeine suggest that the CNS areas involved in the control of cardiovascular function may be under the influence of endogenously released purines. (Supported by a grant from the American Heart Association of Michigan).

61.6 TRANSIENT HYPOTENSION FOLLOWING AREA POSTREMA LESIONS IN THE RAT. Skoog, K.M. and Mangiapane, M.L. Dept. of Pharmacology, University of Rochester, Rochester, NY 14642.

The dog literature suggests that the area postrema (AP) is an important site at which circulating angiotensin II acts to regulate arterial pressure. In contrast, some investigators have suggested that the rat AP is not involved in cardiovascular responses to AII. Recently, however, Mangiapane et al. (1985) have found that ablation of the AP renders the rat unable to sustain chronic angiotensin II hypertension (although pressor responses to acute AII are unaffected). In view of the proximity of the AP to the nucleus of the solitary tract (NTS), we examined in the present study the acute and chronic effects of AP ablation on resting arterial pressure, baroreflex control of heart rate, and lability of arterial pressure. Arterial pressure was recorded and analyzed via an IBM PC-based analog-to-digital data acquisition system. Rats were first given femoral arterial and venous catheters. Two days later, arterial pressure and heart rate were recorded for one hour, immediately after which the AP was ablated under very short-acting pentothal anesthesia. Immediately after the ablation, both sham and AP-ablated rats were replaced in their cages. Pressure and heart rate were recorded for the next 12 hours, and again one hour each day for the next seven days. Although pre-lesion pressures were the same in both groups, the AP-ablated rats had significantly ($p<.001$) lower arterial pressures in the 12 hour period following the lesion, and on each of the following two days. By the third post-lesion day, there was no difference in pressures between the two groups. Ablation of the AP also produced a significant ($p<.02$) bradycardia which was present in the first 12 hours following the lesion, and also on the third post-lesion day. Group differences were greatest during the 12 hr immediately following sham or AP lesions. During this period arterial pressure of the AP lesion rats dropped 15.9 $\pm$ 2.4 mm Hg vs. a drop of 2.0 $\pm$ 1.3 mm Hg in sham lesion rats. Heart rate decreased 38.7 $\pm$ 14.6 beats per min in AP lesion rats and increased 15.8 $\pm$ 11.5 beats per min in sham lesion controls during the same period. Baroreflex control of heart rate was not impaired in AP-ablated animals, nor was lability of arterial pressure increased. These results indicate that: (1) AP ablation produces a transient hypotension; (2) in spite of the proximity of the NTS, AP ablation does not impair the baroreflex, produce increased lability of arterial pressure, or cause neurogenic hypertension. We conclude that the antihypertensive effects of the AP lesion occur without significant damage to the baroreflex function of the NTS. (Supported by NIH grant HL32981.)

61.7 MECHANISM OF THE VENTRICULAR TACHYARRHYTHMIAS THAT OCCUR AFTER TOPICAL APPLICATION OF KAINIC ACID TO THE AREA POSTREMA OF THE CAT. R.A. Gillis*, J. Dias Souza* and P.J. Gatti* (SPON: D.A. Eagles), Dept. of Pharmacol., Georgetown Univ. Schls. of Med. & Dent., Washington, DC 20007.

We recently found that the neuroexcitotoxin, kainic acid, applied topically to the area postrema of chloralose-anesthetized cats causes ventricular tachyarrhythmias (Fed. Proc. 43: 699, 1984). These arrhythmias were associated with increases in sinus rate and arterial blood pressure. The cause of the arrhythmias was not clear but may be related to constriction of the coronary arteries since Somberg (Circulation 68, Suppl. III, 31, 1983) reported that electrical stimulation of the area postrema causes an increase in coronary vascular resistance. The increase in resistance was mediated by increased sympathetic activity as it was counteracted by alpha-adrenergic blockade. To determine whether ventricular tachyarrhythmias evoked by bilateral topical application of kainic acid (40 mM) were due to coronary constriction, we monitored blood flow from the left anterior descending coronary artery of 5 anesthetized cats.

We also monitored the ECG and the arterial blood pressure. Topical application of kainic acid produced an increase in coronary vascular resistance (43 + 13%, $P < 0.05$), S-T segment changes, and ventricular tachyarrhythmias. The increase in coronary vascular resistance was noted first (at 2.5 min after drug application) followed by changes in S-T segment (at 3.4 min) and arrhythmias (at 4.1 min). All of these effects were prevented by pretreating animals with the alpha-receptor blocking agent, phentolamine (n = 4). These results suggest that chemical excitation of area postrema neurons produces arrhythmias because of coronary constriction, and that this constriction is mediated by the sympathetic nervous system and alpha-adrenergic receptors on coronary vessels.

61.8 MICROINJECTION OF VASOPRESSIN OR VASOPRESSIN ANTAGONIST INTO THE AREA POSTREMA ALTERS BAROREFLEX FUNCTION IN THE ANESTHETIZED RABBIT. E.M. Hasser*, D.O. Nelson, J.R. Haywood, K.P. Undesser*, R.J. Applegate* and V.S. Bishop. Dept. of Pharmacology, Univ. of Texas HSC, San Antonio, TX 78284.

Arginine vasopressin (AVP) has been shown to augment arterial baroreflex inhibition of renal sympathetic nerve activity (RSNA), an action which is dependent upon an intact area postrema. Low level electrical stimulation of the area postrema also appears to inhibit RSNA and to enhance baroreflex inhibitory effects. This study was designed to examine the RSNA response to small volumes of AVP microinjected directly into the area postrema and to evaluate the effects of microinjection of the V_1 AVP antagonist $d(CH_2)_5TYR(ME)AVP$ (AVPX) on the AVP augmentation of baroreflex function. Rabbits were anesthetized with urethane and instrumented with arterial and venous catheters, and recording electrodes on the renal nerve to record RSNA. The brainstem was exposed, and a glass microelectrode containing artificial CSF, AVP or AVPX was visually inserted into the area postrema. Artificial CSF or AVP (4-10 ng) in a volume of 4-10 nl was injected via a micropressure injection system, and RSNA responses monitored. Following a recovery period, increasing doses of either AVP (2-50 mU/kg/min) or phenylephrine (PE) (0.5-10 µg/kg/min) were infused intravenously for one minute at each dose, and baroreflex curves relating arterial pressure (MAP) and RSNA were constructed for both drugs. AVPX (100 ng in 10 nl) was then injected into the area postrema and infusions of AVP and PE repeated. Microinjection of AVP into the area postrema resulted in a significant inhibition of RSNA, while the same volume of artificial CSF had no effect. Intravenous administration of AVPX (10-15 µg/kg) blocked the inhibitory effects of AVP microinjected into the area postrema. Effects of AVPX injected into the area postrema on baroreflex function are shown below.

SLOPES OF MAP-RSNA RELATIONSHIP

	AVP	PE
Control	9.6±1.2	4.4±0.3
AVPX	5.3±0.7	4.3±0.3

When given intravenously the slope of the MAP-RSNA curve was significantly greater for AVP infusions than for PE. Microinjection of AVPX into the AP normalized the slope of the MAP-RSNA relationship for AVP to that of PE without altering the baroreflex response to PE infusion. Intravenous administration of the same amount of AVPX did not alter the AVP curve. Results suggest that AVP acts at the area postrema to augment baroreflex inhibition of RSNA. This effect appears to be mediated via the V_1 AVP receptor. Supported by NIH grants HL12415-15 and HL32834-01, and AHA #G-581.

61.9 CARDIOVASCULAR FUNCTION IS MARKEDLY ALTERED BY PICOMOLES OF L-GLUTAMATE INJECTED INTO RAT BRAINSTEM. D.O. Nelson, J.L. Feldman, D.R. McCrimmon, and H.L. Cohen*. (SPON: R.E.W. Harrison). Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

The nucleus tractus solitarius (NTS) is a major brainstem site involved in integration of afferent signals related to the neural regulation of cardiovascular function. Several studies utilizing electrical stimulation or large injections of concentrated L-glutamate (GLU) have attempted to functionally map the NTS and surrounding structures. In our previous studies, injections of picomole quantities of GLU (0.2-10 nl) revealed an underlying functional heterogeneity of the NTS. This study investigated the effects of glutamate activation of discrete NTS subregions on the processing of cardiovascular-related information within this structure.

Urethane-anesthetized rats were instrumented to record arterial pressure (BP) and heart rate (HR). Multibarrel micropipettes, filled with 10mM GLU, 100mM glutamate diethyl ester (GDEE), saline or marking dye and connected to a micropressure injection system permitting 100pl resolution, were inserted into the NTS region. GLU injections (0.2-10nl) into the medial and lateral NTS caudal to the obex produced rapid, dose-dependent decreases in HR and BP. At discrete sites, 1nl injections routinely produced 40 mmHg decreases in BP and 50-150 bpm decreases in HR. At some sites effects on BP with no observed change in HR were elicited. Depressor responses increased with depth, beginning 300 um below the brain surface increasing to a maximum at approximately 800 um. At greater depths the responses rapidly attenuated. Marked dose- and site-dependent increases in BP and HR were produced following injections into the commissural nucleus. Maximal responses were obtained approximately 800 um below the brain surface in the medial and caudal aspects of this region. Responses to GLU were attenuated or blocked by simultaneous injection of GDEE.

Our results indicate that glutamate activation of neurons within discrete regions of the NTS markedly alters BP and HR. Further, we hypothesize a heterogenous organization of the NTS with respect to cardiovascular regulation.

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61.10 DORSAL MOTOR NUCLEUS OF THE VAGUS: SELECTIVE MU- AND KAPPA-OPIOID RECEPTOR MEDIATED CARDIOVASCULAR RESPONSES. A.H. Hassen. Dept. Physiology, West Virginia School of Osteopathic Medicine, Lewisburg, West Virginia 24901.

Previous studies have described the selective cardiovascular responses elicited by mu- and kappa-opioid receptors in the nucleus of tractus solitarius (NTS) and nucleus ambiguus (NA) regions of the rat hindbrain; mu-agonists increased mean arterial pressure (MAP) and heart rate (HR) following microinjection into either region whereas kappa-agonists depressed MAP following NA microinjection. The present study describes the unique cardiovascular responses elicited by receptor-selective opioid agonists following microinjection into another nucleus which is important in cardiovascular regulation, the dorsal motor nucleus of the vagus (DMN).

Male Sprague Dawley rats were anesthetized with urethane, the trachea intubated to permit artificial ventilation and the femoral artery and vein cannulated. MAP, HR and blood gas were monitored by standard recording devices. Each animal was placed in a stereotaxic device and the obex was exposed by removing the medial portion of the occipital bone. A pulled glass capillary (OD 14-42u) filled with either 0.9% NaCl (vehicle) or opioid solution was stereotaxically inserted into the DMN (AP: obex, L: 0.5 mm; V: 0.8 mm below the dorsal surface of the brainstem) or the commissural nucleus of the NTS (AP: obex, L: 0.5 mm; V: 0.3 mm below the dorsal surface of the brain stem). Each animal was monitored for 15 minutes prior to a single microinjection (100 nl) of vehicle or opioid solution. Naloxone was administered 15 min. after the microinjection and the animal was observed for an additional 10 min. Fast green was used to mark the injection site which was identified by microscopic examination of frozen sections.

The mu-agonist DAGO elicited a dose-related (3 pmol-3 nmol) increase in blood pressure following DMN microinjection (+1±2 - +28±2 mmHg). Although the high dose did not change HR, the lower dose tended to decrease HR (-23±11 BPM). Animals pretreated with atropine methyl nitrate (ATMN) exhibited a greater increase in MAP accompanied by tachycardia (+56±15 BPM) following DAGO injection into the DMN. This contrasts with the lack of effect by ATMN on the tachycardia elicited by DAGO microinjected into the NTS. The kappa agonist U50,488H (30 nmol) lowered HR (-54±10 BPM) without affecting MAP following DMN microinjection; the bradycardia was attenuated by pretreatment with ATMN. It is concluded that mu- and kappa-receptors in the DMN increased vagal activity to the heart.

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61.11 QUANTITATIVE LOCALIZATION OF CARDIOVASCULAR AREAS OF CAT BRAIN USING COMPUTER RECONSTRUCTED 3D IMAGES OF GLUCOSE UTILIZATION.
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The use of a microcomputer for image processing can greatly aid in mapping glucose utilization in the brain. The use of pseudocolor display techniques and reconstruction algorithms allow data gathered by [14C] 2-deoxyglucose (GU) mapping techniques as well as from other autoradiographic techniques to be meaningfully displayed and analyzed. We have developed software which has allowed us to relate quantitatively reconstructed GU data directly to the cat cytoarchitectonic atlas. The analysis of the autoradiographic data is initiated by digitizing the films using a scanning microscope equipped with a photomultiplier tube having a resolution to 10 microns square. The optical density data is stored on disc and then converted to GU values using the Sokoloff equation and appropriate measurements of plasma parameters and [14C] standards. The majority of our analysis programs work with two-dimensional GU images. A program was recently developed to reconstruct the brain using a series of up to 140 serial sections of scanned brain sections. Once alligned a vertical or horizontal cursor can be used to slice through the reconstructed brain and a computer generated horizontal or sagittal image will be displayed. This new image can then be analyzed quantitatively in a manner identical to the original transverse images. Before the slice is created, the sections which will make it up must be aligned. This is done by choosing three corresponding points on each of the two images. Using this information, the program rotates the outline of the second image so as to provide a best fit and displays it over the first image. The operator can then translate and rotate the outline, alligning it visually. The second section is then rotated to fit the first and the process is repeated for each section until all are in register. Slices are then created using a weighted average smoothing algorithm in which the data value in the new slice at a particular point is determined by the average of the corresponding data points in the two sections which lie in front of and behind the point, inversely weighted by their respective distances to the point. This algorithm does not require that the existing sections be evenly spaced throughout the length of the new slice, nor does it limit the length of the new slice to a multiple of the number of sections which make up the slice. We have written another program which allows the user to trace one or more areas on a GU data section, either reconstructed or original, and compute the mean and standard deviation over that area. This program can also interface with a high resolution sonic digitizing tablet which utilizes a photograph of the corresponding histological section that represents the anatomy of the scan being analyzed. Using a scaling program one can outline a histologically defined area within the photograph using the cursor from the sonic digitizer and the same area will be analyzed on the computerized scan. This type of analysis greatly reduces the experimentor bias in the interpretation of the so-called "hot spots" in the autoradiographic record. In addition another program allows the user to select any point on the scan using a movable cursor and displays the GU and location in stereotaxic coordinates, which is computed from the step size of the microscope and location of a reference point. These two programs can be used together to provide a quantitative mapping of GU in the cat brain. The theoretical accuracy of the process is determined both by the shrinkage of the histology in the autoradiological process, as well as by the resolution of the scanner. Most sections are scanned at a resolution of 100 microns/pixel, allowing localization of a point within 100 microns in a particular animal. This accuracy is not as useful in making comparisons between animals because of differences in brain size; scaling programs under development will allow comparison between two brains with this accuracy. The programs do allow the publication of data in tabular and statistical as well as graphical form, and allow the possibility of a quantitative composite map of brain activity. This analysis has been extremely useful in identifying areas of the cat brain that are influenced by physiologically and electrically stimulated cardiovascular reflexes. (Supported by NIH Grants HL27968, NS18037, RCDA HL00959, and the VA).

61.12 CENTRAL PROJECTIONS OF VAGAL AFFERENTS IN THE CAT: A [14C]2-DEOXYGLUCOSE MAPPING STUDY. D.R. Kostreva
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Fourteen adult cats 2-4 kg were anesthetized with pentobabital sodium and placed on positive pressure ventilation. EKG was monitored along with systemic blood pressure. A femoral artery was cannulated for blood measurements of glucose, PCO_2, PO_2, pH and scintilation counting. A femoral vein was cannulated for injection of the single bolus of 100 uCi/kg of [14C]2-deoxyglucose. The cervical vagi were exposed in all animals. The cats were divided into two groups. One group had the cervical vagi sectioned bilaterally and the left cut central end of the vagus was placed across a pair of stimulating electrodes connected to a stimulator. The parameters were a frequency of 10 Hz, a pulse width of 0.5 ms and a current strength of 5 mA. These parameters produced a 20-50 mmHg pressor response in the cat. A pattern of periodic stimulation was used so as not to fatigue the response. In most of the non-stimulated control cats the vagi were sectioned, however, in a few they were exposed surgically but left intact. Ventilation was adjusted to insure an arterial PCO_2 as close to 23 mmHg as possible. The animals were then given a single bolus injection of [14C]2-deoxyglucose. The stimulated group were stimulated periodically. During the 45 minute period, 16 paired blood samples were taken for scintilation counting and blood glucose measurements. The animals were then sacrificed and the brains removed and frozen rapidly. The brains were then serially sectioned using a cryostat, and covered with film for autoradiography. After 12 days of exposure the films were developed and scanned and converted to glucose utilization values using the Sokoloff equation. The results indicate that stimulation of vagal afferents unilaterally in the cat result in primarily an ipsilateral increase in glucose utilization by the medial subnucleus of the nucleus tractus solitarius. Other areas of the brainstem that appear to be effected by stimulation of vagal afferents are effected bilaterally, these include the external cuneate nuclei, and the inferior olives. This study demonstrates that stimulation of vagal afferents not only produces profound physiological effects but that these effects also produce marked changes in glucose utilization by specific nuclei and synaptic regions of the brainstem. (Supported by NIH Grants NS18037, HL27968, RCDA HL00959 and the VA)

61.13 ROSTRAL VENTROLATERAL MEDULLA MEDIATES THE CENTRAL HYPOTENSIVE ACTION OF PROPRANOLOL. P.J. Privitera*, A.R. Granata, D.J. Reis, R.L. Tackett* and T.E. Gaffney*. Depts. of Pharmacology & Medicine, Medical Univ. of South Carolina, Charleston, SC 29425 and Cornell Univ. Med. Coll., New York, NY 10021.

We previously observed in the dog that the hypotensive response to centrally administered propranolol was correlated with elevations in cerebrospinal fluid norepinephrine levels. Furthermore, central α-adrenergic blockade prevented the propranolol-induced hypotension (Science 213: 911,1981). Collectively, these studies supported the concept that the centrally mediated hypotensive response to propranolol results from drug-induced release of norepinephrine which then stimulates central α-adrenergic receptors to decrease peripheral sympathetic nerve activity and lower arterial pressure. The present studies were designed to localize the central site(s) at which propranolol acts to produce its hypotensive effect. In pentobarbital-anesthetized dogs, perfusion of propranolol (25 ug/kg/min for 30 min) through the brain ventricular system resulted in 25 $\pm$ 8 mmHg decrease in arterial pressure. Selective perfusion of the same drug dose through the fourth ventricle resulted in a comparable hypotensive response (-24 $\pm$ 5 mmHg) whereas perfusion through the forebrain ventricles (lateral-third ventricle) resulted in a much reduced effect (-8 $\pm$ 2 mmHg). These results suggested that the central hypotensive action of propranolol was localized to the hindbrain region. Experiments were then conducted in urethane-anesthetized rats to determine the effect of bilateral microinjections of propranolol into the region of the rostral ventrolateral medulla (RVL) containing adrenergic neurons of the C1 group. This region was selected because it is known to contain noradrenergic nerve terminals and participate in cardiovascular regulation. In addition, we have previously demonstrated that tyramine, a drug which releases catecholamines from adrenergic nerve terminals, produces hypotension and bradycardia when injected into the RVL (Fed. Proc. 43(3):400, 1984). Bilateral microinjections of propranolol (0.25 to 2.0 nmoles) into the RVL produced an initial pressor response followed by a sustained decrease in arterial pressure which persisted for the course of the experiments (120-180 min). Heart rate was not consistently altered by the drug. The injection site was verified pharmacologically by injecting tyramine, 10 nmole, at the end of each experiment and observing a decrease in arterial pressure and heart rate and by histologic localization of the injection track. In those experiments in which propranolol produced no hypotensive response, tyramine likewise was without effect suggesting that the response to propranolol is anatomically localized to the C1 area in the RVL. The results suggest that an action of the drug in the C1 region of the RVL contributes to the hypotensive effect of centrally administered propranolol.

61.14 STIMULATION OF NEURONS OF C1 AREA OF THE ROSTRAL VENTRAL LATERAL MEDULLA INCREASES REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. M.D. Underwood*, C. Iadecola and D.J. Reis (SPON: T. Meikle). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Neurons in an area of the rostral ventral lateral medulla (RVL), containing adrenaline neurons of the C1 group (C1 area), maintain normal levels of blood pressure (Ross et al., J. Neurosci., 4:474, 1984) and mediate baroreceptor reflexes (Granata et al., Am. J. Physiol., 248:H547, 1985). We sought to determine whether RVL neurons also participate in the regulation of the cerebral circulation.

Studies were undertaken in 56 adult male Sprague-Dawley rats anesthetized with chloralose (40 mg/kg, s.c.), paralyzed (tubocurarine) and artificially ventilated. Arterial pressure (AP), heart rate and blood gases were monitored and controlled. The RVL was stimulated electrically (1 sec on/1 sec off; 100 Hz, 100 uA, 10 min) with microelectrodes or chemically by microinjection of the excitatory amino acid, kainic acid (KA). The evoked hypertension was controlled to keep AP in the autoregulated range of regional cerebral blood flow (rCBF). rCBF was measured using 14C-iodoantipyrine as indicator with bilateral dissection of 11 brain regions. Placements of electrodes or cannulae were always localized histologically post-mortem.

In controls rCBF ranged from 57 ml/min x 100g in corpus callosum to 105 in inferior colliculus. Unilateral electrical stimulation of RVL increased rCBF ($p < 0.05$, n=6) bilaterally and symmetrically in inferior colliculus (160% of unstimulated control), superior colliculus (150%), hypothalamus (135%), thalamus (164%), caudate nucleus (143%), hippocampus (140%), frontal cortex (152%), parietal cortex (136%), occipital cortex (138%), and corpus callosum (132%). Bilateral adrenalectomy did not change resting rCBF (n=5) nor the elevations elicited by electrical stimulation (n=5). KA microinjected into RVL unilaterally (n=6) or bilaterally (n=4) at a dose producing sustained elevation in AP (5 nm in 100 nl), elicited changes in rCBF comparable in magnitude and pattern to those elicited by electrical stimulation, suggesting that the responses are elicited from local neurons and not fibers of passage in the C1 area. Bilateral electrolytic lesions placed in the RVL area after spinal cord transection with AP maintained in rCBF autoregulated range by i.v. phenylephrine infusion, had no effect on resting rCBF (n=6) nor on the magnitude of increases in rCBF elicited by elevation of arterial pCO_2 to 64.4 + 0.5 mmHg (n=5). We conclude that: (a) excitation of neurons in RVL increases rCBF globally: this increase is not attributable to substances released from the adrenal glands, (b) in contrast to their tonic role in the systemic circulation, neurons in RVL do not contribute to maintaining resting rCBF and do not participate in the cerebrovasodilation elicited by hypercarbia. Neurons within the RVL, possibly those of the adrenergic C1 group, can initiate a global cerebrovasodilation, largely through neural pathways intrinsic to the brain. (Supported by NIH Grant numbers HL18974 and NS03346 and by US DOD DAMD 1784C4185.)

61.15 DEPRESSION OF SYMPATHETIC PREGANGLIONIC NEURONS (SPGNs) BY METHYLDOPA IS PREVENTED BY INHIBITION OF PNMT. Parley W. Madsen and Donald N. Franz. Department of Pharmacology, University of Utah, Salt Lake City, Utah 84132.

The possibility that the central vasodepressor effect of methyldopa is produced by methylepinephrine (mEPI) instead of methylnorepinephrine (mNE) is supported by studies showing that mEPI is highly bound to central alpha-2 receptors (Goldberg et al., Eur. J. Pharmacol. 69:95, 1981) and depresses SPGNs by potent activation of alpha-2 receptors (Guyenet and Stornetta, Brain Res. 235:271, 1982). In further support of this hypothesis, we have previously found that a selective PNMT inhibitor, SKF 64139, prevents the marked depression of SPGNs by reserpine (5 mg/kg) 3 hr after infusion of 150 mg/kg of methyldopa (Soc. Neurosci. Abstr. 8:426, 1982). The depressant effect of methyldopa is attributed to synthesis and release of mEPI from bulbospinal EPI pathways that terminate in the SPGN neuropil. The present study was designed to avoid the use of reserpine to reveal the depressant effect of methyldopa and to use a more selective PNMT inhibitor, LY 134046 (Fuller et al., Biochem. Pharmacol. 30:1345, 1981), to prevent this depression.

Sympathetic discharges, recorded from upper thoracic preganglionic rami, were evoked by stimulation of descending excitatory pathways in the cervical dorsolateral funiculus (intraspinal) or thoracic intercostal nerves (spinal reflex) in unanesthetized spinal cats. After pretreatment with 20 mg/kg of carbidopa, methyldopa (100 mg/kg) gradually depressed sympathetic discharges evoked through each pathway to about 50% of control values by 2 hr. The depression produced by methyldopa was rapidly antagonized by yohimbine (0.5 mg/kg). Pretreatment (4 hr) with LY 134046 (20 mg/kg) completely prevented the depression of transmission by methyldopa/carbidopa. Instead, the evoked responses were gradually enhanced to greater than 200% of control. The effect of LY 134046 was not due to blockade of alpha-2 receptors because clonidine (25 ug/kg) produced its typical depression that was rapidly antagonized by yohimbine.

The ability of PNMT inhibitors to prevent depression of SPGNs by methyldopa supports the proposal that mEPI released from bulbospinal EPI pathways is the functional inhibitory metabolite of methyldopa at this site. The enhancement may be due to release of NE or mNE from descending excitatory NE pathways. (Supported by HL-24085, GM-07579, and Utah Heart Association)

61.16 NEURONS CONTAINING THE ADRENALINE-SYNTHESIZING ENZYME PNMT IN THE CAT BRAIN. P.J. Gatti*, D.A. Ruggiero, R.A. Gillis, M. Anwar*, W.P. Norman, D.H. Park and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021 and Depts. of Pharmacology and Anatomy, Georgetown Univ., Washington, D.C. 20007.

Neurons containing the adrenaline synthesizing enzyme, PNMT (phenylethanolamine-N-methyltransferase) are located in the ventrolateral (VLM) and dorsomedial (DMM) medulla in rat but have not yet been mapped in the cat. In this study we demonstrate, for the first time, the distribution of neurons containing PNMT in the brainstem of cat. Adult cats were anesthetized (pentobarbital (40 mg/kg i.p.)) treated with cerebroventricular injections of colchicine (0.5 mg/200 ul), reanesthetized and perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer. In control animals vehicle alone was administered intraventricularly. Sections (40 um) were cut on a sliding microtome and then stained with antibodies to PNMT by using peroxidase-antiperoxidase immunocytochemistry. Twenty-four hours after colchicine injections, cells containing PNMT were intensely labeled in VLM and DMM. In VLM, labeled cells extended from the level of the facial nucleus to the calamus scriptorius. Most cells were stained at the level of the rostral third of the inferior olive in an area equivalent to the nucleus reticularis rostroventrolateralis of rat (Ross et al., J. Comp. Neurol. 228, 1984). Farther caudally, cells were adjacent to the lateral reticular cerebellar relay nucleus, and at the level of the calamus, lined the ventral surface. In DMM, cells were restricted to the caudal nucleus tractus solitarii (NTS) including subpostremal, medial and commissural subdivisions.

In this study we have observed major differences in the distribution of PNMT labeled cells in cat when compared to data from rat. In cat: 1) the cell group in VLM was more elongate vertically, and not clearly differentiated into subdivisions; 2) few or no cells were labeled in the rostral DMM (i.e. in rostromedial NTS, periventricular grey, medial longitudinal fasciculus or nucleus paragigantocellularis dorsalis); 3) few cells with horizontal axes of orientation or dendrites entering the raphe were labeled; 4) the organization of PNMT labeled neurons in the caudal NTS differed topographically and extended farther caudally into the commissural region; and 5) unlike rat, cells were clearly labeled in the area postrema and subpostrema.

We conclude: 1) in cat (as in rat) neurons are labeled with PNMT in two areas of medulla associated with visceral (e.g., cardiorespiratory) control; and 2) species differences exist between cat and rat in the organization of the PNMT cell systems.

61.17 A STUDY OF PRESUMPTIVE NEURONAL INPUTS TO THE A5 NORADRENERGIC CELL GROUP. Christopher E. Byrum* and Patrice G. Guyenet, (sponsor C.E. Creutz). Univ. of Virginia School of Medicine, Department of Pharmacology, Charlottesville, VA 22908.

The A5 noradrenergic (NE) cell group is located in the ventrolateral reticular formation of the pons. These cells project massively to the intermediolateral cell column of the spinal cord and play a still incompletely understood role in central autonomic regulation. The purpose of the present experiments was to determine which brain areas project to the portion of the reticular formation in which these cells are located.

Small deposits of wheat germ agglutinin-conjugated HRP (HRP-WGA) were made in the rostral portion of the A5 NE cluster by either iontophoresis or pressure injection. The overlap of the deposit with the A5 cell group was confirmed by photographing the location of fluorescent NE cells in vibratome sections obtained throughout the injection area (Faglu technique) before the sections were processed for HRP localization. Since the main A5 cell cluster lies within the rubrospinal tract, the presence or absence of neuronal labeling in the red nucleus was taken as evidence for or against damage to axons of passage.

In all cases where the HRP-WGA deposit (diameter 0.75 to 1.5 mm) overlapped with the A5 NE cell group and did not damage the rubrospinal tract (N=5), significant retrograde labeling was found in the following structures: Kölliker-Fuse nucleus, ventral midbrain periaqueductal gray (PAG), posterior parvocellular part of the paraventricular nucleus of the hypothalamus, lateral and perifornical hypothalamic areas, zona incerta, dorsal parabrachial area, vestibular complex, ventrolateral medullary reticular formation, intermediate and commissural portions of the nucleus tractus solitarius, and locus coeruleus.

Although it is unclear which of these inputs to the A5 area make synaptic contact with A5 NE cells, the nature of the inputs strongly supports the assertion that the A5 area is primarily involved in autonomic regulation.

61.18 STIMULATION OF THE NORADRENERGIC CELLS OF THE LOCUS COERULEUS IN RAT DECREASES BLOOD PRESSURE. A.F. Sved. Neurology Service, V.A. Medical Center, East Orange, NJ 07019, and Dept. Neurosci., UMDNJ, Newark, NJ 07103.

Previous studies have demonstrated that electrical stimulation of the locus coeruleus (LC) elicits a marked increase in arterial pressure (AP). However, the conclusion that stimulation of the LC produces a pressor response is based totally on electrical stimulation, which activates nerve cells in a region as well as the fibers passing through that region. In the present study, we examined the effect of stimulating specifically the noradrenergic cells of the LC on AP. Male Sprague-Dawley rats anesthetized with α-chloralose (60 mg/kg iv) following induction with halothane had cannulas placed in a femoral artery and vein and were mounted in a stereotactic instrument. Burr holes were drilled through the skull to allow placement of electrodes and micropipettes into the LC. Electrical stimulation of the LC (50 μA, 50 Hz, 10 sec train of 0.2msec pulses) increased AP more than 30 mmHg. This pressor response to electrical stimulation was not effected by pretreatment of the LC with 6-hydroxydopamine (2μg in 0.2 μl injected slowly into the left LC of rats treated with pargyline 10 days before study); the 6-hydroxydopamine treatment destroyed all (>90%) noradrenerigic neurons in the region of the LC, as demonstrated by histofluorescence using the FAGLU method. In contrast to electrical stimulation, microinjection of the neuroexcitatory agent L-glutamate into the LC decreased AP. The depressor response to L-glutamate was dose-related with 0.5nmol (in a 0.1ul injection volume) being the minimum dose to elicit a significant response (-10±2 mmHg, n=6) and 4.0 nmol producing the maximal response, a decrease in AP of 34±4 mmHg (n=6). The depressor response to 2.0 nmol L-glutamate injected into the LC was totally prevented by pretreatment with 6-hydroxydopamine. In addition, carbachol (1.0 nmol), a cholinergic agonist which has ben deomonstated to increase the activity of LC neurons, decreased AP (-16±3, n=5) when injected into the LC. These results suggest that stimulation of the LC decreases AP and that the pressor response elicited by electrical stimulation is not due to stimulation of noradrenergic cells of the LC.

Supported by funds from the Foundation of the University of Medicine and Dentistry of New Jersey and the Veterans Administration.

61.19 EFFECT OF BLOOD PRESSURE ALTERATIONS ON NEUROTRANSMITTER TURNOVER IN LOCUS COERULEUS AS MEASURED BY IN VIVO ELECTROCHEMISTRY. D. Bhaskaran,* and C.R. Freed, Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

Locus coeruleus contains the majority of noradrenergic cell bodies in the brainstem and may play a role in blood pressure (BP) regulation. Our laboratory has reported changes in neurotransmitter turnover in nucleus tractus solitarius (NTS) and dorsal raphe nucleus (DRN) following manipulations of BP with drugs (Neurosci. Abs. 91.1, 1984, Life Sci. 34: 1581, 1984). We have now studied the effect of drug-induced hypertension and hypotension on neurotransmitter metabolism in the LC. Urethane anesthetized male Sprague-Dawley rats weighing 250-350 g had carbon paste in vivo electrochemical (EC) electrodes placed in LC and linear sweep voltammetry was performed from -0.2 V to +0.5 V at a rate of 10 mV/sec using a DCV-5 cyclic voltammetry apparatus (Bioanalytical Systems). Peaks were seen at 0.15 V and at 0.28 V. Studies with alpha-methylparatyrosine, fusaric acid, and pargyline showed that the first peak was produced by extracellular fluid DOPAC while the second peak was 5-HIAA. Phenylephrine was infused i.v. to raise BP by 50 mm Hg and nitroprusside was used to reduce BP by 20 mm Hg. To confirm the EC findings, other groups of rats were decapitated during and after hypertensive and hypotensive drug infusions and the LC assayed for norepinephrine (NE), dopamine (DA), DOPAC, serotonin (5-HT) and 5-HIAA using HPLC with EC detection. Results showed that during phenylephrine-induced hypertension, there was no significant change in extracellular fluid DOPAC concentration during the first 40 min of drug infusion. However, as the hypertension continued for 60 min, the signal increased to 160 % of baseline and the increase persisted even after the infusion. The extracelluar fluid concentration of 5-HIAA increased with the onset of the infusion and remained elevated at 210 % of baseline after the infusion. Nitroprusside-induced hypotension did not change the catechol peak but it did lead to a 190 % increase in 5-HIAA. Direct tissue assays confirmed that DOPAC and 5-HIAA were changed as predicted by the EC electrodes. Tissue NE and 5-HT did not change in any experiment but tissue DA was decreased during nitroprusside infusion. These experiments show that hypertension and hypotension lead to changes in catecholamine and indoleamine metabolism in locus coeruleus. We have found in NTS and DRN that the carbon paste in vivo EC electrode measures extracellular fluid NE and 5-HIAA. By contrast, LC appears to have DOPAC and 5-HIAA as the primary electrochemically active species measured by carbon paste and carbon fiber electrodes (Brain Res. 273: 197, 1983). Changes in DOPAC are associated with hypertension but not hypotension. DA and 5-HT in LC may be important for cardiovascular regulation.

61.20 THE ORGANIZATION OF DORSAL MEDULLARY PROJECTIONS TO THE AMYGDALOID CENTRAL NUCLEUS AND PARABRACHIAL NUCLEUS IN THE RABBIT. B.S. Kapp, J.S. Schwaber,[1] C.G. Markgraf* and T. Bilyk-Spafford.* Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405 & [1]Dupont Central Research Neurobiology Group, Glenolden, PA 19036.

Both the amygdaloid central nucleus (ACE) and the parabrachial nucleus (PBN) have been implicated in cardiovascular regulation in the rabbit (Kapp et al., 1982; Hamilton et al., 1981). Since both areas are reciprocally connected, and receive direct projections from the nucleus of the solitary tract (NTS)/vagal dorsal motor nucleus (DMN) complex (Ricardo & Koh, 1978; Nordgren, 1980), they appear to be important components of a larger forebrain system involved in cardiovascular regulation. The present experiment was designed to further examine the anatomical organization of this system by determining the extent to which neurons of the NTS/DMN complex which project to the ACE also project via collaterals to the PBN.

Twenty New Zealand rabbits received injections of Fast Blue (FB; 10%; 40-150 nl) aimed at the ACE. Two to four weeks later, injections of Bisbenzimide (Bb; 40-80 nl) were aimed at the lateral parabrachial region. One to three days thereafter the animals were perfused, and 30 µm frozen sections were taken throughout the rostral-caudal extent of the NTS/DMN complex and examined using fluorescent microscopy for the presence of single and double-labeled neurons.

Less than two percent of labeled neurons within the NTS/DVN complex contained both FB and Bb. Neurons containing either FB or Bb demonstrated a similar, predominantly ipsilateral topographical distribution, with the majority located caudal to obex. At caudal levels FB and Bb labeled neurons were located within the DMN and commissural subnucleus of the NTS. More rostrally, labeled neurons were located within the DMN as well as within the parvocellular, medial, dorsomedial and intermediate subnuclei of the NTS.

To assure that retrogradely-labeled neurons in the NTS were not a function of transport by injured fibers of passage, injections of ^{3}H-leucine/proline were made into the NTS/DMN complex caudal to the level of obex in five rabbits. Transported label was concentrated within the medial component of the ACE as well as within the PBN where it was most dense lateral to the brachium conjunctivum.

These results suggest that separate populations of neurons within the NTS/DMN complex project to the ACE and PBN, and that these populations demonstrate similar topographical distributions within the dorsomedial medulla. These distributions are quite similar to the distribution of catecholamine-containing A2 neurons recently demonstrated to project to the hypothalamic paraventricular nucleus in the rabbit (Blessing et al., 1982). Supported by NS16107

61.21 ADRENAL MEDULLARY HORMONES AND BETA-ADRENORECEPTORS ARE INVOLVED IN THE GLOBAL CEREBROVASODILATION ELICITED BY STIMULATION OF THE DORSAL MEDULLARY RETICULAR FORMATION IN RAT. P.M. Lacombe*, C. Iadecola, M.D. Underwood*, K. Chida* and D.J. Reis. (SPON: M.E. Ross). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of the dorsal medullary reticular formation (DMRF) in rat, globally increases regional cerebral blood flow (rCBF) and metabolism. The finding that acute removal of the adrenal glands markedly reduces the elevations in rCBF suggests that part of the vasodilation depends upon substance(s) released from adrenals (Iadecola et al., Soc. Neurosci. Abstr. 10, 168, 1984). Although the most likely candidates are the adrenal catecholamines (CA), other substances released from the adrenal cortex or medulla, could be involved. In this study we sought to determine: (a) whether these substance(s) are released from the medulla and (b) whether beta-adrenergic receptors are involved in the cerebrovasodilation. Rats were anesthetized (chloralose 40mg/kg), paralyzed (tubocurarine) and artificially ventilated. Arterial pressure (AP) and heart rate were monitored and blood gases were kept in physiological range. DMRF was stimulated (50 Hz; 50 uA; 1 sec on/1 sec off) and AP kept in the autoregulated range for rCBF. rCBF was measured by the 14-C-iodoantipyrine technique in 13 dissected brain samples. In unstimulated rats (n=5) rCBF ranged from 64±4 (ml/100g x min) in corpus callosum to 110±11 in inf. colliculus. Acute total adrenalectomy (n=5) did not change resting rCBF. In intact rats (n=5) DMRF stimulation elicited an increase in rCBF in all areas with maximal responses in cerebral cortex (frontal cortex, Fcx to 206%; parietal, Pcx to 208%; occipital, Ocx to 189% of control; $p < 0.01$). After acute total adrenalectomy (n=6), DMRF stimulation increased rCBF only in Fcx (144%), Pcx (161%) and Ocx (171%) ($p < 0.02$), the increases being significantly lower than those obtained in intact stimulated rats ($p < 0.05$; analysis of variance). Comparable effects were elicited by acute removal of only the adrenal medulla (Fcx 164%, Pcx 164%, Ocx 158%; $p < 0.05$ from unstimulated and from intact stimulated). Administration of the beta-adrenergic receptor blocker propranolol (1.5 mg/kg, i.v.), 30 min prior to rCBF measurement, did not affect resting rCBF (n=5), however it abolished the increase in rCBF elicited by DMRF stimulation in intact animals, with a regional pattern and magnitude similar to that elicited after adrenalectomy or adrenal demedullation (rCBF increases: Fcx 144%, Pcx 146%, Ocx 136%; $p < 0.05$ from unstimulated and from intact stimulated). We conclude that substance(s) released from the adrenal medulla contribute to the cerebrovasodilation elicited by DMRF stimulation. The cerebral vasodilation is, in part, mediated by beta-adrenergic receptors, presumably within CNS. It is therefore probable that CA mediate the adrenal-dependent component of the cerebral vasodilation elicited by DMRF stimulation. However, since CA do not cross the intact blood-brain barrier, the mechanism through which they increase CBF and metabolism remains to be established. (Supported by NIH NS03346)

61.22 A VASODEPRESSOR RESPONSE ELICITED BY CHEMICAL STIMULATION OF INTRINSIC NEURONS OF ROSTRAL FASTIGIAL NUCLEUS (THE FASTIGIAL DEPRESSOR RESPONSE). K. Chida*, C. Iadecola, M.D. Underwood*, and D.J. Reis (SPON: R.P. Friedland). Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of the rostromedial pole of the cerebellar fastigial nucleus (rFN), in rat as in other species, elicits highly coordinated cardiovascular (the fastigial pressor response) and behavioral responses. Since electrical stimulation excites both neurons and their processes, these responses could originate from excitation of neurons within rFN or from stimulation of neuronal processes originating elsewhere and passing through rFN. In this study we sought to determine by chemical stimulation whether the hypertension and tachycardia elicited from rFN stimulation in rat, are due to excitation of local neurons. An unexpected vasodepressor response was found. Rats were anesthetized (chloralose, 40 mg/kg), paralyzed and artificially ventilated. Arterial pressure (AP) and heart rate (HR) were continuously monitored and recorded. rFN was stimulated electrically, with 8 sec trains of pulses (duration: 0.5 msec), or chemically, by the excitatory amino acids kainic acid (KA) , l-glutamic (l-glu), l-aspartate (l-asp), dl-homocysteate (dl-hom) locally microinjected (volume 100 nl) through micropipettes. Placements were histologically verified. Electrical stimulation of the rFN (n=17) produced a stimulus-locked elevation in AP and HR, graded with the intensity and frequency of the stimulus (threshold current: 14 ± 1.2 uA, at 50 Hz). At 5 times threshold AP rose 46 ± 2 mmHg. Microinjection in rFN of l-glu (n=5), at doses effective in elevating AP when microinjected in the rostral ventrolateral medulla (0.01-100 nmole), or l-asp (1-10 nmole) (n=5) had no effect. Chemical stimulation of the rFN with KA (n=10) produced a sustained (< 10 min), dose-dependent (0.05-5 nmole) reduction in AP and HR (AP reduction: 56 ± 6 mmHg, HR reduction: 103 ± 9, at 5 nmole; $p < 0.001$). Microinjection of KA (5 nmole) into the overlying cerebellar vermis (n=5), dentate nucleus (n=5) or fourth ventricle (n=4), failed to evoke a similar response. Microinjection of dl-hom (1-100 nmole) (n=5) also elicited a dose-dependent reduction in AP and HR (AP reduction: 34 ± 7 mmHg at 100 nmole; $p < 0.01$. To destroy the intrinsic neurons in rFN sparing the fibers of passage, the excitotoxin ibotenic acid was unilaterally injected in rFN (15 ng in 1.5 ul). In 7 days, neuronal perikarya completely disappeared in the rostral and intermediate portion of FN, with extensive gliosis. Electrical stimulation of the lesioned rFN (n=3), elicited elevations in AP and HR, not different from those obtained from the rFN of intact rats (AP elevation at 5 x threshold (<100 uA): 47 ± 4 mmHg; $p > 0.05$ from intact group). We conclude that: (a) the cardiovascular changes elicited by electrical stimulation of the rFN are not due to excitation of neurons intrinsic to rFN, but, most likely, to excitation of neuronal processes, originating elsewhere; (b) neurons in rFN responsive to KA and dl-hom, but not l-glu or l-asp, can initiate a potent and heretofore unrecognized vasodepressor response, the fastigial depressor response.

61.23 CEREBRAL CORTICAL NEURONS ARE REQUIRED FOR THE CORTICAL CEREBROVASODILATION ELICITED BY STIMULATION OF THE FASTIGIAL NUCLEUS IN RAT. C. Iadecola, S. Arneric, L.W. Tucker, H. Baker and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Electrical stimulation of the cerebellar fastigial nucleus (FN) increases local cerebral blood flow (lCBF) globally in brain and in cerebral cortex, independently of local metabolism (Nakai et al., Brain Res. 260, 35, 1983). Lesions of the basal forebrain (BF) abolish the cortical vasodilation (Iadecola et al., Brain Res., 279, 41, 1983) suggesting that the cortical vasodilation is mediated by pathways originating in or passing through BF. However it is not known whether these pathways affect CBF directly or via interposed cortical interneurones. To determine whether local neurones in cerebral cortex participate in the cortical cerebrovasodilation elicited by FN stimulation, rats were anesthetized with halothane and a local lesion produced by using the excitatory neurotoxin ibotenic acid (IBO) (10 ug in 1 ul) microinjected into the left primary sensory cortex. Five days later, rats were anesthetized (chloralose), paralyzed and artificially ventilated. Blood gases were controlled and arterial pressure was kept within the CBF autoregulated range. FN was electrically stimulated (1 sec on/1 sec off) at 50 Hz and 100 uA. lCBF was measured autoradiographically in 30 brain regions by the 14-C-Iodoantipyrine technique. IBO microinjection resulted, within 5 days, in lesions highly reproducible with respect to size, location and chemistry. Histologically, the lesion was characterized by neuronal loss over a cortical area of 3-5 sq. mm with variable gliosis and capillary proliferation. HRP-WGA (n=3) microinjected into the lesion, was transported retrogradely into the neurons of the BF and ventrobasal thalamus, indicating that major afferent pathways to the lesioned area were preserved. Biochemically, the activity of glutamic acid decarboxylase (GAD) was reduced to 26±9% of control (n=7; $p < 0.01$), while the activity of choline acetyltransferase was unchanged. These observations indicate loss of intrinsic GABAergic and probably other neurons, with preservation of the afferent cholinergic fibers,mostly from BF. IBO treatment (n=5) did not alter resting cortical lCBF when compared with the contralateral side (113±25 vs. 123±7; ml/100g x min; NS) nor with the corresponding area of unlesioned controls (125±8; NS). FN stimulation (n=6), in untreated rats increased lCBF significantly and symmetrically in most brain regions. However, lCBF did not increase within the lesion (122±8% of control; NS) in contrast to the lCBF in contralateral homologous site wherein it rose to 171±9% ($p < 0.005$). The absence of vasodilation within the lesion was not due to vasoparalysis since hypercarbia (pCO_2: 63±4 mmHg) (n=5) increased lCBF in the cortical target area bilaterally and symmetrically (lesion: 330%, contralateral: 327%; NS).

We conclude that local cortical neurones do not substantially contribute to resting lCBF or to the cerebrovascular reactivity to CO_2. However such neurons are required for cortical cerebrovascular vasodilation elicited by FN stimulation. (Supported by NIH NS03346 and DOD DAMD17-84-C-4185)

61.24 PUTATIVE PATHWAYS LINKING THE FASTIGIAL PRESSOR AREA AND THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IN THE RAT. A.Del Bo and A.Rosina*, Clin.Medica IV,Univ.di Milano and Ist.Fisiologia Centri Nervosi, CNR, Milano, Italy.

Electrical stimulation of the pressor site of the fastigial nucleus (FN) in the rat induced secretion of vasopressin (VP) into the plasma which could be modulated by afferents from the baroreceptors (Del Bo et al., Circ.Res.,54:248, 1984). Aim of the present study was to disclose the pathways which might subserve such a functional control of rostral FN on neurosecretory cells in the hypothalamus.

A technique which combines retrograde and anterograde fluorescent tracing(Rosina,Neurosci.Lett.,33:217,1982) was used. In urethane anesthetized rats the anterograde tracer Fast blue (FB) was microinjected (0.1 µl, 2% w/v) into the left rostromedial FN while the paraventricular nucleus of the hypothalamus (PVN) was centered on both sides with the retrograde tracer Diamidino yellow (DY) (0.1 µl, 1% w/v). After 10 days survival, animals were perfused with 20% formalin buffered at pH 7.2. 25 µ frozen sections were analyzed under a Leitz Ploemopak,by a filter system providing excitation light of 360 nm.

FB axons and axon terminals leaving the rostral FN through the superior and inferior cerebellar peduncles distributed bilaterally to several regions in the brainstem: the vestibular complex, the nucleus reticularis gigantocellularis, the nucleus parasolitarius, the lateral reticular nucleus. FB ascending fibers and terminals were found through the periventricular and central grey, the locus coeruleus and parabrachial complex. Brainstem DY neurons projecting to PVN were traced in the ventral medulla, tractus solitarius, raphe nucleus, dorsal tegmental nucleus, locus coeruleus, central grey and parabrachial nucleus. Among the above mentioned structures, which are known to be involved in direct and reflex control of circulation, FB terminals and DY cells were found to overlap in only two regions: scattered in the ventral medulla and more numerous in the locus coeruleus-parabrachial complex. The observed effect of FN stimulation on VP secretion may well be mediated by polysynaptic pathways but the present data suggest that a disynaptic link may also be possible.

61.25 CORTICOTROPIN-RELEASING FACTOR: EFFECTS ON BAROREFLEX CONTROL OF HEART RATE. L.A. Fisher. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Corticotropin-releasing factor (CRF), by virtue of its central nervous system (CNS) distribution and actions, is suspected of being a physiologic mediator of the integrated endocrine and autonomic responses to stress. Simultaneous elevations of mean arterial pressure (MAP) and heart rate (HR), indicative of altered baroreflex function, are observed both during exposure to various physical and psychological stressors (e.g., exercise, cold) and after intracerebroventricular (icv) administration of CRF. Therefore, the CNS effects of CRF on baroreflex control of HR were examined in detail by constructing complete stimulus-response curves relating MAP to pulse interval (PI; reciprocal of HR) over a wide range of MAP.

All experiments utilized conscious, unrestrained male rats (200-250 gm) fitted with lateral cerebroventricle cannulae and femoral artery and vein catheters. After measurement of basal MAP and HR, the rats received icv injections of saline (10 µl) or CRF (1.5 nmoles). Baroreflex testing began 10 min later, when MAP and HR reached stable plateaus. MAP was raised or lowered by alternating intravenous infusions (1 min, 20 sec duration) of graded doses of phenylephrine and nitroprusside. MAP and HR were recorded just prior to the onset and again during the last 20 sec of each infusion. MAP and HR were allowed to recover to preinfusion levels between each infusion. After conversion of HR to PI, all resting and response values were fitted to an S-shaped curve with nonlinear regression analysis.

The relationship between MAP and PI was clearly modified after CRF treatment. As previously demonstrated, CRF-treated rats displayed concurrent elevations of MAP and HR causing the MAP-PI curves to be shifted downward and to the right. Moreover,response range and reflex gain were markedly reduced while threshold MAP and MAP at midrange were increased following CRF treatment. These changes in curve parameters suggest that CRF alters transmission in CNS pathways involved in processing baroreceptor information. The pronounced effects of CRF on baroreflex function are likely to cause, acutely, enhanced cardiovascular performance and thus support the rise in metabolic rate that attends various stressful conditions. However, if such cardiovascular activation is sustained chronically, the potential for deleterious consequences is great.

61.26 ORGAN BLOOD FLOW DISTRIBUTION FOLLOWING CENTRAL INJECTIONS OF ANGIOTENSIN II IN CONSCIOUS RATS. R. Rettig, F.C. White* and M.P. Printz*. University of California, San Diego, School of Medicine, La Jolla, CA 92093.

Intracerebroventricular (icv) injections of angiotensin II (ANG II) are well documented to elicit pressor responses and bradycardia in rats and other species. The exact hemodynamic mechanisms underlying these responses have not yet been completely identified. The dipsogenic effect together with a recent report of a decrease in splanchnic nerve activity following central ANG II stimulation have been interpreted to suggest that ANG II elicits a centrally integrated behavioral and autonomic response pattern that resembles a classical "feeding response" and that is associated with a redistribution of organ blood flow away from the skeletal musculature and in favor of the mesenteric vascular bed.

In order to test this hypothesis we have measured blood flow in several vascular beds prior to and 2 min after icv injections of ANG II (100 ng) in 10 adult, conscious, freely moving Sprague-Dawley rats, using the tracer microsphere technique (diameter of spheres: 15 ± 3 µm; labels: Ce-141, Sn-113). Ten additional rats received icv injections of vehicle only. Central ANG II injections reduced blood flow significantly in the skin (-59%), stomach (-57%), bronchial circulation (-46%), small intestine (-43%), kidneys (-22%) and colon (-11%). There were no significant blood flow changes in the skeletal musculature, heart and brain, whereas blood flow increased slightly in the hepatic artery vascular bed. Injections of vehicle only did not significantly alter blood flow in any of the vascular beds, except for an unexplained increase in hepatic artery flow.

These data suggest that the central ANG II-induced pressor response in conscious rats is mediated to a major extent by vasoconstriction in the mesenteric vascular bed and the kidneys, whereas skeletal muscle blood flow does not change significantly. This organ blood flow distribution pattern is not compatible with the classical "feeding response".

61.27 INTERRUPTION OF THE THALAMIC MD-PREFRONTAL PROJECTION HAS NO EFFECT ON STIMULATION-ELICITED CARDIAC CHANGES OR LEARNED BRADYCARDIA IN THE RABBIT. Shirley L. Buchanan and D. A. Powell. WJB Dorn VA Hospital, and University of S.C., Columbia, SC 29201

New Zealand albino rabbits, anesthesized with ketamine and thorazine, received knife cuts (Kopf Scouten assembly) either medial or lateral to the mediodorsal nucleus (MD) of the thalamus. A third group of control animals received sham cuts. A stimulating electrode was implanted in all animals. After a 1 to 2 week recovery period cardiovascular responses elicited by electrical stimulation through the previously implanted electrode were assessed in conscious animals. Heart rate (HR), blood pressure (BP), electromyographic activity (EMG) and respiration were measured in response to 2-sec trains of biphasic stimulation (40-200 μA). In addition, the HR component of the Orienting Reflex (OR) was measured in response to 10 presentations of a 4-sec, 75 db, 1216 Hz tone. HR was measured on a beat-by-beat basis for 10 beats prior to tone onset, for the duration of the tone, and for 10 beats following tone offset. Following OR assessment, rabbits received 2 sessions of differential classical conditioning, during which either the 1216 Hz tone, or a 304 Hz tone, was consistently paired with a 3 mA paraorbital electrical shock. HR was measured as during orienting assessment.

As previously found, electrical stimulation of MD elicited large bradycardiac responses, accompanied by pressor responses and respiration increases. Neither medial nor lateral knife cuts appeared to affect this response. The OR to the tone stimulus was a HR deceleration, which declined across trials. The HR CR was also a bradycardiac response, with larger decelerations occurring in response to the tone followed by shock (CS+) than to the non-reinforced tone (CS-). Both the OR and the CR were intact following either medial or lateral cuts. Since these cuts interrupt efferent fibers from MD to prefrontal cortex, these results suggest (a) that the autonomic changes elicited by electrical stimulation of MD are not mediated by MD-prefrontal connections, and (b) similarly, that the bradycardia elicited by Pavlovian conditioning contingencies is not dependent on the integrity of these connections.

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61.28 APPLICATION OF GLUTAMATE AND CARBACHOL TO THE MEDIODORSAL NUCLEUS OF THE THALAMUS ELICITS AUTONOMIC CHANGES IN CONSCIOUS RABBITS. D. A. Powell and Shirley Buchanan, Neuroscience Lab., WJB Dorn VA Hospital and Univ. of South Carolina, Columbia SC

Chronic indwelling cannulas were implanted in the mediodorsal nucleus (MD) of the thalamus in albino rabbits under ketamine and thorazine anesthesia. After a 1-2 wk recovery period the medial ear artery was cannulated under a local anesthetic and several autonomic measures, including heart rate (HR), mean blood pressure (BP), respiration, and electromyographic activity (EMG) were recorded subsequent to the injection of μl amounts of glutamate, GABA and carbachol. All drugs were dissolved in .9% saline. At the conclusion of testing the animals were sacrificed by nembutal and perfused transcardally with saline and 10% formalin. Histological verification of cannula tips was then assessed after cryostat sectioning and staining with thionin.

It was found that dose-related decreases in HR and increases in BP were obtained with both glutamate and carbachol at doses in the nmol and μmol range. However, GABA did not have an effect when injected into MD. Some injections also produced increases in respiration and EMG. Control injections through cannulas chronically implanted in the overlying hippocampus or adjacent ventricles revealed a different pattern of responding, suggesting that the effects obtained with MD placements were due to chemical stimulation of MD. Moreover, assessment of diffusion of methylene blue around the cannula tip in acutely sacrificed animals revealed that at the injection and time parameters employed, the drug remained in MD. Saline injections produced only a minimal effect, and control injections of carbachol and glutamate into other thalamic nuclei, i.e. AV and the reticular nucleus, did not produce similiar autonomic changes.

Responses elicited from MD in the present experiment were similar, but not identical, to those produced by electrical stimulation of MD. They suggest that the bradycardia and pressor responses produced by electrical stimulation are due to stimulation of MD cells and not fibers of passage. One troublesome feature of these findings, however, is that the changes produced were somewhat smaller than those produced by electrical stimulation. These differences may reflect the fact that a smaller population of MD cells is activated by chemical stimulation compared to those activated by electrical stimulation.

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61.29 CORRELATION BETWEEN RESPIRATORY AND CARDIAC AUTONOMIC NERVE ACTIVITIES. M. Kollai* and K. Koizumi. Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, NY 11203

The aim of the study was to obtain information about the nature of relationships between respiratory and cardiac autonomic nerve activities in spontaneously occurring situations and in those evoked by superior laryngeal nerve stimulation. The time course of activities recorded from phrenic, expiratory-intercostal muscle, cardiac sympathetic and parasympathetic nerves was analyzed in chloralose anesthetized and artificially ventillated dogs. We found that: 1) The pattern of cardiac autonomic nerve discharges in relation to the respiratory cycle varied in different experiments, depending on how transition from inspiration to expiration occurred; this was detected by monitoring changes in phrenic activity; 2) When the amplitude of integrated phrenic discharges varied, a similar pattern of variations appeared in the sympathetic but not in the vagal discharges; 3) During the rising phase of slow wave blood pressure oscillations, phrenic firing increased during inspiration and was maintained at a moderate level also during expiration; simultaneously sympathetic activity level was enhanced and vagal activity level was diminished; 4) Stimulation of laryngeal afferents produced total silence in phrenic activity and steady firing of the expiratory muscle nerve as well as discharges of both vagal and sympathetic nerves. The alternating discharge pattern between the two cardiac autonomic nerves previously present was lost.

From these observations it has been concluded that the primary factor in creating different autonomic activity patterns is the changing qualities of central inspiratory activity; its time course defines the period when vagal activity is inhibited, and its intensity determines whether and when the sympathetic activity is facilitated. (Supported by grant from USPHS, NS-00847).

61.30 CAN ARTERIAL CHEMORECEPTORS INFLUENCE BARORECEPTORS? J. T. Hansen. Dept. Cellular and Structural Biology, Univ. Texas Health Science Center, San Antonio, TX 78284.

Peripheral arterial chemoreceptors, such as the carotid and aortic bodies, lie in close proximity to baroreceptor regions located on the carotid sinus and aortic arch. The carotid body and sinus are innervated by the carotid sinus nerve, which possesses both chemosensory and baroreceptor afferents which terminate in the NTS. These two cardiovascular receptors generally are viewed as separate reflex systems; the carotid body initiates respiratory reflexes in response to changes in blood gases while the baroreceptors mediate cardiac and vasomotor reflexes in response to an elevated blood pressure. However, recent ultrastructural evidence demonstrates that these two receptor systems share a common blood supply (McDonald, D.M., and Larue, D.T. J. Neurocytol., 12:117, 1983). Vascular cast preparations of the carotid sinus show that the blood supply to the sinus vasa vasorum comes from small arterioles in the carotid body. This vasa vasorum is intimately associated with clusters of baroreceptor nerve endings on the carotid sinus. Additionally, isolated glomus-like cells, typical of those seen in the carotid body, occasionally are observed in the sinus wall adjacent to baroreceptor nerve endings. The carotid body glomus cells contain catecholamines and several neuropeptides, which may be released onto adjacent nerve endings or into the carotid body vasculature. Therefore, these neuromodulatory substances may have access to the baroreceptor nerve endings and smooth muscle of the carotid sinus via the vasa vasorum, or directly via paracrine secretion from isolated glomus-like cells in the sinus wall.

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61.31 ROLE OF THE CAROTID SINUS BARORECEPTORS AND THE INTERNAL AND EXTERNAL CAROTID ARTERIES IN THE CEREBRAL ISCHEMIC RESPONSE OF THE RABBIT. William G. LeBlanc,* Barry E. Hurwitz,* Philip M. McCabe & Neil Schneiderman. (SPON: Lincoln Potter) Dept. Psych., Univ. of Miami, Coral Gables, FL 33124

The cerebral ischemic response (CIR) in the rabbit, produced by interruption of the blood supply to the head, consists of elevated arterial pressure, bradycardia, apnea, and a shift in regional blood flow. It is normally produced by permanently occluding the two vertebral arteries and temporarily occluding the two common carotid arteries (CCAs). Since previous studies have typically involved occlusion of the CCAs below the carotid sinus (CS), questions remain concerning the relative roles played by the internal (ICAs) and external (ECAs) carotid arteries and about the influence of the CS baroreceptors upon the reflexive bradycardia.

It has been reported that the carotid sinus (CS) baroreceptors are not involved in the reflexive bradycardia of the CIR (Dampney et al., 1979, Circ. Res., 45, 48-57). However, we found that after aortic nerve transection, the contribution of the CS baroreceptors was masked when the CIR was produced by occluding the CCAs below the CS, but was observed when the CIR was produced by occluding the ICAs and the ECAs separately at a point above the CS.

Although there are anastomoses between the ICAs and the ECAs, no blood flow from the ECAs into the intracerebral circulation is observed during resting conditions (Scremin et al., 1982, J. Cerebral Blood Flow & Metabolism, 2, 55-66). In contrast, we found that these anastomoses are patent under certain occlusive conditions, allowing blood flow from the ECAs to the intracerebral circulation. Simultaneous occlusion of ICAs and ECAs elicited a CIR. Release of either one ICA or one ECA during the CIR resulted in a reversal of the CIR. Occlusion of only the ICAs or ECAs was not sufficient to produce a CIR. When the ICAs but not the ECAs were occluded, blood flow from the ECAs was sufficient to raise intracerebral arterial pressure to a level capable of preventing the initiation of the CIR or reversing the effects of an ongoing CIR. Dye injected into one ECA did not enter the cerebral circulation when one ICA was patent, but did enter the cerebral circulation when both ICAs were occluded, suggesting that flow can occur from the ECAs to the intracerebral circulation given a favorable pressure gradient.

Supported by AHA (FA505F), NHLBI (HL04726), and NINCDS (NS18749) research grants.

61.32 ADRENAL MODULATION OF CEREBRAL VASODILATION ELICITED BY ELECTRICAL STIMULATION OF THE CENTROMEDIAN-PARAFASCICULAR COMPLEX IN RAT. S. Mraovitch, F. Lasbennes,* Y. Calando* and J. Seylaz.* Lab. of Physiologie et Physiopathologie Cérébrovasculaire, INSERM U 182, CNRS UA 641, Université Paris VII, Paris, France.

Recently it has been discovered that electrical stimulation of the cerebellar fastigial nucleus (FN) increased cerebral cortical blood flow (Nakai et al., Am. J. Physiol. 343 (Heart Circ. Physiol. 12) H:226-H235, 1982). Since monosynaptic projections from FN were found to terminate in the centromedian-parafascicular complex (CM-Pf) but not in the cortex, it is conceivable that increase in cortical cerebral blood flow (Cx CBF) elicited by FN stimulation may be in part mediated via diffuse CM-Pf projections through the cortex. In the present studies we sought to examine in rat whether electrical stimulation of the CM-Pf would modify regional Cx CBF (rCBF) and if so, whether the changes are mediated through neural pathways intrinsic to brain. Rats were anesthetized (chloralose), paralyzed (tubocurarine) and artificially ventilated. Arterial pressure (AP), heart rate and body temperature were continuously monitored and blood gases were measured and controlled. CM-Pf was electrically stimulated with intermittant trains (1 sec on/1 sec off) at 200 Hz and with an intensity (50-100 μA) corresponding to 3x the threshold current required for a 10 mm Hg AP elevation. At all times, AP was maintained within the autoregulated range ($<$150 mm Hg) for rat rCBF. rCBF was measured in right and left samples of 10 brain regions by the ^{14}C-iodoantipyrine technique with regional dissection. In all animals, histological localizations of stimulated sites were carefully reconstructed. In unstimulated and sham operated rats (n=7) rCBF ranged from 40$\pm$3 (ml/100g/min) in corpus callosum to 99$\pm$16 in inferior colliculus. During stimulation of CM-Pf (n=7) rCBF increased bilaterally in all cerebral regions ranging from + 118% in parietal cortex ($p<0.001$) to +38% ($p<0.001$) in cerebellum. rCBF was significantly increased in medulla, pons, inferior colliculus, superior colliculus, frontal cortex, occipital cortex, caudate-putamen and corpus callosum. The cerebral vasodilation persisted after unilateral transection of the cervical sympathetic trunk without differences in rCBF between innervated and denervated sides. In 6 rats, stimulation of sites adjacent to the CM-Pf, including the posterior nucleus of the thalamus, and ventrobasal nucleus of the thalamus, failed to modify rCBF. In contrast, acute adrenalectomy (n=6) significantly ($p<0.05$) decreased elevated rCBF during CM-Pf stimulation in all cortical regions (frontal -36%, parietal -34%, and occipital -27%) and in caudate nucleus (-37%). We conclude: (1) electrical stimulation of CM-Pf increase CBF, (2) the response is anatomicaly specific and not affected by ipsolateral cervical sympathectomy, (3) adrenal hormones probably catecholamines and circulating glucocorticoids considerably contribute to cerebral vasodilation elicited by CM-Pf stimulation. Thus, excitation of neurons originating in, or fibers passing through the CM-Pf can elicit a powerful cerebral vasodilation which appears to be mediated by intrinsic neural pathways and modulated by the adrenal hormones.

61.33 ACUTE MYOCARDIAL DAMAGE AND PLASMA CATECHOLAMINES AFTER OCCLUSION OF THE MIDDLE CEREBRAL ARTERY IN THE CAT. K.E. Smith*, V.C. Hachinski, M.D. Silver*, C. Gibson and J. Ciriello. Departments of Physiology, Clinical Neurological Sciences and Pathology, University of Western Ontario, London, Canada N6A 5C1.

Focal cerebral ischemia in humans has been shown to result in increases in plasma norepinephrine levels and cardiac arrhythmias (Stroke, 12:200, 1981; Stroke, 13: 838, 1982). In addition, systemic administration of catecholamines has been shown to cause myocardial damage (Arch Pathol., 93:356, 1972). Furthermore, we have recently shown that plasma levels of norepinephrine are elevated after occlusion of the middle cerebral artery (MCA) (Stroke, 6:136, 1985). Taken together, this evidence suggests that cerebral ischemia may produce myocardial damage as a result of activation of the sympathetic nervous system. In the present study, experiments were done in chloralosed, paralyzed and artificially ventilated cats to investigate the effects of occluding the left MCA on acute cardiac damage and its relationship to plasma catecholamine levels. The hearts were removed from the perfused cats and examined for histopathological changes. After occlusion of the MCA for 8-10 hours, 43% (8) of the hearts examined (n=19) were found to have either acute myocardial necrosis (4/8), focal hemorrhage (3/8) or both (1/8). The levels of plasma norepinephrine (+46 ± 20%) and dopamine (+143 ± 73%) were increased significantly ($p<0.025$) from pre-occlusion values (-4 ± 10% and -14 ± 19%, respectively) in these cats with acute myocardial damage compared to the cats without acute myocardial changes. These data suggest that a rise in plasma catecholamines due to increased sympathetic activity following MCA occlusion may be a causal factor for myocardial damage. (Supported by the Heart & Stroke Foundation of Ontario).

61.34 BLOOD FLOW IN THE CAUDATE NUCLEUS HAS DIFFERENT REGULATORY MECHANISMS THAT IN OTHER STRUCTURES. E. Pinard,* S. Puiroud,* and J. Seylaz* (Spon: G. Bilotto). Lab. of Physiologie et Physiopathologie Cérébrovasculaire, INSERM U 182, CNRS UA 641, Université Paris VII, Paris, France.

In view of the discrepancy related to the effect of amines or other vasoactive agents on cerebral blood flow (CBF), further studies were performed in 16 rabbits by means of a new method based on mass spectrometry (Seylaz et al., Am. J. Physiol. 245, H513-H518, 1983). This technique enables local, quantitative and continuous measurement of partial pressures of oxygen and carbon dioxide (PO_2, PCO_2) and local, quantitative and sequential (each 3 minutes) measurement of CBF. Helium was chosen as tracer gas. CBF measurements by means of this technique were validated by a systematic comparison with the results obtained in the same experimental conditions with the ^{14}C-ethanol tissue sampling technique. Several days before the experiment, gas sampling cannulas were chronically implanted in two different structures: either caudate nucleus (n=7), or thalamus (n=5), or parietal cortex (n=5), or putamen (n=8), or hippocampus (n=8). The day of the experiment, the animal was freely breathing and not anaesthetized. The cannulas were connected to the mass spectrometers, arterial pressure (AP) was recorded, and blood gases were controlled.

Previous studies (Aubineau et al., Brain Res., 61, 153-161, 1973) from our laboratory had demonstrated that: 1) isoprenaline increased blood flow in caudate nucleus (CN) and not in thalamus, 2) noradrenaline markedly decreased blood flow in CN without affecting thalamic or cortical blood flow, without any disruption of the blood brain barrier. The tentative conclusions of these studies were that these effects were due to the heterogeneity of adrenergic receptors distribution on cerebral vessels with a much higher density in the carotid territory. It was unlikely that a metabolite proposed by Berne et al. (Circ. Res. 35, 262-271, 1974) to be a link between metabolism and blood flow had regional effects on CBF, comparable to those of adrenomimetics. Nevertheless, our results expressed as mean $\pm$ SEM are the following: intravenous injection of adenosine at a dose (0.1 mg/kg/min) which induced a small decrease in AP -8.7$\pm$0.8%) caused a highly significant vasodilation in the CN (+48.0$\pm$3.0%), did not affect significantly blood flow in thalamus, cortex and putamen and had little effect on hippocampus blood flow (+12.5$\pm$4.0%).

In conclusion, the specificity of the vascular reactivity of the CN to sympathomimetic agents has been also found for adenosine. In view of the weak permeability of the blood brain barrier (BBB) to this nucleoside it is tempting to bring together the mechanism of action of all these vasoactive agents: a possible mechanism could be an indirect action via receptors located in structures devoid of a BBB and specifically related by intrinsic nervous pathways to the cerebral vessels of the CN.

61.35 CALCIUM CHANNEL BLOCKER SPECIFICITY FOR CEREBRAL VS. PERIPHERAL RESISTANCE ARTERIES IN VITRO. F.W. Marcoux and J.M. Weaver*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The action of calcium channel blockers on vascular smooth muscle is now well documented. Indeed, clinical trials are ongoing as efforts to establish therapeutic utilities for these inhibitors of vasoconstriction. Few studies, however, have compared the activities of these compounds on arterial resistance segments from different vascular beds. We examined in vitro the activity of 4 structurally different calcium channel blockers, diltiazem, flunarizine, nimodipine and verapamil, on cerebral and peripheral arterial resistance segments which were taken from the same rat. Middle cerebral (MCA) and mesenteric arterial resistance (MTR) segments (<200μ in diameter) were mounted in parallel myographs and equilibrated in artificial CSF. The arterial segments were constricted with their respective EC_{90} concentrations for K+ and then exposed in a non-recirculating manner to increasing concentrations of one of the calcium channel blockers (10^{-10} - 10^{-4}M in ½ log increments). Concentration-inhibition curves constructed from the geometric mean $IC_{5,10,50,90}$ and 95_S for each calcium channel blocker in MCA and MTR segments were compared.

All 4 calcium channel blockers inhibited K+-induced constrictions in a concentration dependent fashion. Diltiazem and verapamil inhibited K+-induced constrictions in MCA and MTR segments in 2 phases, affecting first the secondary plateau phase of the contractile response and then, at higher concentrations, the initial peak phase. With diltiazem, the initial peak phase of the contractile response was inhibited more potently in MTR than in MCA segments. With verapamil, the secondary plateau phase of the contractile response was inhibited more potently in MTR than in MCA segments. Flunarizine and nimodipine showed single phase inhibition of the K+-induced response and both were approximately 5 times more potent in MCA than in MTR segments.

Thus, diltiazem and verapamil inhibited K+-induced vasoconstriction in a fundamentally different way than did flunarizine and nimodipine. Flunarizine and nimodipine showed clear specificity for middle cerebral vs. mesenteric arterial segments. Whether these differences in activity demonstrated with resistance arterial segments invitro will contribute to our understanding of the relative therapeutic benefits for each of the drugs in vascular disease will require further study, both experimentally and in the clinic.

61.36 INNERVATION OF CEREBRAL BLOOD VESSELS BY PEPTIDERGIC NERUONS Kunio Nakai,Toru Itakura*, Kazuo Nakakita*,Hideyoshi Yokote*, Yutaka Naka*, Ichiro Kamei,Harumichi Imai* and Norihiko Komai*. Dept. of Neurological Surgery,Wakayama Med. College,Wakayama 640 Japan

Evidences that peptide-containing neurons as well as aminergic neurons may be involved in regulating the cerebral blood flow have recently been cumulating(Edvinsson,TINS.,5.425,1982;8:126,1985; Eckenstein and Baughman.Nature,309:153).Present study further investigated peptide-containing neurons along the cerebral blood vessels comparing with other neurotransmitter system such as noradrenaline using PAP-immunohistochemistry and hydrogen clearance method.Guinea pigs weighing 200-300 g were perfused through ascending aorta with 4% paraformaldehyde , 0.5% glutaraldehyde and 0.2% picric acid in cold phosphate buffer(pH=7.4) under deep anesthesia with Nembutal (50 mg/kg). After postfixation pial arteries and cryocut brain sections(20μ) were placed in 0.1% triton X-100 in phosphate buffered saline until the day of immunostaining using anti-VIP,-SP or dopaminebeta hydroxylase(DBH) as primary antibodies. In general nerve fibers within vascular adventitia showed denser distribution in the anterior part of circle of Willis. Nerve plexus gradually decreases its density as caliber of the vessel decreases. VIP immunopositive fibers along pial arteries characteristically showed spiral running pattern throughout the intracranial pial arteries. Ultrastructural observation of VIP positive fibers along pial arteries demonstrated that about 80% of VIP positive fibers within vascular adventitia lay very close to vascular smooth muscles(within one micron).Within the cerebral cortex VIP positive cell bodies and fibers are numerous (Morrison et al.,Brain Res.,292:269,1984). Some of those VIP neurons were found very close to intraparenchymal blood vessels. In contrast, SP immunopositive fibers along pial arteries run in a mesh-work pattern and lay relatively apart from the vascular smooth muscles. Neither a nerve terminal nor a cell some immunostained with SP was found within the cerebral cortex. DBH immunopositive fibers along pial arteries showed mainly spiral pattern in the arteries near the bifurcation of middle cerebral arteries, although they tended to show mesh-work running pattern as the vessel caliber decreased. Some of DBH immunopositive fibers within the cerebral cortex showed close contact with intraparenchymal blood vessels.

Using hydrogen clearance method we have observed a significant increase of regional cerebral blood flow(r-CBF) by intracortical injection of VIP,a significant decrease by injection of noradrenaline and no significant change by SP.

Above results suggest that VIP neuron plays an important role in regulating cerebral blood flow by increasing it through a direct action on both pial and parenchymal vessels and further suggest a possibility of VIP being a useful therapeutic drug for an ischemic stroke of the central nervous system.

61.37 RECOVERY OF CARDIOVASCULAR FUNCTION AFTER CHEMICAL SYMPATHECTOMY WITH 6-HYDROXYDOPAMINE. S.J. Fluharty*, M.J. McCann*, S.A. Meyers*, R.R. Vollmer*, M.J. Zigmond and E.M. Stricker. Depts. of Biological Sciences, Pharmacology and Psychology, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Catecholamine (CA)-containing neuronal systems can sustain substantial damage without severe disturbance of function. We have proposed that such plasticity results from adaptations to the injury that occur in residual elements of the damaged system, and have been evaluating this hypothesis by examining cardiovascular function in pithed rats after systemic treatment with 6-hydroxydopamine (6-HDA). Although sympathetic innervation of the heart was decreased by >90% for several weeks, chronotropic responses to thoracolumbar stimulation were normal within 3 days of 6-HDA treatment. Bilateral adrenalectomy had no significant effect on chronotropic responses elicited by 20 sec pulses of increasing frequency, either in control animals or in 6-HDA-treated rats. Restoration of function therefore may have resulted from enhanced synthesis and release of norepinephrine (NE) from surviving sympathetic terminals. Indeed, tyrosine hydroxylase activity in undamaged neurons of the heart was increased 3-fold relative to NE levels at this time. Moreover, blockade of CA biosynthesis with alpha-methyltyrosine (AMT; 300 mg/kg) substantially reduced the heart rate responses in 6-HDA-treated rats with intact adrenals, whereas it had no such effect in control animals.

Similar results have been obtained using continuous preganglionic stimulation with 0.5 Hz for 20 min. Heart rate increased rapidly during the first 5 min, and by 20 min it was elevated by almost 150 beats/min in control animals (more than twice the increase after 20 sec of stimulation with 0.5 Hz). AMT treatment had little effect on this response either in control animals or in adrenalectomized rats. Although prolonged preganglionic stimulation also increased heart rate in 6-HDA-treated rats, to levels that were 60% of normal despite cardiac NE depletions greater than 95%, this tachycardia was reduced significantly by AMT treatment and it was eliminated completely if AMT was given to 6-HDA-treated rats that also had been adrenalectomized.

These results suggest that biochemical adaptations allowing enhanced synthesis and secretion of CA within residual sympathetic nerves may have an important functional impact on the heart after near-total denervation induced by systemic 6-HDA treatment. Moreover, they indicate that adrenal medullary CAs make a significant contribution to cardiac responsivity after 6-HDA treatment when the capacity for NE synthesis in the surviving sympathetic nerves had been exhausted.

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61.38 **THE INTRACARDIAC INNERVATION OF THE CARDIAC VENTRICLES IN THE RAT - A RETROGRADE FLUORESCENT TRACER STUDY.** **B.J. Pardini, P.G. Schmid, and D.D. Lund. Veterans Administration Medical Center and Cardiovascular Center, Department of Internal Medicine, University of Iowa, Iowa City, Iowa 52240.**

Prior studies from this laboratory have described the topographic location and number of intracardiac ganglion cells in the rat - sites of termination for cardiac vagal preganglionic neurons (Soc. Neurosci. Abstr. 10:607, 1984). The ganglion cells are located in four distinct regions of the heart, all above the atrio-ventricular groove: 1) between the superior vena cava and aorta, 2) superior and posterior to the interatrial septum, 3) posterior to the left atrium, and 4) posterior to the right atrium. The high degree of interanimal reproducibility and localization of the ganglion cells to these topographic areas suggest a certain amount of anatomic organization. The purpose of the present experiments was to determine if the organization of the intracardiac ganglion cells extended beyond the location of their somata to include the sites at which they terminate. The fluorescent dyes, Fast Blue and rhodamine-labeled microspheres, were used to retrogradely label cell somata which projected to the free wall of the left ventricle in the rat. Equithesin-anesthetized rats were intubated and respired by positive pressure. After a right thoracotomy, multiple injections of Fast Blue or rhodamine-labeled microspheres were made in the ventricular wall with a glass micropipette attached to a Hamilton 10 ul syringe. Three to four injections of 1 to 2 ul each were made into the ventricular wall. After closure of the chest, the rats were extubated, and allowed to survive for 48 to 96 hrs. Rats were perfused with saline followed by 10% buffered formalin; hearts were excised and 25 um frozen sections were cut on a Cryostat. Sections were viewed through an Olympus microscope with fluorescent attachment. After injections into the left ventricle, clusters of labeled ganglion cells were seen in the area of the interatrial septum and posterior to the left atrium (areas 2 and 3, above). No labeled somata were seen in the vicinity of the aorta and superior vena cava (area 1) or posterior to the right atrium (area 4). Preliminary experiments indicate that there are no labeled cells in the parasympathetic preganglionic areas of the medulla, providing evidence that vagal preganglionic neurons probably do not terminate in ventricular regions which are void of ganglion cells, and that dye did not spread to atrial regions or through the blood to artifactually label neurons. The data indicate that individual loci of intracardiac ganglion cells may have organized projections to specific sites of termination at the neuroeffector junction in the ventricle.

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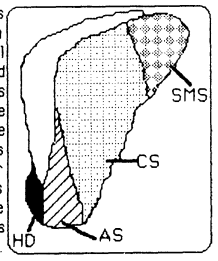
62.1 REGIONAL SPECIALIZATION OF THE BASAL GANGLIA: THE CORTICOSTRIATO-PALLIDAL SYSTEM IN THE RAT OLFACTORY TUBERCLE. D.S. Zahm[1] and Lennart Heimer. Department of Neurology, Univ. of Virginia School of Medicine, Charlottesville, VA. 22908. [1]Currently: Department of Anatomy, St. Louis Univ. Sch. of Medicine, St. Louis, MO. 63104.

The stability in the fundamental organization of the cortico-striatopallidal (CSP) system, despite the diversity of relationships of its various levels with different parts of the brain, leads us to suspect that CSP circuitry provides a cellular mechanism indispensible to neural processing as it is carried out by nearly the entire forebrain. Understanding, however, has been impeded by the absence of a suitably simple model in which input-output relationships might be investigated. The part of the CSP system in the olfactory tubercle (OT) is unique because of the direct and profound influences exerted upon it by responses to olfactory stimuli. OT striatum receives a dense projection from mitral and tufted cells in the olfactory bulb and a massive input from primary olfactory cortex. Other afferents which buttress OT striatal character include tyrosine hydroxylase and cholecystokinin immunoreactive fibers from the midbrain, serotonergic fibers from the raphe, and projections from the amygdala and thalamic intralaminar nuclei.

We have provided additional details about this most ventral part of the basal ganglia using contemporary light and electron microscopic tract tracing methods in combination with immunocytochemical analysis. Despite its diverse input, the output of OT striatum as demonstrated by transported Phaseolus vulgaris-leucoagglutinin appears to end topographically and exclusively in typical striatopallidal synaptic configurations within the polymorph layer of the OT and subcommissural ventral pallidum (VP). Correspondingly, VP in the polymorph OT has fewer efferent targets than more dorsal, subcommissural parts of VP, globus pallidus (GP), or entopeduncular nucleus (EP), projecting significantly only to mediodorsal thalamus and the midbrain ventral tegmental area. As the whole extent of VP, by virtue of dense terminal staining with antisera against glutamate decarboxylase, enkephalin, and substance P, is regarded as neurochemically distinct from GP and EP, VP in the OT polymorph layer can be further distinguished because it lacks terminal staining against neurotensin antiserum, which is a moderate to heavy marker of the subcommissural part of VP and a medial-most strip of GP. Although the origin of neurotensin axon terminals in pallidum requires further investigation, the absence of that marker in the most ventral part of VP further supports our impressions that it is organized more simply than more dorsal parts of the CSP system. Further investigations into the potential offered by the OT as an experimental model of basal ganglia cellular mechanisms are warranted. Supported by NIH grants NS17743 and NS 01799.

62.2 **Functional compartments of the cat striatum.** **A.Jayaraman.** Dept. of Neurology, Louisiana State University School of Medicine, NewOrleans, Louisiana, 70112,USA.

A precise knowledge of the organization of the projections from functionally defined cortical areas is fundamental to our understanding of the functions of the striatum (and the basal ganglia). Recent studies have shown that the rat caudatoputamen may be divided into a medial limbic and a lateral nonlimbic compartment. A detailed analysis of the projections from cytoarchitectonically defined cortical areas suggests that the nucleus accumbens (NA) and caudate nucleus (CN) may have several functional subcompartments. Stereotaxic injections of 0.05 µl of 2% WGA-HRP were placed into the NA and CN in 27 cats. After 24 -36 hrs, the animals were deeply anesthetized, sacrificed, the 40µ thick brain sections were processed for HRP histochemistry. These sections were studied using dark- and brightfield microscopy. Injections in medial NA resulted in selective labeling of cells of CA 1 and subicular regions thereby confirming the hypothesis that the medial NA is the **"hippocampal district"(HD)** of the striatum. Lateral NA injections led to labeling of cells in the prelimbic cortex (area 32), infralimbic cortex (area 25), the entorhinal cortex (area 27), the perirhinal cortex (areas 35 and 36), and the agranular insular cortex. These cortical areas are known to be reciprocally connected with each other. The agranular insular cortex and the infralimbic cortex, the cortical representation areas for gustatory, cardiac, respiratory, and gastric responses, project monosynaptically to the nucleus of tractus solitarius, a major center for visceral responses in the brain stem. This connectivity pattern suggests that the lateral NA receives major projections from cortical areas that are concerned with visceral responses. This sector of the striatum may be called the **"autonomic" or "visceral striatum"(AS)**. Medial caudate injections labeled cells of area 6 aß, the ventro- and dorsolateral orbital cortex, the granular insular cortex, the ventral retrosplenial cortex, the cingulate gyrus, areas 7, 21a, 21b of the suprasylvian gyrus, the dorsoposterior and ventral regions of the posterior ectosylvian gyrus, and the posterior sylvian gyrus. The medial CN is called the **"cognitive striatum"(CS)** since it receives projections from these complex multimodal association areas of frontal, parietal, insulotemporal, and occipital cortex,which are involved in integrative, language and cognitive functions. Since the medial CN receives projections from several cortical areas which have neurons with oculomotor properties, viz., area 6 aß (the frontal "oculomotor" area), area 7, insular cortex, this area of the striatum may also subserve oculomotor functions. The lateral CN receives major projections from the primary motor cortex (area 4), and less dense projections from areas 6 aß, 3,1, and 2 and this region is called as the **"sensory-motor striatum" (SMS)**.



Supported by NS 16609.

62.3 **CIRCUITRY OF THE PEDUNCULOPONTINE NUCLEUS REVEALED WITH FLUORO-GOLD. EVIDENCE FOR A PALLIDAL-TEGMENTAL-SPINAL PROJECTION.** By L.C. Schmued and J.H. Fallon, Dept. of Anatomy, Univ. of California, Irvine.

This study employed a novel fluorescent dye useful for demonstrating retrograde axonal transport. This tracer exhibits numerous advantages over other similarly used compounds in terms of sensitivity, extent of cellular label, permanence, emission intensity, limited uptake by intact axons, compatability with other histochemical techniques, and wide latitude of survival times. This dye, Fluoro-Gold (Fluorochrome Inc. P.O. Box 4983, Englewood, Colorado 80155) was pressure injected as a 3.5% solution. Survival times ranged from one day to one month. Short survival times resulted in gold colored granules in the cytoplasm with frequent axonal label. Longer survival times resulted in extensive staining of dendritic processes.

Initially, the gray matter of the upper thoracic spinal cord of albino rats was injected with Fluoro-Gold. Many nuclei known to project to the spinal cord contained retrograde label. Of particular interest was the retrograde label seen in the pontine tegmentum region corresponding to the areas described physiogically as the mesencephalic locomotor region and anatomically as the pedunculopontine nucleus.

This area was subsequently injected with the dye to determine its afferents. The following regions were found to project to these mesencephalic regions: substantia nigra (especially in pars lateralis, and to a lesser extent, in pars compacta and pars reticulata, subthalamic nucleus, zona incerta, globus pallidus (ventral caudal portion), entopeduncular nucleus, bed nucleus of the stria terminalis, and central nucleus of amygdala.

The third phase of this study involved injecting the above structures with an anterograde tracer (^{3}H-adenosine or PhA-L lectin) to verify these connections. Tissue injected with ^{3}H-adenosine was processed for autoradiography and resulted in silver grains over potential postsynaptic neurons. Tissue injected with PhA-L lectin was processed for immunocytochemistry and demonstrated entire axons and terminals.

The last phase of the experiment involved combining anterograde transport from the aforementioned pallidal regions with retrograde dye transport from the spinal cord. In these cases, anterogradely labeled axons from the globus pallidus or substantia nigra could be seen in close proximity to, and sometimes running alongside of the dendrites of cells in the pedunculopontine nucleus retrogradely labeled from spinal cord injection. Although it is not possible to prove the existence of synaptic terminals with light microscopy, this evidence supports the notion of a direct motor output from pallidum to mesencephalon to spinal cord. Conceptually, these pathways may provide a significant route whereby information exits loops of the extrapyramidal motor system to ultimately innervate motor neurons of the spinal cord. Methodigically, we feel this work demonstrates the superiority of Fluoro-Gold as a retrograde tracer. Supported by grant NS 15321.

62.4 PROJECTIONS FROM THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS TO THE THALAMUS IN THE RAT. B.M. Spann and I. Grofova. Department of Anatomy, Michigan State University, E. Lansing, MI. 48824.

In the rat, the nucleus tegmenti pedunculopontinus (PPN) has been demonstrated to correspond to the mesencephalic locomotor region. The nucleus has been shown to have reciprocal connections with various basal ganglia nuclei and to give rise to a descending projection to the spinal cord. In addition, it provides a prominent cholinergic input to the intralaminar nuclei of the thalamus. Several retrograde transport studies have indicated that the PPN-thalamic connection is both crossed and uncrossed but neither the extent of crossing nor the presence of axonal collateralization have been established.

In the present study a series of experiments were carried out to determine the distribution of thalamic projecting neurons in PPN and to test for the presence of collateralization. In the first set of experiments 0.05µl of 2% HRP-WGA solution was stereotaxically injected into the parafascicular nucleus (Pf) and also involved other intralaminar nuclei. The majority of labeled neurons were located ipsilaterally in a dorsolateral region of PPN which has been identified by Sugimoto and Hattori as the nucleus tegmenti pedunculopontinus pars compacta (PPNc), (Neuroscience, 11, 931-946, 1984). The present study confirmed the presence of a prominent contralateral PPN projection to thalamus. Of the labeled neurons projecting to intralaminar nuclei, 21% were located contralaterally while the remaining 79% were ipsilateral. Furthermore, the contralateral neurons exhibited the same distribution pattern as that seen ipsilaterally. Thalamic projecting cells were either fusiform or multipolar in shape and varied in sizes from 19 to 42µm along the longest axis.

In the second set of experiments Granular Blue (GB) and Diamidine Yellow Dihydrochloride (DYD) were stereotaxically injected ipsilaterally and contralaterally, respectively in the Pf of the rat. The dyes also diffused to adjacent thalamic regions but did not extend to subthalamus. The distribution and morphology of the labeled neurons were similar to that described above. While approximately 85% of the labeled neurons in PPNc projected ipsilaterally to the intralaminar nuclei, the remaining 15% projected contralaterally. Furthermore, no distinct separation of the two populations of PPNc projection neurons was observed. Less than 5% of the PPNc projection neurons possessed collaterals branching to both the ipsilateral and contralateral intralaminar nuclei.

(Supported by NIH Grant NS 19483)

62.5 TOPOGRAPHIC DISTRIBUTION OF CONNECTIONS FROM THE PRIMARY MOTOR CORTEX TO THE CORPUS STRIATUM IN THE OWL MONKEY. H.J. Gould, III and R.H. Whitworth, Jr.* Dept. of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70119.

Projections from motor cortex to corpus striatum were studied in a new world monkey, Aotus trivirgatus. Microstimulation mapping techniques were used to identify specific portions of the primary motor map (Gould et al., '83, Soc. Neurosci. Abst., 9:309). Identified stimulation sites were subsequently injected with wheat germ agglutinin conjugated to horseradish peroxidase. After survival times of 36 to 48 hours, the animals were perfused and the brains were removed from the skull, blocked and cut frozen in the coronal plane. Sections processed with tetramethyl benzidine for peroxidase activity were mounted on slides and examined with the light microscope.

All injection sites were confined primarily to cortical Area 4 and were responsive to current levels between 1.0 and 5.0 μA when presented in trains of 24 pulses. Each pulse in a train was 0.2 msec in duration and was presented at a frequency of 300-350 Hz. The major projections from the primary motor cortex to the corpus striatum terminate bilaterally in the putamen. The general topographic orientation of the representation of body parts presents with the hindlimb located dorsally, adjacent to the cortical white matter and the head ventrally. The most ventral portion of the putamen is free of labelled terminations. The densest terminations are organized into bands that extend between the subcortical white matter laterally and the internal capsule throughout the rostral to caudal length of the putamen. More broadly distributed, sparser terminations are also found to form a much more complex pattern. Contralateral projections to the putamen are found in the same general distribution but are much less dense and appear to be somewhat more restricted in rostrocaudal extent.

Dense terminations in the caudate nucleus are discontinuous and restricted to cell islands located along the medial aspect of the internal capsule. Less dense terminations are confined to fewer cell islands along the medial aspect of the contralateral caudate nucleus. Sparse labelling is also observed in the dorsal-most aspect of the ipsilateral claustrum.

These results are consistent with those reported earlier for old world monkeys (Kunzle, '75, Brain Res., 88:195; Jones et al, '77, J. Comp. Neurol., 173:53; Liles and Updyke, '85, Brain Res., in press) and suggest that there is a general topographic organization in the primate putamen. The differences in the pattern of dense versus sparse terminations within the putamen may reflect differences in the function of the corticostriate projection.

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62.6 SOMATOMOTOR, OCULOMOTOR AND HIGH-ORDER PATHWAYS THROUGH BASAL GANGLIA OF CAT. I.M. Jeffers and C.R. Olson, Psychology Department, Princeton University.

INTRODUCTION. The aim of the experiments reported here was to characterize neural connections between the basal ganglia and cortical areas serving somatomotor, oculomotor and high-order functions. In neuroanatomical tracer experiments, connectional topography was analyzed at each stage of the loop leading from cerebral cortex to caudate nucleus to entopeduncular nucleus to ventral thalamus to frontal cortex.

(1) Cortico-caudate topography. In 7 cats, we placed deposits of nuclear yellow (NY) and bisbenzimide (Bb) at separate sites in the head of the caudate nucleus, then analyzed the distribution of retrogradely labeled cells in cerebral cortex. Dorsolateral caudate injections gave rise to retrograde labeling of primary motor cortex and of cortical areas to which it is linked. Dorsomedial injections gave rise to retrograde labeling of the medial frontal eye field and of cortical areas projecting to it. Ventromedial injections labeled prefrontal cortex and related cortical areas.

(2) Caudate-entopeduncular topography. In the same cases, we charted anterogradely labeled axon terminals in the entopeduncular nucleus. Tracer deposits in dorsolateral, dorsomedial and ventromedial divisions of the caudate nucleus produced terminal labeling in lateral, intermediate and medial divisions of the entopeduncular nucleus respectively.

(3) Entopeduncular-thalamic topography. In 5 cats, we placed deposits of NY and Bb in ventral thalamus, systematically varying the mediolateral placement of the deposit. More medial thalamic deposits gave rise to more medial retrograde labeling in the entopeduncular nucleus.

(4) Thalamo-cortical topography. In 8 cats, we placed deposits of NY and Bb in a cortical zone encompassing the ventral bank of the cruciate sulcus and the adjacent medial face of the frontal pole. As injection-sites moved from somatomotor cortex (deep in the cruciate sulcus) through the medial frontal eye field (close to the sulcal lip) to prefrontal cortex (on the medial face of the hemisphere), labeled cells in the ventral thalamic complex assumed progressively more medial locations.

CONCLUSION. Topographic connectional patterns revealed by this study are compatible with the notion (first proposed for primate by DeLong) that cortical areas with somatomotor, oculomotor and high-order functions project in parallel through the basal ganglia and thalamus to separate divisions of the frontal lobe serving those functions.

62.7 LOCAL PROJECTIONS WITHIN THE DIAGONAL BAND DEMONSTRATED BY ANTEROGRADE TRANSPORT OF PHASEOLUS VULGARIS - LEUCOAGGLUTININ (PHA-L). H.R. Brashear*, D.S. Zahm*, and L. Heimer (SPON: F.E. Dreifuss). Department of Neurology, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

Studies of axonal transport from neurons in the nuclei of the diagonal band (DB) with tritiated amino acids or wheatgerm agglutinin-horseradish peroxidase have emphasized long projections. Golgi impregnated material, however, reveals that many DB neurons have extensive local axon arborizations and suggests that longer intranuclear connections are present as well. Immunohistochemical demonstration of the anterogradely transported plant lectin Phaseolus vulgaris - leucoagglutinin (PHA-L), introduced by Gerfen and Sawchenko (Brain Research, 290:219-238, 1984), permits further investigation of short DB projections.

Iontophoretic injections of PHA-L were placed in the nuclei of the vertical and horizontal limbs of the DB and the anterogradely transported tracer was localized with the biotin-avidin immunoperoxidase method. The discrete nature of the PHA-L injections frequently allowed the study of neurons limited to the DB, which were impregnated in a Golgi-like manner. Labeled perikarya at the injection sites were medium to large with fusiform to polygonal shapes. The large dendritic arbors of these neurons frequently extended across the entire breadth of the DB. Dendrites and cell bodies had few visible spines. Axons with multiple varicosities extended conspicuously along the longitudinal dimensions of the DB. Some of these axons gave off multiple very short collaterals within the DB. Injections in ventral parts of the DB produced numerous clusters of terminals in dorsal parts of the DB near the medial septal nucleus. Bifurcating axons, one branch of which lacked visible terminals, suggested that some of the terminals present in DB arise from collaterals of longer projections. Other labeled axon segments could be seen ending in multiple terminals close to the injection site. Further study will clarify whether these latter fibers arise as collaterals from projection neurons or if some DB neurons have axonal arborizations which remain entirely within DB. These observations suggest that intrinsic connections play an important role in the organization of the neuronal circuitry of the DB.

62.8 THALAMOSTRIATE PROJECTIONS FROM THE VENTRAL ANTERIOR THALAMIC NUCLEUS IN THE DOG. D. Tanaka, Jr., L. G. Isaacson*, and B.K. Trosko*. Department of Anatomy, Michigan State University, East Lansing, Michigan 48824.

Previous studies using retrograde tracing techniques have demonstrated a projection from the ventral anterior thalamic nucleus (VA) to the neostriatum. However, details concerning the topography and patterns associated with VA thalamostriate terminal fields have not been described. In the present study, we examined the distribution and organization of VA thalamostriate projections and terminal fields using both anterograde and retrograde tracing techniques.

Injections of tritiated leucine and proline were made into the rostrolateral, caudomedial, and caudal-intermediate parts of VA in 3 dogs. In each case, the densest accumulations of label were located adjacent to the internal capsule in the dorsal and lateral parts of the head of the caudate nucleus. Projections from caudal VA were located slightly more caudally and ventrally in the caudate than were those from rostral VA. Dense aggregations of silver grains were scattered throughout areas containing more diffuse and widespread label. At some levels small amounts of label were also noted in the dorsal part of the putamen.

Lectin-conjugated horseradish peroxidase was injected into the head of the caudate nucleus in 4 dogs. Small injections into the dorsolateral corner of the caudate resulted in clusters of retrogradely labeled neurons forming a dorso-ventrally oriented arc in the most lateral part of VA. Larger injections placed mid-laterally in the caudate resulted in a more widespread distribution of neurons located in the mid-region of VA. In both cases, the labeled neurons extended throughout the rostro-caudal extent of VA and into the rostral part of the ventral lateral nucleus. Injections placed medially in the caudate resulted in only a few labeled cells located in the dorsal and medial parts of VA.

These results indicate that VA projects topographically to a widespread region in the head of the caudate nucleus, with the lateral and mid-portions of VA projecting heavily to the dorsal and lateral caudate and medial VA projecting sparsely to medial caudate. Since there is evidence to suggest that VA, the basal ganglia, and cortical area 6 may form part of a neural circuit involved with the sequencing of motor activities, it is of particular interest that the densest VA thalamostriate projections appear to terminate in the same region of the caudate that receives the densest projection from area 6.

(Supported by NIH Grant NS16991 and BRSG funds to the College of Veterinary Medicine).

62.9 INTERRELATED ORGANIZATION OF CORTICOCORTICAL AND CORTICOSTRIATAL PROJECTIONS: A MULTIPLE RETROGRADE LABELING STUDY IN THE CAT. R.S. Fisher, M.K. Boylan, M.S. Levine, C.D. Hull, and N.A. Buchwald. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Pl. Los Angeles, CA 90024.

Multiple retrograde labeling methods were used to demonstrate the axonal branching and crossing of the corticocortical and corticostriatal projections of 10 adults cats. Single markers (lectin-bound horseradish peroxidase, nuclear yellow and/or fast blue) were pressure-injected into area 4 of the precruciate gyrus of the neocortex (Cx) and into one or both of the caudate nuclei (Cds). Labeled neurons were demonstrated by combined brightfield and epifluorescent illumination in the uninjected area 4 of the Cx.

Both single and multiple labeled neuronal somata were most prevalent in area 4 of the uninjected Cx. All possible combination of labeling including triple-labeled cells (3 collaterals: commissural corticocortical fibers, decussated corticostriatal fibers and undecussated corticostriatal fibers) were evident. Both the corticocortical and corticostriatal projections originated from a single line of small-to-medium size pyramidal neurons (somatic areas = 129$\pm$ 5μm^2; nuclear areas = 33 $\pm$2 μm^2). These somata were located in both the supra- and infragranular laminae and composed up to 40-50% of all Cx neurons in the regions of the greatest density of labeled cells.

Thus, corticofugal projections to the neocortex and neostriatum demonstrated a rich variety of complex axonal branching and decussation. Collateralized afferents to the Cx and Cd arise from cortical as well as subcortical neurons. The branched corticofugal projections are important because they may subserve intra- as well as interhemispheric connectivity. Supported by USPHS Grant AG 01558, HD 05958.

62.10 THE ROLE OF N-METHYL-D-ASPARTATE (NMDA) MEDIATED EXCITATION, AND CHOLINERGIC MUSCARINIC AND GABAERGIC NEUROTRANSMISSION WITHIN THE RAT VENTROMEDIAL THALAMIC NUCLEUS (VM) IN MOTOR CONTROL. T. Klockgether*, M. Schwarz*, L. Turski*, K.-H. Sontag*. (SPON: J. Noth). Max-Planck-Institute for Exp. Medicine, Hermann-Rein-Str. 3, 3400 Göttingen, F.R.G.

The rat ventromedial thalamic nucleus (VM) is a point of convergence of several anatomical pathways involved in motor function. This nucleus receives GABAergic fibers from the substantia nigra pars reticulata, cerebellar fibers, which possibly use acetylcholine and cortical fibers which use glutamate as their transmitter. The VM is the source of a widespread projection to almost the entire neocortex. To investigate the role of neurotransmitters within the VM in motor control behavioural tests were performed in rats receiving microinjections of transmitter specific drugs into the VM. In addition, electromyographic (EMG) signals were registered from several muscles in awake, unrestrained rats.

The GABA agonist muscimol (MSC) (10-50 ng) and the N-methyl-D-aspartate (NMDA) antagonist 2-amino-7-phosphonoheptanoic acid ((-)AP7) (25-250 ng) injected bilaterally into the VM induced a state of immobility which is generally referred to as catalepsy, whereas the muscarinic antagonist N-methyl-scopolamine (SCOP) (0.5-5.0 µg) was devoid of a behavioural effect. Both, the (-)AP7 (100 ng) and the MSC (25 ng) induced catalepsy were found to be dose-dependent and site specific. The (-)AP7 (100 ng) induced catalepsy was antagonized by both, NMDA (100 ng) and the GABA antagonist bicuculline (BIC) (100 ng). The MSC (25 ng) induced catalepsy was antagonized by both, BIC (100 ng) and NMDA (100 ng).

EMG analysis did not reveal relevant differences between the behavioural state induced by injection of MSC or (-)AP7 into the VM. In both cases, animals displayed a tonic EMG-activity in the gastrocnemius muscle which is considered to reflect limb rigidity. The animals were unable to initiate limb movements or locomotion due to an inability to produce phasic EMG bursts. On the other hand, the muscular reactions which serve to maintain or reinstate the animals static equilibrium were intact.

The present results suggest that the balance between NMDA mediated excitation and GABAergic inhibition within the VM represents a critical factor (1) for the development of limb rigidity and (2) for the animal's motility. The failure of SCOP injected locally into the VM to induce a behavioural effect may be interpreted in terms of a weak cholinergic muscarinic tone in the nucleus.

The study was supported by a grant from the Deutsche Forschungsgemeinschaft (So 136/3-1).

62.11 THREE-DIMENSIONAL RECONSTRUCTION OF THE TOPOGRAPHY OF CORTICO-STRIATAL PROJECTIONS IN THE RAT. J.K. Chapin, M. Sadeq, W.K. Smith, D.S. Schlusselberg, and D.J. Woodward. Dept. of Cell Biology and Anatomy, U. of Texas Health Sci. Cntr., Dallas, Tx. 75325.

The three-dimensional topography of the cortico-striatal projection system in the rat was investigated through use of the orthogradely transported lectin PHA-L. Pressure or iontophoretic injections of PHA-L were made in electrophysiologically and cytoarchitectonically defined subregions of the frontal and parietal cortices. A microscope-computer based system for acquisition and display of neuroanatomical data was used for 3-D reconstruction of the pattern of terminal labelling observed in serial coronal sections.

Overall, injections in the frontal cortex produced a distinctly different pattern of terminal labelling in the striatum than injections in the parietal cortex. Frontal cortico-striatal projections typically coursed posteriorly from the injection site, and terminated in the ventro-lateral quadrant of the striatum. Parietal cortico-striatal projections coursed anteriorly from the injection site and terminated in the dorso-lateral striatal quadrant.

For example, the 3-D pattern of cortico-striatal terminal labelling from the MI forelimb cortex (in caudo-lateral frontal cortex) resembled a rostro-caudally elongated wedge extending posteriorly from the region of the injection site. This wedge was formed by sprays of axons spreading ventro-laterally from the MI corticofugal bundles descending through the striatum. In more caudal coronal sections this labelling could in many cases be observed to divide into an inner core and an outer shell. By contrast, the 3-D pattern of labelling resulting from injections in the caudo-lateral SI cortex resembled an elongated shell following the dorso-lateral border of the striatum up to 4 mm anterior to the injection site. More medially placed SI cortical injections labelled this outer shell, plus a concentric inner shell. Within their respective target zones, projections from both parietal and frontal cortices followed a medio-lateral and a rostro-caudal topography, ie. more rostro-medial cortical injection sites projected to more rostro-medial sites in the striatum.

In conclusion, this investigation revealed markedly different patterns of projection to the striatum from the frontal cortex, as compared with the parietal cortex. Nevertheless, within these categories, a traditional rostro-caudal and medio-lateral topography was maintained. Supported by grants NS18041, AA03901, and the Biological Humanics Foundation.

62.12 COMPUTER-AIDED MAPPING AND RECONSTRUCTION OF THE STRIATO-PALLIDAL PROJECTION IN THE RAT. V.B. Domesick, P.A. Paskevich* and S.W. Matthysse. Depts. of Anatomy and Psychiatry, Harvard Medical School and McLean Hospital, Belmont, MA 02178.

Findings reported during the past decade support a dualism in the innervation of the rostral striatum of the rat. Whereas a large, ventromedial part of the region receives substantial, partly overlapping projections from a variety of structures implicated in the circuitry of the limbic system (hippocampal formation, amygdala, frontocingulate cortex, ventral tegmental area), a smaller dorsolateral sector of the rostral striatum receives only sparse limbic afferents but is densely innervated by the sensorimotor cortex (for a review see Kelley, Domesick and Nauta, 1982). Concurrently, the globus pallidus has been redefined - morphologically as well as histochemically - to include a rostroventral extension, the ventral pallidum of Heimer and Wilson (cf. Haber and Nauta, 1982). The present communication reports an attempt to determine the extent to which the dualism in the innervation of the striatum corresponds to the subdivision of the globus pallidus into a dorsal and ventral pallidum.

In order to study the striato-pallidal projection by the aid of autoradiographic tracing methods, small iontophoretic injections of ^{3}H-Proline^{3}H-leucine were placed throughout the caudatoputamen and nucleus accumbens. The results were mapped and reconstructed on standard rat brain sections by computer-aided methods.

For the purpose of charting the autoradiographic data, an Apple IIe microcomputer was used to control an interactive video imaging and display system which allows us to superimpose computer-generated diagrammatic and densitometric displays over the video transmitted microscopic images. The data thus obtained were used to provide contours for the reconstruction of consecutive sections with a second system using a digitizing tablet linked directly to a 32 bit super mini-computer. The software required to perform three-dimensional reconstructions, calculate volume, surface area, and statistical analysis were developed by us to suit our specific needs.

The results show that the topography of the striato-pallidal projection reflects the dual organization of the striatum. The dorsolateral, non-limbic sector was found to project to the classically defined globus pallidus whereas the limbic, ventromedial sector projects to the ventral pallidum with preservation of the medial to lateral and dorsal to ventral topography. These findings suggest the existence of two parallel trans-striatal conduction lines, one through the dorsal pallidum, the other through the ventral pallidum. Supported by grants NIMH 5P01 MH31154 and NIH 1R01 NS19945.

62.13 **A COMPUTER MODEL OF THE NEOSTRIATAL SPINY NEURON BASED ON HIGH VOLTAGE ELECTRON MICROSCOPIC MEASUREMENTS OF CELL SHAPE.** C.J. Wilson, Dept. of Anatomy, Univ. Tenn. Ctr. Hlth. Sci., 875 Monroe, Memphis TN 38163.

Neurophysiological studies of rat neostriatal spiny cells both in striatal slices and in vivo have yielded puzzling results when analyzed by peeling exponentials from transients recorded at the onset or offset of small intracellular current steps. These experiments give estimates of electrotonic dendritic length ranging from about 1.4 to 1.8 length constants. Because spiny neuron dendrites in the rat range from 150 to 200 µm in length, this implies a length constant in the range of 80 - 150 µm for these neurons. Dendritic diameters for these cells are between 0.35 µm and 1.0 µm over most of their length. Assuming 75 ohm/cm^2 for the intracellular resistivity, these results suggest that the dendritic membrane should have a resistance in the range of 200 to 1500 ohm-cm^2. This range of values produces whole neuron input resistance values that are generally less than that experimentally observed for these cells (20-40 MΩ), and matches the transient data only if very high values of membrane capacitance are assumed (3-6 µF/cm^2). A possible source of error in these calculations is the failure to include a possible contribution from dendritic spines in both the steady state and the transient responses.

These issues were reexamined using a sample of neurons intracellularly injected with horseradish peroxidase after intracellular recording in vivo. The stained neurons were postfixed in osmium tetroxide, and embedded in plastic in preparation for sequential light and electron microscopic study. The entire dendritic tree was reconstructed from serial 50 µm vibratome sections, and the number of dendrites, their lengths, and the occurrences of branch points noted. Selected regions of the cells were then sectioned at a thickness of 5 µm on an ultramicrotome, and sections were placed in folding grids for direct examination in the High Voltage Electron Microscope at 750 or 1000 keV. The resulting images had sufficient resolution to avoid all ambiguities arising from overlapping of spines and silhouetting by the dendrite and allowed measurement of dendrites and spines at 0.01µm precision. These measurements were used in a computer simulation of the steady state and transient responses of the neurons to brief pulses and steps of intracellularly applied current. The spine membrane was found to account for 50 to 75% of the total membrane surface area at all positions along the spiny part of the dendritic tree. As a result, the effect of the spines could be approximated for steady state analysis by increasing the dendritic surface area but maintaining a constant dendritic cross sectional area. The resulting steady state model of the spiny neuron yielded more realistic values for the predicted input resistance of the cells and higher estimates of the membrane resistance. A similar improvement in the model for transient responses was obtained. These results suggest that a large part of the discrepancy between theoretical and observed responses of spiny neurons to intracellularly applied currents may be due to the passive electrical properties of the spines. Supported by NIH grant NS20743.

62.14 SLOW SODIUM AND I_A CURRENTS IN NEOSTRIATAL NEURONS. E. Galarraga, J. Bargas* and J. Aceves*. Dept. Physiology and Neuroscience. Centro de Investigación del IPN. 07000 México, D. F. México.

In the previous Meeting, we presented evidence indicative of the participation of the Ca-activating K conductance determining the firing pattern of neostriatal neurons. Here we have explored the participation of the slow sodium current and of I_A on the firing of these neurons. Using conventional microelectrode techniques, intracellular electrical activity of neostriatal neurons was recorded in slices of rat neostriatum. Long outward current pulses of threshold strength produced a slow and progressively increasing depolarizing potential that, after a long latency, evoked cell firing. TTX (1 µM), but not Co, blocked the slow depolarizing potential. 4-aminopiridine (1 mM) drastically shorthened firing latency and augmented the slow depolarizing potential, which when not reaching firing threshold was seen as a slow oscillation of the membrane potential. The oscillation was not blocked by Co. When the depolarizing pulse was preceded by a conditioning hyperpolarization, the latency for cell firing was prolongued. 4-AP blocked the effect of the conditioning hyperpolarization. In the presence of TTX (1 µM) the depolarizing pulse preceded by conditioning hyperpolarization produced a slow hyperpolarizing potential instead of the slow depolarizing one. TEA (10 mM) did not block the slow hyperpolarizing potential. Conditioning hyperpolarization significantly reduced frequency of neuronal firing. 4-AP also drastically reduced frequency of neuronal firing, but the interval of the two first responses was decreased, and occasionally only slow depolarizing potentials were observed intercalated between the fired potentials. These results strongly suggest that both slow sodium and I_A currents are present in neostriatal neurons, and that both of them play a significant role in determining the firing pattern of the neurons. (Supported by PCCBBNA-020884 grant from CONACyT of México).

62.15 ^{14}C DEOXYGLUCOSE MAPPING STUDIES OF SOMATOSENSORY STIMULI: REGIONAL EFFECTS IN RAT BASAL GANGLIA. L.L. Brown and L.I. Wolfson*. Department of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.

Previous anatomical, electrophysiological, lesion and behavioral studies suggest that the striatum receives sensory information from cortex which may be important for movement feedback, movement planning, learned movements and the orienting response. We used two simple somatosensory stimuli to determine whether and where they affected basal ganglia metabolism, and whether the magnitude of the response in ventrobasal thalamus and primary cortex was preserved in striatum.

^{14}C Deoxyglucose experiments were carried out in three groups of awake, partially restrained rats: no stimulus control (n=8), tactile stimulus (left side, stroke of the forelimb, 1/2sec, 20g force with a modified von Frey hair; n=3) and passive movement stimulus (left forelimb extension-flexion, 1/4sec; n=3). Glucose utilization (umols/100g/min) and R-L% difference ([R-L/L] x 100) were calculated.

Three striatal regions at each of three anterior-posterior planes were analysed. In the anterior and MID striatum (A8620u - A7890u, Konig and Klippel), the tactile stimulus caused a contralateral increase in glucose utilization over controls (R-L% difference = 21±9%) in a specific dorsal region. The passive movement stimulus caused an increase in glucose utilization (R-L% difference = +32±10%) in a posterior (A7470u) and lateral region of striatum, different from the region of the tactile stimulus effect. Passive movement also increased glucose utilization in the globus pallidus (R-L% difference = +8±4%). Both sides of the substantia nigra compacta and a ventral striatal region were affected bilaterally by both stimuli (+40%), but the reticulata region was affected by the tactile stimulus only. For the tactile stimulus group, the ventrobasal thalamus, sensory cortex and striatum showed similar R-L% differences: +24±8, +22±5 and +21±9% respectively. Although data from a paralysed control group are not yet available, the data emphasize the importance of somatosensory stimuli for basal ganglia function, and emphasize that there is localization of function within basal ganglia which will simplify further analyses of striatal function and the relationship of striatal transmitters to function.

62.16 CYTOCHROME OXIDASE ACTIVITY IN THE RAT NEOSTRIATUM: A LIGHT AND ELECTRON MICROSCOPIC STUDY. G.A. Graveland*, L. Schiff* and M. DiFiglia (Spon: J. Nathanson). Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Histochemical studies have shown that the enzyme cytochrome oxidase (CO), which participates in oxidative phosphorylation, can serve as a useful marker for examining metabolic demands in different regions of the central nervous system and within different populations of neurons. CO histochemistry was used to to determine the relative distribution of metabolic activity in neostriatal neurons. Rats (N=5) were anesthetized and perfused with 2% paraformaldehyde and 1-2% glutaraldehyde in phosphate buffer (ph 7.3). Vibratome sections were incubated in 0.15% diaminobenzidine (DAB) for 15 min to 2 hrs. Control sections incubated in DAB plus .1M sodium cynanide failed to exhibit the histochemical reaction. At the light microscopic level a heterogeneous distribution of CO reaction product was observed within caudate neuropil and was characterized by regions of high activity, 200-800 µm in size, surrounded or adjacent to areas of lower activity. At the ultrastructural level CO reaction product was localized to membranes and intracristae spaces of mitochondria. The majority of reactive mitochondria (those containing the densest precipitates of reaction product) were found within distal spiny dendrites in all caudate regions. In areas of high activity the mitochondria within bundles of myelinated fibers and in many axon terminals were also highly reactive whereas those in neuronal somata, primary dendrites and glial cells and processes exhibited relatively little or no activity. The distribution of CO activity was examined quantitatively using a protocol modified from Carroll and Wong-Riley (J Comp Neurol, 222:1984). Mitochondria were rated for their density of CO reaction product as dark, moderate, light, or non-reactive in caudate neuropil taken from CO rich (1506µm^2) and poor (1500µm^2) zones. Results showed that within dendrites greater proportions of the mitochondria were reactive in caudate regions with high CO activity (N=844; 44% dark, 14% moderate, 25% light, and 17% non-reactive) than in regions of low CO activity (N=842; 11% dark, 17% moderate, 39% light and 33% non-reactive). Similarly, greater proportions of the mitochondria localized to axon terminals were reactive in CO rich zones (N= 350; 47% dark, 24% moderate, 20% light and 9% non-reactive mitochondria as compared to areas of low CO activity (N=256; 9% dark 8%, moderate, 23% light, and 60% non-reactive).

The variability in CO activity in caudate may be related to previous anatomical and immunohistochemical observations which show its heterogeneous organization. The processing of numerous synaptic inputs by neostriatal spiny dendrites may be responsible for its high metabolic demands. Supported by grant NS 16367(MD).

62.PO EVIDENCE IN THE CAT THAT THALAMOSTRIATAL FIBERS INNERVATE STRIOSOME OR MATRIX TISSUE ACCORDING TO THEIR SITE OF ORIGIN. C.W. Ragsdale, Jr. and A.M. Graybiel. Psychology Dept., MIT, Cambridge, MA 02139.

By histochemical criteria striosome and matrix compartments can be distinguished in the mammalian striatum. This compartmentalization is observed in the innervation patterns of the major fiber-systems afferent to the striatum. It has not been clear, however, whether, like midbrain and cortex, the thalamus sends fibers to both striosome and matrix compartments; correlative studies of thalamostriatal connections have been limited to those of the posterior intralaminar complex. We report here on the thalamostriatal projections of a range of thalamic nuclei traced from a total of 24 deposits of tracer substances, either a horseradish peroxidase-wheat germ agglutinin conjugate, [35]S-methionine or a mixture of tritiated proline and leucine, made in 14 cats. Adjacent sections from the striatum were processed alternately for autoradiography or peroxidase histochemistry to demonstrate transported label and for acetylcholinesterase (AChE) activity or met-enkephalin- or substance P-like immunoreactivity to show the striosomes. Injection sites were analyzed with reference to maps of thalamostriatal neurons prepared by Beckstead (1984) and Jayaraman (1985).

Injections of the paraventricular nucleus labeled fibers in the caudate nucleus (CN) that traveled in a strip along the ventricular edge of the nucleus and formed patches in the medial and ventral CN. These patches coincided with AChE-poor striosomes identified in adjoining sections. Deposits placed in midline thalamic sites including the rhomboid nucleus also labeled fibers that selectively innervated striosomes in the medial and ventral CN. By contrast, large injections of the posterior intralaminar complex elicited widespread fiber-labeling in the striatum that appeared in the medial CN to be broken up by holes. Comparisons with adjacent sections stained for AChE demonstrated that these gaps in labeling matched the AChE-poor zones. We conclude that the thalamostriatal system is not limited to matrix tissue, that there are thalamic sites that selectively innervate striosomes and that in medial striatum the distributions of fibers from posterior intralaminar and anterior midline thalamus must be at least in part complementary.

Labeled thalamostriatal fibers often were arranged in inhomogeneous patterns that could not be completely accounted for by the histochemical staining. Of special note were fibers labeled by several deposits in the posterior intralaminar nuclei which formed complex patterns in matrix tissue and which, particularly in the dorsal and lateral CN, tended to circumscribe and sometimes to invade the striosomes. Clear instances of 'avoids' of substance P-rich striosomes in the dorsolateral CN (part of the sensory-motor-afferented striatum) were seen following large injections placed near the transition zone between the posterior nuclear group and the ventrolateral nucleus.

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BASAL GANGLIA: IMMUNOCYTOCHEMISTRY

63.1 VARIATIONS IN CHOLINE ACETYLTRANSFERASE DISTRIBUTION WITHIN RAT VENTRAL STRIATUM: AN IMMUNOCYTOCHEMICAL STUDY. P.E. Phelps and J.E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The ventral striatal region, defined by Heimer and colleagues to include the substriatal gray, striatal cell bridges, the olfactory tubercle, and nucleus accumbens, contains high levels of acetylcholine and its synthesizing enzyme, choline acetyltransferase (ChAT). Correlated light and electron microscopic studies were conducted to describe variations in ChAT-positive (ChAT+) terminal-like staining that may represent additional neurochemically distinct areas of the ventral striatum. The specificity of the ChAT monoclonal antibodies and the methods used in this investigation have been previously demonstrated.

Light microscopic findings indicated significant differences in the intensity of ChAT+ punctate staining in ventral striatum that ranged from moderate to extremely dense. Most of the substriatal gray and striatal cell bridges, as well as the lateral part of accumbens, contained a moderate number of thin beaded ChAT+ fibers and punctate structures. The most dense ChAT+ fiber and punctate staining was observed within a stripe that extended along the lateral edge of the substriatal gray into the olfactory tubercle, as well as in dense areas along the striatal bridge between accumbens and the tubercle. In counterstained sections, these intensely stained regions were often associated with clusters of ChAT-negative somata. Similar associations of extremely dense ChAT+ staining with unlabeled cell clusters were observed adjacent to all islands of Calleja. Dense concentrations of ChAT+ beaded fibers were also observed among the granule cells of the islands. An intermediate level of ChAT+ staining was found within the molecular and multiform layers of the tubercle, but relatively little was observed within the dense cell layer, giving the tubercle a laminated appearance. Nucleus accumbens also varied in ChAT+ staining, with a medial, oval-shaped region adjacent to the major island of Calleja being more dense than the lateral portion. EM observations made in substriatal gray and the dense stripe revealed that some of the beaded ChAT+ fibers, initially seen by LM, formed synaptic contacts, and were particularly numerous in the striatal stripe. The ChAT labeled boutons contained pleomorphic vesicles, and those forming synapses were usually associated with symmetrical junctions, most often contacting dendrites. ChAT+ boutons also synapsed with unlabeled medium-sized somata characterized by sparse cytoplasm and either smooth, or in the striatal stripe, indented nuclear membranes. These results suggest that variations in density of cholinergic terminals may define unique subregions of the ventral striatum. Supported by NIH grant NS18858.

63.2 ULTRASTRUCTURAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN RAT BASOLATERAL AMYGDALA. J. Carlsen* and L. Heimer (SPON: G.R. Hanna). Department of Neurology, University of Virginia, Charlottesville, VA 22908

It has been amply demonstrated, that the basolateral amygdaloid nucleus (BL) receives a dense cholinergic innervation from the ventral forebrain cholinergic system. Immunocytochemical localization of choline acetyltransferase (ChAT) was used in this study to identify the ultrastructural morphology and synaptic associations of cholinergic terminals in the BL.

A very dense homogenous pattern of ChAT-positive punctate structures and fine varicose fibers were observed throughout the BL. Electron microscopy demonstrated that many of the punctate structures were axon terminals, many of which established symmetric contacts with soma, dendrites or spines. Occasionally, an asymmetric cholinergic axodendritic contact was observed. Whereas both pyramidal and non-pyramidal neurons (nomenclature, according to Millhouse, O.E. and J. de Olmos, Neuroscience, 10: 1269 (1983)) seemed to receive ChAT-positive axodendritic contacts, axosomatic cholinergic synapses were apparently restricted to non-pyramidal neurons.

Furthermore, a group of hitherto unrecognized small ChAT-positive cells (10-14 µm largest diameter) were observed in the BL. Electron microscopy confirmed the presence of small immunoreactive neurons, and revealed that they received both symmetric and asymmetric ChAT-negative synapses on their soma. These features tend to classify them as non-pyramidal intrinsic neurons.

The BL apparently receives a dual cholinergic innervation, one originating in the ventral forebrain, and another in an intrinsic group of cholinergic neurons. A similar dual cholinergic innervation has recently been demonstrated in the cerebral cortex. Likewise, the ultrastructural characteristics of the cholinergic synapses in the BL share many features with those of the cerebral cortex (Houser, C.R., et al., J. Comp. Neurol. 234: 17 (1985)).

Supported by Danish Medical Research Council grant 12-4983 and USPHS grant NS 17743. Anti-ChAT, courtesy of F. Eckenstein and P. Salvaterra.

63.3 ULTRASTRUCTURAL IDENTIFICATION OF CHOLINERGIC TERMINATIONS ONTO THALAMOSTRIATE PROJECTION NEURONS IN THE DOG. L.G. Isaacson and D. Tanaka, Jr. (SPON: S.T. Sakai) Department of Anatomy, Michigan State University, East Lansing, Michigan 48824

Cholinergic projections from nucleus tegmenti pedunculopontinus (PPN) to the centrum medianum-parafascicular complex (CM-Pf) of the thalamus have been demonstrated in the rat using the retrograde transport of ^{3}H-choline (Sugimoto and Hattori, 1984) as well as in the dog using a combined immunocytochemical and WGA-HRP retrograde labeling technique (Isaacson and Tanaka, 1985). In addition, Sugimoto and Hattori (1984) have shown PPN projections which terminate onto thalamostriate projection neurons in the CM-Pf complex. However, these studies have not directly demonstrated whether cholinergic terminals establish synaptic contacts with thalamostriate neurons. The present study utilizes a combined immunocytochemical and WGA-HRP retrograde labeling technique in an attempt to examine the ultrastructural morphology of cholinergic terminals within CM-Pf and to localize cholinergic terminals making synaptic contact with thalamostriate neurons in the dog.

Thalamostriate projection neurons were localized using the cobalt chloride-glucose oxidase reaction following WGA-HRP injections into the caudate nucleus. The ChAT-immunoreactive terminals (i.e. cholinergic terminals) were visualized using the Avidin-Biotin technique.

Cholinergic terminals within CM-Pf exhibited immunoreactivity associated with the cytoplasmic matrix and synaptic vesicle membrane. The majority of these cholinergic terminals contained populations of pleomorphic synaptic vesicles. Intermediate-type contacts were frequently observed onto dendritic as well as somatic profiles, some of which were identified as belonging to retrogradely labeled thalamostriate projection neurons. This study provides direct evidence of cholinergic terminations onto thalamostriate projection neurons within the CM-Pf complex. In conjunction with previous reports, the results of the present study suggest that thalamostriate neurons in the CM-Pf complex may receive direct cholinergic input from the nucleus tegmenti pedunculopontinus.

(Supported by NIH Grant NS16991 and BRSG funds to the College of Veterinary Medicine)

63.4

SEE SESSION 62.PO

63.5 DIFFERENTIAL DISTRIBUTION OF M1 AND M2 MUSCARINIC BINDING SITES IN THE STRIATUM OF THE ADULT AND IMMATURE CAT. M.A. Nastuk and A.M. Graybiel. Dept. of Psych. & Whitaker Coll.,MIT, Cambridge MA 02139.

Distributions of cholinergic antagonist binding sites in the developing striatum are characterized by dense patches of binding in register with dopamine islands. However, in the mature striatum few clear patches of heightened binding density are visible in sections incubated with ^{3}H-propylbenzilylcholine mustard (^{3}H-PrB; Nastuk and Graybiel'83). This diminished patterning suggested that the early patchiness of binding could be a consequence of developmental events rather than reflecting a nascent functional compartmentalization persisting to adulthood. Alternatively, patterning at maturity could have been masked in the material prepared with ^{3}H-PrB, a nonselective ligand. In an attempt to resolve this question, we examined with ^{3}H-film autoradiography the distributions of muscarinic receptor subtypes in the adult cat's striatum and in a series of fetal and neonatal cats (E40-P4). Binding conditions were chosen to identify M1 and M2 sites according to protocols of Hammer et al.'80 (and pers. comm.) and Potter et al.'84: M1 sites were defined as those labeled with ^{3}H-pirenzepine (^{3}H-PZ;10nM), and M2 sites were defined as those bound with ^{3}H-N-methylscopolamine (^{3}H-NMS;0.3nM) in the presence of 100nM unlabeled PZ (gift of R.Hammer). Binding assays were performed in striatal tissue; K_d's were 7.6nM (^{3}H-PZ) and 0.42nM (^{3}H-NMS), and levels of nonspecific binding were low.

Autoradiograms of mature striatum showed distinct patches of heightened binding density in sections incubated under M1 but not M2 conditions. Patches of putative M1 binding sites matched acetylcholinesterase (AChE)-poor striosomes. Putative M2 binding appeared virtually uniform. At all fetal ages sampled (E40-E58) M1 and M2 sites were distinctly clustered, and both sets of clusters corresponded to AChE-rich patches and to patches rich in tyrosine hydroxylase immunoreactivity (i.e., to dopamine islands). During the perinatal period, both M1 and M2 distributions changed. M2 binding became uniform while M1 binding became less crisply patchy and transiently less tightly linked to local differences in the AChE staining pattern (itself undergoing maturational changes) before becoming resolved into the adult distribution. In all animals there were regional differences in the degree of patterning of M1 binding. For example, in the mature cat the patches were rarely prominent in the dorsolateral caudate nucleus and rarely crisp in the putamen.

The present finding of heightened striosomal muscarinic binding as seen with M1 but not M2 binding conditions suggests that striatal cholinergic function is heterogeneous at maturity. Further, the ontogenetic linkage between dopamine islands and clusters of M1 and M2 subtypes, and the demonstration that dopamine islands match striosomes in the adult cat (Jiminez-Castellanos & Graybiel, in press), suggests that residual clustering of striatal M1 sites may be related to the compartmental distribution of a subset of dopamine containing fibers.Supported by NSF BNS83-19547&the Seaver Inst.

63.6 QUANTITATIVE AUTORADIOGRAPHY OF GABA RECEPTORS IN THE CAT MOTOR THALAMUS. K. Kultas-Ilinsky, J. Fogarty* and I. Ilinsky, Dept. of Anatomy, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Ventral anterior (VA) and ventral medial (VM) thalamic nuclei are known to receive a massive inhibitory input from basal ganglia (BG) in contrast to the VL whose major input is excitatory and from the cerebellum. Recently it has been demonstrated that intensity and distribution of GAD-immunoreactivity differs in individual subdivisions of the cat motor thalamus (Kultas-Ilinsky et al.,1985. J. Neurosci. in press). Thus, VA and VM contain numerous GAD-immunoreactive axo-dendritic terminals of BG origin whereas GAD-immunoreactive structures in the VL are represented mainly by dendro-dendritic synapses. In light of these findings it was of interest to determine the distribution of different GABA receptors in the cat motor thalamus and to learn whether they correlate with the known topography of BG projections and organization of intrinsic neuronal circuitry. The binding of three ligands specific to different GABA receptors was studied: ^{3}H-muscimol(^{3}HM)-an agonist of high affinity $GABA_A$ sites, ^{3}H-Baclofen(^{3}HB)-an agonist of $GABA_B$ receptors and ^{3}H-flunitrazepam, (^{3}HF)-benzodiazepine agonist that binds to low affinity $GABA_A$ sites. The autoradiographs of tissue sections were digitized using a computer image analysis system calibrated to convert the optical density values into concentrations of ^{3}H per g of tissue.

The density and distribution of the three types of GABA binding sites were compared in different subdivisions of the motor and adjacent nuclei of the rostral thalamus. The highest density of ^{3}HM and ^{3}HF binding sites was found in the midline and limbic nuclei whereas the density of ^{3}HB binding in the midline nuclei was lower and comparable to that in VA and VM. The lowest density of all three binding sites was in intralaminar nuclei and the reticular nucleus did not display any measurable binding. Although overall density of receptors in the motor nuclei was always less than in midline and limbic regions the following distribution pattern was noted: medial and posterolateral VM consistently displayed a high density of binding sites for all three ligands; the VL had the highest number of ^{3}HF-receptors; anterolateral VM and VA had the lowest number of ^{3}HM binding sites, moderate density of ^{3}HF binding sites and high number of ^{3}HB binding sites. The results suggest that the distribution of $GABA_B$ receptors correlates the best with the known topography of GABAergic BG projections in the motor thalamus, whereas low affinity $GABA_A$ binding sites are more likely coupled to GABAergic local neuronal circuits. The hypothesis is now being tested in experiments with unilateral lesions in BG.

Computer image analysis was performed in LONI at Dept. Neurol., Washington Univ., St. Louis. Supported by grants from American Parkinson Disease Association and NIH #NSRO119280.

63.7 A COMBINED GOLGI, DEGENERATION, AND IMMUNOCYTOCHEMICAL STUDY OF THE VENTRAL PALLIDUM IN RAT. L. Záborszky, T. Tömböl*, W.H. Oertel, and L. Heimer. Department of Neurology and Clinical Neuroscience Research Center, University of Virginia, Charlottesville, VA; 1st Department of Anatomy, Semmelweis University, Hungary; and Department of Neurology, Technical University, Munich, FRG.

In order to understand the neural circuits of the ventral pallidum, an attempt was made to characterize some of its neurons in terms of three dimensional structure and afferent connections with special reference to GABA-ergic input.

Lesions were made in the rat nucleus accumbens and olfactory tubercle. Some of the animals received 100 µg colchicine in the lateral ventricle. After a survival time of 30 hours, the animals were perfused by a buffered picric acid-paraformaldehyde fixative (Somogyi and Takagi, 1982). Vibratome sections containing the ventral pallidum were first processed for glutamic acid decarboxylase (GAD) or choline acetyltransferase immunohistochemistry. The sections wer then assembled into agar blocks and reprocessed for Golgi impregnation (Freund and Somogyi, 1983). Impregnated cells were photographed in ventral pallidal areas known to receive projections from the nucleus accumbens or from the olfactory tubercle. The sections were gold toned according to Fairen et al. (1977) and embedded into resin.

The impregnated cells had a triangular or fusiform shape, measuring 25 µm (long diameter). The three to four long primary dendrites were characterized by a smooth outline with occasional beaded or undulated portions. Some of the slender dendrites were unbranched, whereas other divided into two-three branches close to the cell body. Only the initial portion of the axons was impregnated. In a few cases we could observe GAD-positive puncta outlining the distal portions of the dendrites.

An electron microscopic analysis is now being performed in order to determine if Golgi-impregnated neurons do receive GAD-containing and/or degenerating terminals from the ventral striatum. We are also analysing the relative distribution of degenerating terminals on ChAT- and GAD-containing neurons.

Supported by USPHS Grant No. 17743.

63.8 STRIATAL(-LIKE) GABA-ERGIC NEURONAL POPULATIONS IN RAT OLFACTORY TUBERCLE, CENTRAL AND MEDIAL AMYGDALOID NUCLEI. W.H.Oertel and E.Mugnaini. Dept.Neurol.,Technical Univ.,Munich,FRG and Dep.Biobeh.Sci.,U.Conn,Storrs, CT 06268, USA.

It was recently demonstrated by immunocytochemistry for glutamic acid decarboxylase(GAD),the biosynthetic enzyme for GABA that the vast majority of neurons in rat striatum, i.e. in caudate/putamen and accumbens nucleus, are represented by two classes of GAD-positive neurons (Oertel and Mugnaini, Neurosci.Lett.,47:233,1984): A minor category consists of medium to large size neurons.The second category represents the vast majority of the striatal medium size spiny neurons. In the present study, we have investigated the distribution of GAD-immunoreactive nerve cell bodies in the olfactory tubercle (OT), which is considered as the most ventral part of the striatum by Heimer (1978), in the islands of Calleja (IC) and in the amygdala.

Untreated rats and rats pretreated topically with colchicine (10-20 µg) 12-40 hr before sacrifice were used. The animals were perfused with a zinc formaldehyde fixative. Floating cryostat sections were processed for GAD-like immunoreactivity with the PAP-method.

In untreated rats,few medium to large size neurons were GAD-immunoreactive in OT, the central amygdaloid nucleus (AC), the medial amygdaloid nucleus(AM) and the massae intercalatae(MI).The vast majority of medium size neurons in the polymorph layer and pyramidal layer of OT, the neurons in the granule cell clusters in IC and the vast majority of neurons in AC, AM and MI were GAD-immunoreactive in optimally stained specimens of untreated rats and were consistently GAD-positive in specimens of colchicine pretreated animals. The remaining amygdaloid areas contained a medium to low density of GAD-positive neurons.

The data indicate that OT, AC, AM and MI and the granule cell clusters in IC bear some resemblence to striatal areas in respect to the types and distribution of GABAergic neurons. In addition, these areas receive catecholaminergic input. As OT shares a high acetylcholinesterase activity with the caudate/putamen and accumbens nucleus, our data support the view of Heimer (1978) that OT is the most ventral subnucleus of the striatum . In contrast, AC and AM exhibit low acetylcholinesterase activity and therefore are suggested to be subsumed under the denominator "striatal-like" areas.

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63.9 ^{3}H-QNB BINDING ON SUBSTANCE P-, AND SOMATOSTATIN-IMMUNOREACTIVE STRIATAL NEURONS. M.A. Ariano & S.L. Kenny.* Department of Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

^{3}H-QNB is a reversible antagonist of muscarinic acetylcholine receptors (mAChr), and has been used to detect binding sites of this neurotransmitter system *in vitro* (Yamamura & Snyder, 1974). Localization of mAChr is very high in the striatum (Nonaka & Moroji, 1984), and we have used a modification of the *in vitro* autoradiographic receptor binding method of Kuhar (Wamsley, et al, 1981) to determine ^{3}H-QNB binding on individual striatal cell bodies. Immunohistochemical characterization of these striatal neurons has been determined prior to analysis of ^{3}H-QNB in an attempt to correlate the distribution of mAChr on perikarya reactive for the undecapeptide, substance P, or the inhibitor of growth hormone release, somatostatin. Both of these compounds have been previously described within neurons of the striatum (Fonnum, et al, 1974; Brownstein, et al, 1977; DiFiglia & Aronin, 1982; Beal & Martin, 1983).

The brains of male Sprague-Dawley rats (200-250 gm) were rapidly removed and frozen in a cryostat at -25°C in the coronal plane. 8 µm sections were thaw-mounted onto chrom alum-coated glass slides. The detection of immunohistochemical reactivity for the two neuropeptide substances was performed first, using the PAP method of Sternberger (1979). The reaction product was visualized using 3,3'-diaminobenzidene as the chromagen. Tissue sections were thoroughly rinsed, followed by incubation with 0.25-0.5 nM ^{3}H-QNB for 2 h at room temperature (Ariano, 1985). The slides were dipped into NTB-3 emulsion (Kodak), dried, and stored for 5-7 days. Sections were developed in D-19 (Kodak), followed by stabilization in fixer. After extensive washing, sections were covered with glycerol, and examined in bright-field optics using a Zeiss Photomicroscope 3.

Regions of densest immunohistochemical staining were chosen for analysis. Preliminary experiments demonstrate a prevalent clustering of ^{3}H-QNB autoradiographic silver grains in the emulsion overlying substance P- and somatostatin-reactive somata within the striatum. Quantitation of the silver grains is currently under examination, comparing the density of mAChr binding sites over the neuropeptide-reactive cells versus the surrounding neuropil.

This work was supported by a grant from the National Science Foundation, BNS 83-20600. MAA is the recipient of an RCDA, NS-00864.

63.10 THE DEVELOPMENTAL RELATIONSHIP BETWEEN DOPAMINE ISLANDS AND EARLY STRIOSOMAL NEURON CLUSTERS IN THE FETAL CAT STRIATUM. A.M. Graybiel & H. Newman-Gage*, Dept. Psychol. & Whitaker College, MIT, Cambridge, MA 02139. (SPON: F.O. Schmitt).

Striatal neurons lying in the histochemically distinct compartments known as striosomes can be pulse-labeled in the cat by single *in utero* injections of ^{3}H-thymidine at embryonic days E23-30. Such ^{3}H-thymidine labeled neurons are already clustered during the late fetal period, and lie in the striatal compartments innervated by the early-forming "dopamine island" fibers. These findings have raised the possibility of a developmental relationship between the clustering of dopamine island fibers and of striosomal neurons. Here we present evidence that both become clustered during a restricted time-window of development (ca. E35-45), and that there is a progressive and orderly spatial linkage between the two.

Brains from E35-50 fetuses were collected following initial exposure to ^{3}H-thymidine at E24-28. Dopamine-containing afferents were identified with tyrosine hydroxylase (TH) immunohistochemistry and ^{3}H labeled neurons by autoradiography. Striatal cell bodies that were heavily labeled (^{3}H CB) were present at all fetal ages. Light labeling was also prominent up to E45. At E35, TH-positive fibers appeared throughout the putamen, were densest in the cell bridges of the internal capsule, and were just beginning to invade the caudate nucleus (CN). ^{3}H CB had a similar distribution, but a prominent band of ^{3}H CB also appeared along the medial face of the CN immediately next to the ganglionic eminence. The CN had a central core with fewer labeled neurons. By E38, occasional streamers of ^{3}H CB connected the medial band with the ^{3}H CB piled up along the internal capsule, and the arrangement of the ^{3}H CB in the putamen was not uniform. TH immunostaining was distinctly inhomogeneous. Patches of TH extended medially and laterally from the cell bridges and there were hints of clustering in the CN core. By E42, ^{3}H CB were present throughout the width of the CN rostrally, and many were clustered. The medial and lateral ^{3}H CB bands, connected by streamers, still appeared farther caudally. Crisp TH patches were present at all levels, and tended to be aligned with clusters (less crisp) of ^{3}H CB. By E45, ^{3}H CB were organized in distinct clusters in the CN, including in its central core. The ^{3}H CB clusters were well-circumscribed at mid-CN levels but appeared to be still nascent elsewhere in the CN. ^{3}H CB clusters of both types were aligned with TH-positive patches. Faint TH patches appeared in medial regions where ^{3}H CB clustering was not pronounced. At E50, crisp ^{3}H CB clusters matched crisp TH-positive patches. We conclude that from near the inception of striosomal-cell and island-fiber clustering, groups of these elements are in a position to interact. Discrete clustering of the fibers occurs earlier than discrete clustering of the cell bodies but small neuronal clumps are visible as early as fiber groupings can be detected. NSF BNS83-19547 & NIH NS07711-01.

63.11 DEVELOPMENTAL ANATOMY OF DOPAMINE ISLANDS IN THE CAUDATE NUCLEUS OF THE CAT. H. Newman-Gage* and A.M. Graybiel, Dept. of Psych. & Brain Science, MIT, Cambridge, MA 02139. (SPON: Walle J.H. Nauta).

During development, the dopamine-containing innervation of the caudate nucleus (CN) is organized in patches known as dopamine islands. Prenatally, these coincide with patches of dense acetylcholinesterase (AChE) staining. In the adult, the dopamine island regions correspond to the zones of pale AChE staining known as striosomes. There are distinct dorsoventral differences in the islandic innervation and in the neurochemical content of striosomes. We report here an analysis of the development of the dopamine islands in a series of cat fetuses taken at almost daily intervals from embryonic day 27 (E27) to birth as well as in kittens aged P1-P8. The islands were identified with TH antiserum (Dr. T. Joh) and were compared to patterns visible in adjoining AChE-stained sections.

TH or AChE activity was minimal in the CN until after E35. By E38, homogeneous TH staining appeared rostrally but farther caudally faint though definite patches were found in the dorsal CN adjacent to the internal capsule. The non-island (or matrix) TH staining was darkest ventrally. AChE staining was similar to TH staining. At E45, obvious patches of TH and AChE activity were present. These were most distinct laterally, but extended to the medial quarter of the CN. Rostrally, the TH-positive patches appeared broad and often interconnected. In more caudal cross-sections, there were increased numbers of smaller, more discrete patches. The dorsal islands were darker than ventral ones and they appeared to extend farther rostrally and caudally. There were similar rostrocaudal differences in the AChE patches and also a striking extension in background AChE activity from closely surrounding the dense patches to diffusely filling most of the area between dopamine islands. With increasing age, the extent and complexity of the TH and AChE patch systems increased. By E61, TH-positive patches appeared throughout the CN, with dorsolateral patches being darkest and ventral patches most weakly stained. AChE patches showed similar patterns except that ventral AChE patches were not yet present in rostral sections. After birth, matrix TH activity increased and ventrally the TH became more homogeneously dark. AChE-positive regions had begun to coalesce, and ventral staining was as dark as that observed dorsally. By P8, zones lacking AChE stain began to appear though TH-positive patches remained. This was the beginning of the transitional phase to the adult striosomal architecture.

Light microscopic immunohistochemistry of the E58 CN with antibodies to synaptic vesicles (Dr. W. Matthew) indicated a higher density of such vesicles in the dopamine islands. This suggests that synaptogenesis in the island system may precede that in the matrix. Electron microscopic immunohistochemistry substantiated this impression, as TH-positive, vesicle-containing profiles were more common in identified dopamine islands than in the matrix. Supported by NSF BNS83-19547, NIH NS07711-01 and the Seaver Institute.

63.12 TRANSPLANTED DOPAMINE CELLS IN OR PERI-VENTRICULAR REGION GROW BETTER THAN THOSE IN THE CENTRAL PART OF THE CAUDATE NUCLEUS. H. Nishino[1], T. Ono[1], J. Takahashi*[1], M. Kimura*[2], S. Shiosaka*[3] and M. Tohyama*[3]. Dept.[1]Physiol. and [2]Anat., Fac. Med., Toyama Med. Pharmaceut. Univ., Sugitani, Toyama 930-01, and [3]Dept. Neuroanat., Inst. Higher Nerv. Act., Osaka Univ., Osaka 530, Japan.

In several brain sites, transplantation of cerebral tissue or cell suspensions ameliorates functional disturbances that follow electrolytic, chemical or surgical lesions. However, some critical factors (embryo age, transplant volume, injection point etc.) affect the growth of the transplant, and consequently control functional recovery. We report here results of transplanting dopamine (DA) cell suspensions into the caudate nucleus to restore motor balance of rats with unilateral lesions in the nigrostriatal DA pathway, and investigating the relation between growth of the DA cells and behavior recovery.

Unilateral lesions in the nigrostriatal DA pathway of recipient rats were made by microinjection of 6-OHDA. DA cell suspensions prepared from the midbrains of embryos (fetal age, 13-15 days) contained 10^7-5×10^7 cells/ml, more than 90% of which were viable. Transplantations of suspensions (microinjection, total volume less than 10 µl) were made separately in two areas in the head of the caudate nucleus, 1.5mm antero-caudally or latero-medially. Motor imbalance due to lesion or after transplantation was estimated from the number of turns following Met-amphetamine injection (5 mg/kg, i.p.). Motor imbalance (turning to the lesioned side) was ameliorated slightly in the 2nd week and definitely in the 3rd and 4th weeks after transplantation. In the 8th week brains were perfused and removed, and frozen sections were made to stain the tyrosine hydroxylase (TH) activity (PAP method). In behavior recovered rats, TH positive cells were scattered around the injection sites, and it was found that positive cells grew better (extensive axon elongation and dendrite branching) when transplantation was made into the medial part of the caudate nucleus, especially into the periventricular region. The extent of ramification of dendrites and axons was well correlated with the degree of behavior recovery. In well recovered cases, synaptic contacts between the TH positive terminals and recipient caudate neurons were observed.

Data suggest that the growth and ramification of transplanted DA cells is the most critical factor affecting functional recovery, and the amount of growth depends on the site of transplantation.

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63.13 TYROSINE HYDROXYLASE IMMUNOHISTOCHEMICAL ANALYSIS OF THE INFERIOR COLLICULUS IN RATS. U.E. Olazábal[1] and J.K. Moore[2]. Depts. of Psychology[1] and Anatomical Sciences[2], SUNY at Stony Brook, New York, 11794.

Previous work from this laboratory has documented a direct projection from the substantia nigra, pars lateralis(SNL) to the inferior colliculus(IC) in rats and cats(1). In the present study, the chemical characteristics of this pathway were investigated in order to further understand its functional significance. In this immunohistochemical study, rats were anesthetized and perfused with 4% paraformaldehyde and 0.5% zinc salicylate as mordant in 0.45% saline(2). Tissue blocks including the superior colliculus (SC), substantia nigra(SN), and inferior colliculus were removed, sectioned, and processed by the Avidin-Biotin variation of the PAP technique, in order to visualize tyrosine hydroxylase(TH-) and glutamic acid decarboxylase(GAD-) immunoreactivity.

Obtained results demonstrated a plexus of varicose tyrosine hydroxylase-positive(TH+) axons in the IC. This plexus of axons and terminals is confined to the external cortex and rostral pole of the colliculus, and is completely absent from its central nucleus. This study has also confirmed the presence of TH+ neurons in two of the areas known to project to the IC, the central gray (CG), and SNL. Tyrosine hydroxylase is the rate-limiting enzyme in the metabolic pathway leading to the formation of both dopamine and norepinephrine. However, neurons exhibiting TH+ activity in central gray and substantia nigra are known to be dopaminergic rather than noradrenergic(3,4,5). The TH+ cells in the SNL appear to be identical as those described by Hökfelt et al. as zone A9L of the dopamine nucleus system(6). These TH+ SNL neurons have the same size, shape, and distribution as cells which backfill from HRP injections in the IC, while glutamic acid decarboxylase-positive(GAD+) cells in this area are markedly smaller and more elongate.

These findings suggets that TH+ neurons in the substantia nigra, pars lateralis are likely to be the source of the terminal field in the external nuclei of the inferior colliculus. Ongoing experiments are further examining the chemical nature of this system, and its possible role in auditory orienting behavior.(Supported by the Deafness Research Foundation).

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63.14 REGIONAL DISTRIBUTION OF DOPAMINE, TYROSINE HYDROXYLASE AND DOPAC IN RAT STRIATUM. S. L. Rubinstein*, C. Sarvey* and K. Gale (SPON:M. Bradford). Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Biochemical studies of the topographical distribution of dopamine (DA) innervation in the rat striatum have yielded conflicting results. Koslow et al. (Neuropharmacology 13: 1123-1130, 1974) reported a biphasic rostrocaudal distribution of striatal DA with peak DA concentrations occurring at the level of the decussation of the anterior commissure and 1.6 mm rostral to this level. Tassin et al. (Brain Research 107: 291-301, 1976) reported DA concentrations and DA uptake activity to be highest in the rostral-most portion of the striatum and decrease progressively in more caudal striatal regions. The latter authors found peak DA concentrations 1-2 mm from the rostral border of striatum. Since both of these studies utilized tissue micropunches which could introduce a sampling error, we examined the rostrocaudal distribution of DA in the striatum by analyzing complete cross sections (500 u thick) of striatum. In addition, we examined the rostrocaudal distribution of tyrosine hydroxylase (TH) activity and DOPAC concentrations utilizing complete striatal cross sections. Fresh-frozen sections of brains, taken from 350g male Spraque-Dawley rats, were made with a cryostat at -15°C. Analysis of DA and DOPAC was performed using HPLC; TH activity was assayed in the presence of saturating concentrations of substrates using a decarboxylase-coupled assay in which the evolution of $^{14}CO_2$ (from 1-^{14}C-L-tyrosine) is measured (Waymire et al., Anal. Bioc. 43: 588-600, 1971).

The distribution of DA was found to be monophasic, showing a peak located between 2 and 3 mm from the rostral border of striatum; the average peak concentration was 900 pmol/mg protein. The DA concentration gradually decreased in both directions from the peak, declining to concentrations approximately 30% - 50% lower at the rostral and caudal ends of the tissue. The distribution of TH coincided with that of DA, with average peak activity of 1.8 umoles L-tyrosine converted to L-dopa/mg prot/hr. DOPAC concentrations did not covary with DA levels, resulting in different DA/DOPAC ratios at different rostrocaudal levels. In general, the highest DA/DOPAC ratios were found midway through the rostrocaudal extent of the striatum and coincided with the region of highest DA content; these values were approximately 35% higher than the DA/DOPAC ratio found in the tail of striatum.

Supported by HHS grant MH32359 and HHS training grant GM07443

63.15 INTRACELLULAR RESPONSES OF CAT CAUDATE NEURONS TO MICROPRESSURE EJECTION OF SUBSTANCE P, CHOLESYSTOKININ, AND THEIR ANTIBODIES. C.E. Adams, C.D. Hull, M.S. Levine, and N.A. Buchwald. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024.

Immunohistochemical and radioligand binding studies have identified substance P (SP), cholesystokinin (CCK) and their receptors within the basal ganglia (BG). Our investigations of SP and CCK function in BG include an examination of responses of intracellularly-recorded cat caudate neurons to micropressure ejections of SP, CCK, GABA and monoclonal antibodies to SP (A-SP) and CCK (A-CCK). We have observed membrane polarization and/or resistance changes to one or more of the test substances in 56 caudate neurons. Of these 56 cells, 22 cells did not return to original control membrane and/or resistance levels following ejection of a test substance and were not included in our analysis. Ten of the remaining 34 cells were tested with SP. Eight responded with a depolarization of the membrane, 1 exhibited an initial hyperpolarization followed by depolarization while the other showed no membrane change. Fourteen caudate neurons tested with CCK responded with membrane depolarization but only 2 returned to control levels. A decrease in the amplitude of the EPSP and in the amplitude of the inhibitory postsynaptic potential (IPSP) to cortical stimulation was observed in 6/9 caudate neurons tested with GABA. The remaining 3 responded to GABA with a decrease in the amplitude of the EPSP only. Ten out of 13 caudate neurons tested with A-SP exhibited a membrane depolarization. No change in EPSP amplitude was observed. Three of 4 cells examined with A-CCK exhibited membrane depolarization while the fourth showed a hyperpolarization. No changes in EPSP amplitude were observed. Recovery times for recorded membrane and/or resistance changes ranged from less than 20 seconds to as long as 100 seconds after termination of pressure ejection. No consistent interactions resulted from the simultaneous application of a peptide and its antibody. A complex interaction between GABA and SP was observed. SP was found to selectively inhibit spike generation but not the EPSP of cells previously inhibited by GABA. In conclusion, the primary response of cat caudate neurons to application of SP, CCK, A-SP and A-CCK is membrane depolarization, frequently of a prolonged nature. These substances may exert a more complex influence on caudate function as is suggested by the observation of a spike/EPSP dissociation with combined GABA and SP application. Supported by USPHS Grant AG 01558, HD 05958.

63.16 CHARACTERIZATION OF NEUROPEPTIDE Y-IMMUNOREACTIVE NEURONS IN STRIATUM OF CATS AND MONKEYS. Y. Smith, J. Dumas* and A. Parent. Lab. of Neurobiology, Fac. Med., Laval Univ. Québec, Canada.

A detailed study of the distribution of neuropeptide Y (NPY) in the striatum of squirrel monkeys (Saimiri sciureus) and cats was undertaken by means of indirect immunofluorescence and peroxidase-antiperoxidase (PAP) methods.

In monkeys, the NPY-immunoreactivity is homogeneously distributed along the entire extent of caudate nucleus (CD) and putamen (PUT), while in cats marked heterogeneities are noted. In the CD of cats, the NPY-immunoreactive fibers and cell bodies are concentrated in patches of various sizes, which can be readily distinguished from zones of poor NPY-immunostaining. In squirrel monkeys, quantitative measurements reveal that the density of NPY-immunoreactive cell bodies is greater in CD than in PUT, and it increases markedly along the rostrocaudal extent of the striatum. In rostral CD and PUT the densities are 23 cells/mm^2 and 14 cells/mm^2, respectively, whereas the values for caudal CD and PUT are 35 cells/mm^2 and 20 cells/mm^2, respectively. In primate CD and PUT the NPY-positive neurons are either triangular, fusiform or globular, with long and smooth dendrites branching infrequently. Measurements made with a Zeiss modular system for quantitative image analysis show that these NPY-immunoreactive cells belong to a single subset of striatal neurons having a maximum diameter of 19.2 ± 0.1 um and a cross-sectional area of 145.5 ± 0.6 um (Mean ± S.E.M.; N=1238 CD cells and 1169 PUT cells). Furthermore, co-localization studies made with the help of the indirect immunofluorescence technique and Tramu's antibody elution method (Tramu et al., J. Histochem. Cytochem., 26: 322, 1978), demonstrate that the vast majority of NPY-positive striatal neurons in monkey also contain somatostatin (SS). Finally, experiments combining the use of the retrograde tracer WGA-HRP with PAP immunohistochemical method for the visualization of NPY were undertaken to find out if NPY-positive striatal cells are output neurons. The injections of WGA-HRP in substantia nigra and pallidal complex of monkeys and cats produces retrograde labeling of numerous medium-sized striatal neurons, but no HRP granules are found in NPY-immunoreactive cells after such injections. The injection of WGA-HRP in frontal cortex of cats is also ineffective in labeling NPY striatal cells. Thus, the NPY striatal neurons in cats and monkeys do not appear to project outside the striatum.

These results reveal that, in mammalian striatum, NPY is mostly confined to a subpopulation of medium-sized neurons which are intrinsically organized and also contain somatostatin. [The antisera were kindly provided by Drs G. Pelletier (NPY) and R. Benoit (SS). Supported by the MRC of Canada].

63.17 RESPONSES IN DOPAMINE TERMINAL REGIONS TO OLFACTORY AND SOMATOSENSORY STIMULATION IN RATS. Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine and Georgia Mental Health Institute, Atlanta, GA 30306.

Dopaminergic neurons in the ventral tegmentum project to various forebrain regions including nucleus accumbens (NAC), olfactory tubercle (OT) and lateral septum (LS). As an initial step towards investigating possible interactions between sensory input and dopamine (DA) neurotransmission, we recorded responses in these three regions evoked by sensory input and by electrical stimulation of the ventral tegmentum. In adult male rats (n=12) anesthetized with chloral hydrate (400 mg/kg, i.p.), single units were recorded with glass micropipette electrodes filled with a solution of 2M NaCl saturated with fast green dye. The olfactory stimuli (ammonia, cedar oil, ethanol, acrylic solvent, rat urine) were applied with cotton swabs placed 1-2 cm beneath the rat's nostrils for 5-10 sec. The somatosensory stimuli used were light touch, tail pressure and foot pinch. Electrical stimuli were applied via bipolar electrodes in the ventral tegmentum and consisted of trains of monophasic square waves (100-400 uA, 0.2 msec duration, 100 Hz). The rates of spontaneous firing (mean ± SEM) for units in NAC, OT and LS were 8.4 ± 1.9, 14.4 ± 3.0 and 7.3 ± 2.6, respectively. Responses consisted of increases or decreases in spontaneous firing rates and were evoked in each brain structure: 11 of 18 units in OT (61%), 11 of 24 units in NAC (46%) and 3 of 13 units in LS (23%). When responses were observed in NAC or OT units, they could usually be evoked by several of the eight types of sensory stimuli used; for responsive units, the mean number of effective stimuli per unit was 4 in OT and 3 in NAC. However, in LS, only cedar oil or acrylic solvent evoked responses in 3 units. The stimuli producing responses in the greatest number of units in NAC were foot pinch and urine, and in OT they were ethanol and solvent. The pattern and direction of the responses to electrical stimulation varied between units. Again, the highest percentage of units responsive to stimulation was found in OT (67%), compared with 38% for LS and 29% for NAC. When present in the same unit (n=13), responses both to sensory and to electrical stimulation tended to be in the same direction. These results demonstrated that olfactory and somatosensory stimulation can evoke responses in a significant proportion of units in DA terminal regions and that many of these units are also responsive to electrical stimulation of the ventral tegmentum. The relationship between DA and sensory evoked responses is currently being investigated.
(Supported by Grant MH 39783 and by the Georgia Department of Human Resources.)

63.18 **QUANTITATIVE AUTORADIOGRAPHY REVEALS A CORRELATION BETWEEN A HETEROGENEOUS DISTRIBUTION OF DOPAMINE (D-2) SITES AND AChE PATCHINESS IN THE HUMAN STRIATUM.** **J.F. Marshall, J.N. Joyce and D.W. Sapp*. Dept. of Psychobiology, Univ. California Irvine, Irvine, CA 92717.**

Using quantitative autoradiography we have shown that the behaviorally relevant D-2 receptors are organized in a lateral to medial gradient in rat striatum (Joyce et al. Brain Res., 1985) which corresponds to similar gradients of acetylcholinesterase (AChE) stain, cholineacetyltransferase activity, and high affinity choline uptake (Joyce, J.N. and J.F. Marshall, Neurosci. Lett., 53:127, 1985). In human striatum, AChE is also heterogeneously distributed, but takes the form of light and dark patches ('striosomes'). We used quantitative autoradiography to determine whether a corresponding patchy organization of D-2 sites occurs in human caudate or putamen. Thin (20 μM) sections of human striatum, obtained from a 34 year old male with no history of neurological disease, were incubated with [^{3}H]spiroperidol (0.6 - 0.7 nM, 100 Ci/mmol, Amersham) or [^{3}H]sulpiride (19 - 20 nM, 75.4 Ci/mmol, NEN) in the presence of 40 nM ketanserin with or without 1 μM (+)-butaclamol. Adjacent sections were processed for autoradiography or AChE histochemsitry. Autoradiographic sections and a set of ^{3}H-standards were apposed to ^{3}H-sensitive film (LKB). Autoradiographs were digitized, linearized and the resulting images photographed for further analysis, or the density of specific [^{3}H]spiroperidol and [^{3}H]sulpiride binding read from regions of rostral striatum with the aid of an image processor (Altar, C.A. et al., Neurosci. Meth., 100:173, 1984). Clearly identifiable high-density and low-density patches of D-2 binding were visible, and the zones could be followed continuously on adjacent sections for at least 120 μM. The concentration of bound radioligand in the high-density patches was twice that in low-density zones. Importantly, high- and low-density D-2 patches showed a good correlation with, respectively, AChE-rich and AChE-poor patches in adjacent sections stained histochemically. The sections used for autoradiography, and later stained with thionin and luxol-fast, did not show any obvious heterogeneity of cell packing density that corresponded to the patchiness of D-2 receptor density. This work shows that the organization of the D-2 receptor obeys the 'striosomal' pattern of AChE histochemistry, which is consistent with the proposed relationship between this class of dopamine receptors and striatal cholinergic neurons (Joyce and Marshall, ibid).
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63.19 STRIATAL AXONS TO GLOBUS PALLIDUS, ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA COME FROM SEPARATE CELLS: RETROGRADE DOUBLE-LABELING WITH HRP AND FLUORESCENT LATEX MICROSPHERES. R.M. Beckstead. Dept. of Anat., Univ. of Virginia Med. Sch., Charlottesville, VA 22908.

The major input to the two segments of the globus pallidus [in the cat, the globus pallidus (GP) and entopeduncular nuc. (EP)] and the substantia nigra (SN) comes from the striatum (caudate nuc. & putamen). Although there exists little direct evidence, it has been implied in several reports that the striatonigral axons are mainly collaterals of striatopallidal fibers. Two recent reports on the primate brain have shown, using double retrograde dye-labeling, that the nigral and pallidal afferent axons originate from separate striatal cells. I tested this finding and the further question of whether the inputs to GP and EP come from separate striatal cells in the cat using a double retrograde method that combines HRP with rhodamine-fluorescent latex microspheres (FLM). These tracers have the advantages that they do not diffuse widely (especially FLM) from the injection site as do the free fluorescent dyes, are unmistakeably distinguishable from one another, and do not fade under excitation light. In each case, two of the three target cell groups were injected, each with a different tracer, until all three possible combinations of two had been obtained several times. Although none of the deposits of either tracer involved an entire target cell group, many deposits were made that covered a large portion of each. In all cases in which the tracer encroached upon a striatal target, there were cells labeled in the striatum that were of a size and shape that is consistent with the observation that they mainly belong to the category of medium striatal cells. Since the striatal projections to GP, EP and SN are each topographically arranged, the zones of cell-labeling within the striatum varied depending upon the portion of the target nucleus involved by the deposit. Thus, in many cases the fields of striatal cells containing one label overlapped only slightly with those in which cells containing the other label occurred. In other cases, however, there was wide overlap of the striatal zones containing cells marked with either tracer. In all cases, very few double-labeled cells could be found, even where hundreds of cells labeled with either tracer were freely intermingled. These findings confirm that, in the cat as in the primate, the striatal axons to SN arise from cells that are largely separate from the striatopallidal population, and further show that the axons to GP and EP also emanate from different cells. These findings are consistent with the recent observation in the cat (and monkey) that the striatal axons to the two segments of the pallidum are histochemically distinct: those to GP contain mainly met-enkephalin whereas those to EP contain substance P. Since GP, EP and SN all receive GABAergic striatal axons, the present findings suggest that individual GABA cells are selective with respect to their pallidal target.
Supported by NIH (NINCDS) grant NS17827.

63.20 SOMATOSTATINERGIC INPUTS TO THE REGION OF THE A8 CELL GROUP. M-F Chesselet. Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20205.

A dense network of fibers expressing somatostatin (SOM)-like immunoreactivity was observed in the rat in a restricted area of the mesencephalic reticular formation (MRF), corresponding to part of the nucleus cuneiformis. The aspect of the labelling at a light microscopic level was compatible with the presence of SOM-positive terminals in this area and was in sharp contrast with a much sparser fiber labelling in surrounding regions, including the pars compacta of the substantia nigra. This SOM-positive region overlapped with an area of dense CRF-, dynorphin- and enkephalin-like immunoreactivity, suggesting that this zone is innervated by multiple peptidergic systems. In addition, part of the SOM-positive region overlapped with the dorso-lateral aspect of the catecholaminergic cell group A8.

In order to determine the origin of this somatostatinergic projection, retrograde tracing with the fluorescent dye fast blue (FB) was combined with fluorescence immunohistochemistry. After injection of the dyestuff into the SOM-positive region of the MRF, but not in adjacent areas, FB was found to be present in cell bodies expressing SOM-like immunoreactivity in the central nucleus of the amygdala and in the rostral and ventral aspects of the zona incerta (but not in the striatum nor the nucleus accumbens septi). The existence of a projection from the zona incerta to the SOM-positive area of the MRF was confirmed by anterograde transport and immunodetection of the lectin phaseolus vulgaris leucoagglutinin (PHA-L)[1].

Taken together, these results suggest that somatostatinergic neurons from the amygdala and from the zona incerta may contribute to the innervation of a region within the MRF including part of the A8 cell group. This is of particular importance considering the broad field of projections of the A8 neurons which includes areas also innervated by A9 (substantia nigra) and A10 (ventral tegmental area) cell groups[2,3]. Therefore, the projections described here could play a strategic role in the regulation of neuronal activity within the basal ganglia.

1, Gerfen and Sawchenko, Brain Res. 290 (1984) 219-238
2, Deutch et al., Neurosci. Abst. 10 (1984) p9
3, Jayaraman, J. Comp. Neurol. 231 (1985) 396-420

The gift of antisera by Drs. R. Benoit, E. Weber, R. Eskay and T. Joh, and the help of Dr. Gerfen with the PHA-L experiments are gratefully acknowledged. Supported by a fellowship from the Huntington's Disease Foundation of America.

63.21 STRIATAL HETEROGENEITY IN RESPONSIVITY TO NEUROLEPTICS: CORRELATION WITH AFFERENT INPUT. A.Y. Deutch, M. Goldstein, and R.H. Roth. Depts. of Pharmacology & Psychiatry, Yale School of Medicine, New Haven, CT 06510 and NYU Medical Center, New York, NY 10011.

Recent findings indicate the the striatum exhibits anatomical, biochemical, and functional heterogeneity. There are considerable differences across the telencephalic dopamine (DA) terminal field regions in biochemical responsivity to neuroleptics. We have therefore examined heterogeneity in biochemical responsivity to haloperidol (HPD) by assessing regional differences in biochemical indexes of dopaminergic neuronal activation. We have further attempted to correlate the observed pattern of regional responsivity with the sources of dopaminergic afferents to these regions.

Adult male rats were sacrificed following HPD challenge and ten regions of the striatum (CP) punch-dissected from coronal slices. Two measures of DA neuronal activation were assessed: the ratio of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) to DA, as an index of turnover, and accumulation of l-DOPA after decarboxylase inhibition as an index of synthesis. The precise source of DA afferents to the striatal subfields as dissected was evaluated by both a combined retrograde tracer-immunohistochemical method and an anterograde (PHA-L) procedure.

Both the magnitude of the HPD-induced increase in DA turnover and synthesis exhibited a two-fold regional variability. The greatest increase in turnover and synthesis was observed in the rostral dorsomedial sector of the CP, whereas the ventrolateral tail of the CP exhibited the least responsivity to HPD. Overall, a dorsal to ventral and medial to lateral gradient running along an oblique rostrocaudal axis was observed in the biochemical responsivity of the CP to HPD. The precise mesencephalic source of the DA innervation of the CP subfields and the biochemical responsivity of these regions was highly correlated. Areas of the CP exhibiting the greatest degree of change in turnover or synthesis received a DA innervation from the medial half of the A9 cell group; areas exhibiting low responsivity were predominantly innervated by A10 and A8 neurons. Those areas intermediate in responsivity received mixed A9-A8 inputs. Areas of the CP receiving DA afferents from the A8 region and pars lateralis exhibited a relatively low degree of responsivity to HPD, suggesting that the pars lateralis may constitute a functional subset of the A8 cell group; this finding is supported by comparative connectivity of the regions. These data therefore suggest that the A9 DA neurons exhibit the greatest biochemical response to HPD challenge of the mesencephalic DA neurons, the A10 neurons the lowest response, with the A8 neurons occupying an intermediate position. The source of the DA afferents to the CP thus appears to be at least one important determinant of the biochemical responsivity of the CP to neuroleptics, a pattern which remains constant across the striatal, mesolimbic, and mesocortical terminal field regions. Supported by MH-09156 and MH-14092.

63.22 SPONTANEOUS ACTIVITY OF DOPAMINERGIC CELLS IN NUCLEUS TEGMENTI PEDUNCULOPONTINUS, AN AVIAN SUBSTANTIA NIGRA HOMOLOGUE. I. J. Goodman. Dept. of Psychology, West Virginia University, Morgantown, WV 26506-6040.

The avian nucleus tegmenti pedunculo-pontinus (TP), according to evidence regarding its location, connections, neurotransmitter (catecholamine) content and behavioral consequences of its perturbation, appears to correspond to the mammalian substantia nigra. Many details regarding comparative structural and functional similarities and differences await clarification. In an effort to contribute toward that and as part of a program to achieve a better understanding of the role of catecholaminergic structures in avian behavior, the present study investigated characteristics of spontaneous single cell activity in avian TP.

The acute pigeon (Columba livia) preparation, maintained under chloral hydrate (initial dose, 400 mg/kg, i.p.), was employed in this study. Glass micropipettes, filled with 2 M NaCl saturated with fast green, were used to record extracellular unit activity under various drug conditions, i.e., anesthetic alone, anesthetic plus apomorphine (APO, dopamine agonist) and anesthetic plus haloperidol (HAL, dopamine antagonist), all delivered through a cannulated wing vein. These conditions were used as a means of pharmacologically differentiating dopamine and non-dopamine cells within TP, according to criteria established in mammals (Bunney, 1979). The final recording sites in animals were marked by a dye deposit at the electrode tip.

Spontaneously active cells were identified in TP with varying response characteristics across cells. Most cells tended to fire at slower rates (below 15 spikes/sec) with irregular interspike intervals, but with spike durations varying across cells (0.8 - 4.0 ms). Most recorded waveforms were biphasic (pos. - neg.) with a minority being triphasic (pos.-neg.-pos.), usually those with longer durations. The injection of APO (.01 - 0.5 mg/kg) produced a significant decrease in spontaneous firing rates, principally in cells showing longer duration spikes (2-4 ms), multiple spikes (bursts) and lower spike rates (below 8/sec), with inconsistent or no effects seen in other cells. HAL (.01 - 0.5 mg/kg) tended to produce a significant rise in firing rates of cells sensitive to APO and/or with shared electrophysiological response characteristics, while also having inconsistent or no effects on other TP cells. These effects provide a basis for separating TP neurons into dopamine and non-dopamine types, according to electrophysiological and pharmacological criteria. This is not unlike the findings reported for the mammalian substantia nigra. No systematic spatial arrangement of these cell types in TP was established.

63.23 CHARACTERISTICS, DISTRIBUTION, AND INTERRELATIONSHIPS OF SOMATOSTATIN, NEUROPEPTIDE Y, AND NADPH DIAPHORASE NEURONS IN HUMAN CAUDATE NUCLEUS.
N.W.Kowall, R.J.Ferrante*, M.F.Beal,and J.B.Martin. Dept. of Neurology and Neuropathology, Mass. General Hosp and Harvard Med. School, Boston MA 02114.

In the present study we have examined the topography of somatostatin (SS), neuropeptide Y (NPY) and NADPH diaphorase (NADPH-d) neurons in human caudate and their relationship to zones of differential acetylcholinesterase (AChE) activity (striosomes). Twelve blocks of striatum were fixed in neutral buffered formalin at 4 C for 48 hours. Sections were incubated with antisera to SS and NPY followed by amplification and detection using an avidin-biotin peroxidase method or indirect immunoflouresence. Absorption controls were consistently negative. NADPH-d staining was performed as previously reported except for the addition of Triton X-100 (0.8%) and monosodium malate (125mg%) to the incubation medium. These modifications enhanced staining of cell arbors and revealed a heterogeneous pattern of neuropil staining. Large numbers of reactive neurones were uniformly distributed throughout the caudate in bands and clumps which avoided zones of low AChE activity. This was especially striking in AChE, diaphorase double stained sections where neurons often congregated along the margins of high and low AchE activity. The background pattern of neuropil diaphorase activity was identical to that of AChE. The striosomal mosaic was modified in the nucleus accumbens where the elliptical or serpiginous low AChE patches were flattened and oriented into a series of horizontal bands. Colocalization studies, including double diaphorase immunocytochemistry and double simultaneous immunoflourescence, showed that SS, NPY, and diaphorase activities coexist in a single population of neurons in human striatum. The morphology of these neurons is variable and can be divided into 4 categories all of which are typical of aspiny type interneurons. Immunoreactive fibers were more prevalent in ventral caudate and accumbens suggesting that extrinsic projections, possibly from the basal forebrain, may contribute to high levels of SS and NPY in these regions. (NWK is an MRC fellow, supported in part by NS 16367).

63.24 QUANTITATIVE AUTORADIOGRAPHY OF ^{3}H-SPIPERONE BINDING SITES IN RAT STRIATUM FOLLOWING DISCRETE CORTICAL LESION.
J.M. Trugman* and G.F. Wooten, Dept. of Neurology, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

Using quantitative in-vitro film autoradiography we have studied ^{3}H-spiperone (^{3}H-SP) binding in rat striatum following a discrete cortical lesion. A 3x6 mm rectangular burr hole was made overlying the frontal cortex of one hemisphere and a suction lesion of the cortex was made within these confines. The time course and location of degenerating nerve terminals within the striatum were verified using a silver degeneration stain. At 5-7 days post-lesion, degenerating nerve terminals were localized to the dorsal striatum. Rats were killed at 5, 10, and 20 days after cortical lesion. Serial 20 μ sections were taken through the striatum for binding studies. Sections were incubated at room temperature for 30 min with 2 nM ^{3}H-SP in 50 mM tris-acetate-saline buffer, pH 7.4. Adjacent sections for nonspecific binding were incubated with ^{3}H-SP plus 2 μM d-butaclamol. Alternate sections were incubated for 90 min at room temperature in phosphate buffered saline with 1 nM ^{3}H-QNB, with and without 1 μM atropine. Sections were coexposed with calibrated ^{3}H Microscales (Amersham) to LKB Ultrofilm. Films were analyzed densitometrically with a Leitz MPV variable aperture microdensitometer.

Three areas within the body of the striatum were analyzed: dorsal striatum (1.3x.8 mm), ventral striatum (1.3 x .8 mm) and a dorsomedial area of pure gray matter adjacent to the lateral ventricle (.5x.1 mm). Four sections were analyzed per animal with 3-4 animals per time point. Paired t-tests were used to compare specific binding ipsi- and contralateral to the cortical lesion. ^{3}H-QNB binding served as a control for intrinsic striatal damage and revealed no significant asymmetries. The table below summarizes ^{3}H-SP binding expressed as fmoles/mg tissue wet weight. There were no differences at any time point.

		Control n=3	5 days n=3	10 days n=4	20 days n=4
Dorsal Striatum	Control	153±8	137±17	153±21	132±10
	Lesion	148±15	156±13	139±30	134±2
Ventral Striatum	Control	136±23	143±33	151±19	126±9
	Lesion	143±21	153±28	137±14	131±13
Dorsomedial Gray	Control	171±17	159±19	164±32	166±17
	Lesion	161±14	158±36	168±40	169±11

These results suggest that a discrete frontal cortical lesion which causes degeneration of cortical afferents in the dorsal striatum does not alter ^{3}H-SP binding. These results do not support the existence of D-2 receptors on cortico-striatal terminals.

MUSCLE I

64.1 VARIABILITY OF AXONAL CONDUCTION VELOCITY MEASUREMENTS IN MAMMALIAN MOTOR UNITS.
D.A. Gordon, T.M. Hamm, R.M. Reinking*, R.M. Enoka, and D.G. Stuart. Depts. of Physiology and Exercise & Sport Sciences, University of Arizona, Tucson, AZ, 85724.

The conduction velocity (CV) measurement is an important consideration in studies on the properties of mammalian motor units. The purpose of this investigation was to evaluate methods of determining CV of motor axons and to establish the reliability of these methods.

Single motor axons to the functionally isolated tibialis posterior (TP) muscle of the cat hindlimb were activated by suprathreshold stimulation of cut ventral-root (VR) filaments. Action potentials evoked in the TP muscle-nerve were recorded extracellularly and averaged with three bipolar electrodes at the popliteal fossa level. CV measurements were made from VR to muscle-nerve (conventional CV) and between proximal and distal pairs of muscle-nerve electrodes (muscle-nerve CV).

The comparison of CV measurements revealed that the muscle-nerve CV was 5.7% slower, on average, excluding corrections for stimulus utilization time, than the conventional CV estimate. Considerable variability, from a variety of potential sources, was observed in these values. Contributions to variability in CV could be attributed to variability in time and distance measurements as well as differences in stimulus utilization time, variable axonal branching, and variability in regional CV.

The variabilities inherent in distance and time measurements were estimated by making multiple measurements of these parameters. The combined uncertainty in these measurements alone accounted for much of the variability in measured values of conventional and muscle-nerve CV in individual experiments, indicating that uncertainties in the time and distance measurements account for most, but not all, of the variance of CV estimates in motor axons. We are still investigating interexperimental differences in CV estimates which were not well accounted for by these error estimates.

The uncertainties in the conventional and muscle-nerve CV estimates due to errors in time and distance measurements were calculated to be 0.9% and 4.8%, respectively. Comparison of these values suggests that a greater degree of reliability is provided by the conventional measurement of CV in single motor axons, due to the longer conduction distances and time provided by this method. This is in contrast to sensory axons whose morphology differs from motor axons, and in which other methods of measuring CV are preferable (Rindos et al., Experimental Neurology, 86(2): 208-226, 1985).

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64.2 FATIGUE-RELATED CHARACTERISTICS OF THE SOLEUS AND EDL MUSCLES IN SMALL AND LARGE CAGE-REARED FEMALE RATS.
K.A. Volz*, L.L. Rankin*, R.M. Enoka, and D.G. Stuart. (SPON: J.B. Angevine). Depts. of Physiology and Exercise & Sport Sciences, University of Arizona, Tucson, AZ 85724.

Previous studies from this laboratory (Enoka et al., and Rankin et al., Soc. Neurosci. Abstr. 10: 781, 1984) have indicated that the soleus (SOL) and extensor digitorum longus (EDL) muscles of male rats were affected by the size of the cage in which the animal was raised. To continue our investigation into cage-size effects, we have examined some histochemical and fatigue-related characteristics in female rat hindlimb muscle.

Female SD weanling rats were raised for 100-135 days in either a small FDA-approved laboratory cage (SC; 5/cage) or in one 258x larger (LC;15/cage). Subsequently, fatigue-related characteristics were determined in vivo for the muscles SOL and EDL, followed by qualitative histochemical analysis utilizing the stains for NADH and myosin ATPase (Peter et al., Biochem. 11(14): 2627, 1972) and measurement of individual fiber cross-sectional area (CSA) (Van Der Meulen et al., Neurology 27: 355, 1977).

The results indicate that, in contrast to our studies using male rats, the fatigue resistance of SOL and EDL in female rats was not significantly affected by cage size. In addition, cage size did not significantly affect the histochemical composition of either SOL, a predominantly slow-contracting muscle (LC-SOL = 85% SO, 15% FOG; SC-SOL = 78% SO, 22% FOG, n = 4) or EDL, a fast-contracting muscle (LC-EDL = 5% SO, 51% FOG, 44% FG; SC-EDL = 3% SO, 57% FOG, 40% FG, n = 4). The mean CSAs for these fiber types in each muscle were not statistically different either. However, when the mean normalized CSA values of LC-SOL were compared to SC-SOL, a significant difference was revealed ($p < .05$). The same relationship, however, did not hold for EDL, but this is, in part, explained by the significant variation ($F_{12,12} = 11.63$, $p < .0025$) in all fiber-type CSAs for the LC- EDL. Further, when the mean CSAs for type SO fibers in all SOL (LC + SC) were compared to the fatigue index (FI; Burke et al., J. Physiol. 234: 723, 1973), a significant correlation was identified ($r = 0.78$, $p < .05$). Finally, there was no correlation ($r = 0.26$) when the FI of all SOL (LC + SC) were compared to the FI of all EDL.

These results suggest: (1) male and female rats respond differently to the opportunities afforded by cage size, (2) SOL is more affected by cage size than EDL, a consequence perhaps of its presumed usage, (3) the correlation between the SO-CSA of SOL and its FI is developed because of biochemical changes in the SO fibers which enhance fatigue resistance and, (4) since intra-animal fatigue resistance for SOL and EDL is not related, the mechanism of fatigue as demonstrated by the FI may well be different for the two muscles.

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64.3 **RELATION BETWEEN WHOLE-MUSCLE EMG AND FORCE DURING A FATIGUE TEST.** R.M. Enoka, L.L. Rankin*, K.A. Volz*, M.J. Joyner*, and D.G. Stuart. Depts. of Physiology and Exercise & Sport Sciences, University of Arizona, Tucson, AZ 85724.

In an attempt to identify the mechanisms associated with the decline in force during a standarized fatigue test (Burke et al., J. Physiol. 234: 723, 1973), we have monitored, in vivo, the compound muscle action potential (AP) and the isometric force during a 6-min test in which the test muscle was activated by supramaximal intermittent stimulation of its nerve. Accordingly, the soleus (SOL) and extensor digitorum longus (EDL) muscles of male and female SD rats (4.36 and 2.68 N, respectively; 120-150 days) were subjected to a protocol that included 1 Hz trains of 13 stimuli delivered at a rate of 40 Hz. The APs were recorded with a pair of intramuscular stainless-steel electrodes and were quantified by average measurements of the area and "mean" amplitude (MA) for the 13 APs within each train (Stuart et al., Proc. XXIV IUPS XV: 190, 1983). The fatigability of the muscles was quantified by a measure of fatigue resistance (FR): peak tetantic force at 6 min relative to initial force.

By raising the rats in small and large cages, the FR of both test muscles extended over a range of values such that the muscles clustered into significantly different ($p < .001$) groups of relative high, intermediate, and low FR: for SOL, these values ($\bar{X} \pm SD$) were 112 ± 7 (n = 10), 92 ± 6 (n = 7), and $67 \pm 14\%$ (n = 5), respectively, while for EDL, the values were 48 ± 5 (n = 8), 33 ± 5 (n = 8), and $10 \pm 7\%$ (n = 8), respectively. Despite these differences in FR for EDL, the 3 groups exhibited a qualitatively similar relationship between AP area and force during the course of the fatigue test. In all 3 groups, the relationship was characterized by an initial potentiation and a subsequent parallel decline in both AP area and force. Quantitatively, however, the high FR group displayed the greatest potentiation of both force and AP area. Somewhat similar relationships were apparent between MA and peak force for EDL, although MA did not potentiate initially as did AP area. The changes in area and MA during the test were similar for EDL ($r^2 = 0.85$, $p < .01$). In contrast, the high and intermediate FR SOL groups demonstrated a markedly different area-force relationship than the low FR group in that force essentially remained constant while AP area potentiated for the former groups and, conversely, area remained constant while force declined for the low FR group. In addition, the changes in AP area and MA for SOL, while significant ($p < .01$), were less related ($r^2 = 0.35$) than for EDL.

It was apparent that under these conditions (cf. also Sandercock et al., J. Appl. Physiol. 58: 1073, 1985) both AP area and MA changed during the course of the fatigue test. However, we are uncertain at this point as to whether these AP changes reflect a change in the neural drive, a tissue-filtering effect, or some combination of the two mechanisms.

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64.4 **COEXISTENCE OF WHOLE-MUSCLE FATIGUE AND TWITCH POTENTIATION IN RAT HINDLIMB MUSCLE.** L.L. Rankin*, R.M. Enoka, K.A. Volz*, and D.G. Stuart. (SPON: A. Kaszniak) Depts. of Physiology & Exercise & Sport Sciences, Univ. of Arizona, Tucson, AZ 85724.

The degree of potentiation of isometric twitch tension is dependent upon activation history and muscle-fiber type (Burke et al., Brain Res. 109: 515, 1976; Jami et al., J. Physiol. 340: 129, 1983). Comparing whole muscle twitches before (pre) and after (post) a 6-min fatigue test, we observed greater tension in post-twitches than in pre-twitches in some muscles despite significant fatigue developed during the intervening test.

These observations were based upon data acquired during a previously reported study on the effects of cage-size on muscle properties in rat soleus (SOL) and extensor digitorum longus (EDL) (Rankin et al., Soc. Neurosci. Abstr. 10: 781, 1984). The protocol included: (1) 32 twitches at 0.5 Hz; (2) 1 100-Hz tetanus (500 ms); (3) 6-min fatigue test (13 stimuli at 40 Hz repeated 1/s); (4) 8 twitches at 0.5 Hz; and, (5) 2 100-Hz tetani. Fatigue resistance was determined by expressing peak tetanic force at 6 min relative to the initial peak force. Mean values (n = 18) for peak tension (P_t), time-to-peak tension (TPT) and one-half relaxation time (½ RT) were determined for the 8 twitches immediately preceding (pre) and the 8 twitches following (post) the fatigue test.

Twitch potentiation (post P_t/pre $P_t > 1.0$) was observed in 61% of EDL muscles despite a mean 52% decline in peak force during the fatigue test. In contrast, only 33% of SOL muscles potentiated while force declined by 17% during the test. Given their fiber-type compositions (EDL = 3% SO, 59% FOG, 38% FG; SOL = 84% SO, 16% FOG; Ariano et al., J. Histochem. Cytochem. 21: 51, 1973), this difference between EDL and SOL is consistent with the previously reported observation, albeit with a different protocol, that the coexistence of fatigue and potentiation occurred most frequently in FR motor units (FOG fibers), less in FF units (FG fibers) and infrequently in S units (SO fibers) in cat peroneus tertius (Jami et al., 1983). However, in contrast to these investigators, we found positive linear correlations between fatigue resistance and twitch potentiation for both EDL and SOL ($r = 0.94$ and 0.86, respectively).

TPT and ½RT times were significantly longer in post-twitches (123% and 170%, respectively) for EDL, providing further evidence of muscular fatigue (Edwards et al., J. Physiol. 251: 287, 1975). No significant changes in these values occurred in SOL. The change in TPT for EDL in association with potentiation is consistent with the notion that repetitive stimulation can alter the state of activation and hence twitch dynamics (Wilkie, Br. Med. Bull. 12: 177, 1956).

The simultaneous occurrence of whole-muscle fatigue and potentiation suggests that the mechanisms underlying these two phenomena affect different sites within the muscle, but the linear relationship suggests that they are not totally independent of one another.

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64.5 LONG DURATION MUSCLE FATIGUE CAUSED BY IMPAIRED EXCITATION-CONTRACTION COUPLING. R.G. Miller, H.S. Milner-Brown*, D. Hooper*, R.B. Layzer*, & M.W. Weiner*. Department of Neurology, Children's Hospital of San Francisco, San Francisco, CA. 94118 and Department of Neurology, Radiology and Medicine, University of California/ San Francisco, San Francisco, CA. 94143.

The mechanism of long-lasting muscle fatigue has been studied utilizing simultaneous measurements of 31^P Nuclear Magnetic Resonance spectroscopy, surface rectified integrated EMG(RIEMG) and force from the human adductor pollicis. During a four minute maximum isometric contraction to produce muscle fatigue, pH fell from 7.1 to 6.4, phosphocreatine fell to undetectable levels, and mean maximum force fell by 80%; neuromuscular efficiency (NME) (NME=Force (N), in a 5s, 50% maximal contraction) fell to 45% of RIEMG (mV)
the pre-fatigue value immediately after the four minute fatiguing contraction.

During recovery, pH and high energy phosphates returned to control levels within thirteen minutes and maximum force generating capability returned to 96% of control levels within 15 minutes; however, NME recovered more slowly (mean 70% at fifteen minutes and 85% at thirty minutes).

To test our hypothesis that the delayed recovery of NME was due to persistent impairment of excitation-contraction coupling, post-tetantic potentiation of the twitch was examined before and after fatigue. Post-tetanic potentiation (PTP) was 60% in the fresh muscle, less than 25% after fifteen minutes of recovery from fatigue with gradual return toward baseline levels over a time course that paralleled that of NME. Similarly, the response to low frequency stimulation recovered more slowly (85% of control within 21 minutes of recovery) than the response to high frequency stimulation which was nearly complete (97% after twenty-one minutes of recovery).

These observations support our hypothesis that long duration fatigue is independent of muscle pH and inorganic phosphates. A persistent impairment of excitation-contraction coupling is likely based on these observations.

64.6 **ENDURANCE TIME CHARACTERISTICS OF THE DORSIFLEXOR AND PLANTARFLEXOR MUSCLES OF THE HUMAN ANKLE** A. Vahid Shahidi*, R.E. Kearney and I.W. Hunter (SPON: H. Galiana). Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montréal, Québec, Canada, H3G 1Y6.

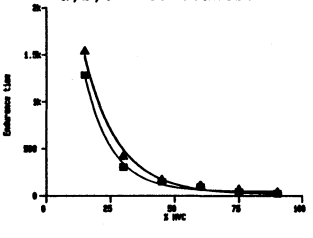
Neuromuscular fatigue may be defined as any reduction in the force generating capacity of the neuromuscular system (Bigland-Ritchie & Woods, Muscle & Nerve, 7:691, 1984). The time for which a specified force can be maintained, the **endurance time**, provides a convenient measure of the fatigue resistance of a muscle. It has been sugggested that the ankle plantarflexors are more resistant to fatigue than the dorsiflexors (Belanger et al., Eur J Appl Physiol, 51:381, 1983), but the relative endurance times of these two muscle groups does not appear to have been documented previously. The purpose of the present study was to determine the endurance times of the ankle dorsiflexors and plantarflexors as a function of the level of contraction.

Subjects were required to match a constant target signal by generating an equivalent ankle torque. Subjects were instructed to stop contracting when they could no longer achieve the target level. Target levels ranging from 15% to 90% of the maximum voluntary contraction (MVC) were examined for both plantarflexing and dorsiflexing contractions. Ankle torque and surface electromyograms from tibialis anterior, triceps surae, and lateral gastrocnemius were sampled at 20 hz throughout the contractions. The endurance time was defined as the time at which the ankle torque first dropped below 95% of the target level.

The figure shows endurance time as a function of contraction level for both plantarflexing (triangles) and dorsiflexing (squares) contractions for a typical subject. Endurance time decreased with increasing contraction level in an approximately exponential manner. Indeed, a simple exponential model fitted to the data provided a good fit; the variance accounted for by this model was always greater than 97.5% for the four subjects studied to date. The excellent quality of the fits obtained is shown by the similarity of the symbols (experimental data) and the smooth lines (predicted values) in the figure.

$$T = ae^{-M/b} + c$$

T = endurance time, (s)
M = % MVC,
a,b,c = constants.



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64.7 REGIONAL DISTRIBUTION OF THE OXIDATIVE CAPACITY AND ITS RELATIONSHIP TO FATIGUE RESISTANCE OF MOTOR UNITS.
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The activity of an oxidative metabolism marker enzyme has been observed to be limited in its predictability of the fatigue resistance(FI) of normal and spinal transected muscle(Baldwin JAP 56:1602,1984). Some disassociation of these two parameters is further suggested by essentially a bimodal distribution of FI in tibialis anterior(TA) units while a population of fibers from this muscle displays a unimodal distribution of succinate dehydrogenase(SDH) activity(Roy Neurosci. Abstr. 73,1984). These results imply that other factors inaddition to the absolute oxidative capacity of a muscle unit determine the FI.

Thirteen TA units were isolated by ventral root splitting and identified by glycogen depletion. SDH activity was measured by a quantitative histochemical technique. The regional distribution of SDH activity was calculated from the mean activity of concentric rings(starting from the edge of the fiber and progressing to the center) and expressed as the slope of the activity of each successive ring(radial distance from edge/SDH activity). The FI was calculated from the ratio of the initial tension relative to the tension at 2 min during 40pps for 330msec stimulation.

Units displayed FI's that ranged from .01-1.0 and SDH activities from 31 picoM/min to 88 picoM/min. Units displayed also one of three consistent and unique distributions of SDH. These are characterized as A)homogenious throughout the fiber, no slope B) mean slope = -0.6 um/picoM/min C) mean slope = -1.0 um/pM/min. Profile C was associated also only with fibers that stained lightly for myosin ATPase(pH 8.8). A non-linear relationship was observed between the mean SDH activity of the unit and FI. However when both the absolute SDH activity and its regional distribution(SDHxSlope) were considered a linear relationship was observed with FI. It appears that the combination of the total SDH activity of a unit's fibers and how that activity is distributed throughout the cross-section of the cells is an important determinant of FI. Further, the regional distribution of SDH activity is related to the physiological classification of unit types in the TA.
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64.8 NON-CORRESPONDENCE BETWEEN ELECTRICAL AND MECHANICAL PROPERTIES OF DIAPHRAGM MOTOR UNITS.
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If the motor unit action potential (MUAP) is a reliable manifestation of the electro-chemical events leading to force generation and if fatigue results from a failure in these events then changes in the MUAP should correspond with force decline. The purpose of this study was to assess the relationship between electrical and mechanical properties of diaphragm motor units during a commonly used fatigue test. In anesthetized cats, MUAP's were recorded from pairs of fine wire electrodes implanted in the sternal, costal and crural regions of the right hemidiaphragm. After freeing the costal margin from the rest of the rib cage, a force transducer was attached in series with diaphragm fibers. The central tendon was clamped near the fiber insertion and fixed to the frame in which the animal was positioned. Following a cervical laminectomy, the spinal cord was transected at C3, and dorsal roots from C4 to C7 were cut bilaterally. While the C5 ventral root was stimulated supramaximally, muscle fiber length was adjusted so that force measurements were made under optimal isometric conditions. Filaments of the C5 ventral root were then dissected until single motor units in the diaphragm were isolated based on criteria of constant evoked MUAP and twitch force responses. Motor units were classified as either fast or slow based on their twitch contraction times and the presence or absence of sag. To assess motor unit fatigability, ventral root filaments were stimulated at 40 pulses/sec in trains of 330ms duration at 1 train/sec. A fatigue index (FI) was calculated as the ratio of force produced after 2 min of stimulation divided by the initial force. During the fatigue test, evoked MUAP's were plotted in a raster display to determine changes in waveform and peak to peak amplitude. In some fast fatigable units (FI < 0.25), changes in the amplitude and/or waveform of the MUAP corresponded with the reduction in force. Similarly, in some slow and fast, fatigue-resistant motor units (FI > 0.75), little change in MUAP amplitude or waveform occured. However, we encountered a significant number of fast motor units whose evoked MUAP's remained unchanged even in the presence of a substantial decline in force. In other motor units, changes in the MUAP occured with very little decrement in force. We conclude that changes in the MUAP during this particular fatigue test do not consistently correspond with the classification of motor unit types. Thus, these data indicate that motor units classified as the same type may have different mechanisms of fatigue.
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64.9 SOMATOTOPIC ORGANIZATION IN THE SEGMENTAL INNERVATION OF THE DIAPHRAGM.
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The purpose of this study was to determine the topographical organization in the segmental innervation of the diaphragm using the method of glycogen depletion. This technique offers the advantage over previously used methods in that the spatial distribution of muscle fibers innervated by stimulated axons can be identified using histochemical techniques. Adult cats were anesthetized and fine wire electrodes were implanted in the sternal, costal and crural regions of the right hemidiaphragm. The costal origin of diaphragm fibers was freed from the rib cage and attached in series with a force transducer. The central tendon was clamped near the insertion of muscle fibers and fixed to the frame in which the animal was positioned. Muscle fiber length was adjusted so that force measurements were made under optimal isometric conditions. Following a cervical laminectomy, the spinal cord was transected at C3 and dorsal roots from C4 to C7 were cut bilaterally. Ventral roots from C4 to C7 were dissected and evoked EMG and/or force responses determined. Responses were primarily limited to the C5 and C6 ventral roots, although smaller responses were occasionally observed by stimulation of C4. To deplete muscle fiber glycogen, the C5 or C6 ventral root was stimulated at 50 to 75 pulses/sec in trains of 100 ms duration at 2 trains/sec. Stimulation was continued until force reached minimal levels (1 to 2 hrs). The diaphragm was then excised and segments from the entire muscle were rapidly frozen. Alternate muscle cross sections were stained for glycogen using the periodic acid-Schiff (PAS) reaction and for myosin ATPase to classify fiber types. Muscle fiber staining intensity in the PAS reaction was quantified using a computer-based image analysis system. Fibers on the non-stimulated side were used as controls. Approximately 3,000 fibers were sampled in each diaphragm. The glycogen content (PAS optical density) of fast-twitch fibers was distributed bimodally, with no overlap between depleted and control fibers. In slow-twitch fibers, a bimodal distribution of glycogen content was also observed, but there was overlap between stimulated and control fibers. Estimation of proportions of fibers,innervated by each ventral root was therefore based on measurements in fast-twitch fibers. Stimulation of C5 resulted in glycogen depletion in about 90% of fibers in the sternal, ventral costal and ventral crural regions. Stimulation of C6 resulted in a similar depletion of fibers in the dorsal costal and dorsal crural regions. There was a gradual reduction in the numbers of fibers innervated by each ventral root moving toward the mid-costal or mid-crural region. An overlap in innervation occured in the mid-costal region with C5 innervating fibers on the abdominal side and C6 fibers on the thoracic side.
Supported by NIH Grant HL29999-03

64.10 INNERVATION RATIO, FIBER SIZE AND SPECIFIC TENSION OF TYPE-IDENTIFIED MOTOR UNITS IN THE CAT TIBIALIS ANTERIOR
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Types of motor units identified by physiological and histochemical profiles differ in the maximum tetanic force (P_o) produced by the single units. These differences might be related to the number of fibers per unit, i.e., innervation ratio (IR), fiber cross-sectional area (CSA), and/or specific tension (SpT). Previous investigations of type-identified motor units, however, have not included direct measurements of each of these factors, and therefore, information regarding the regulation of P_o in different motor unit types is based on indirect estimations.

In this study, the factor(s) responsible for the variability found in P_o of motor units were determined for units of the tibialis anterior (TA). The TA was chosen because it has relatively long fibers. Units in the TA were characterized physiologically as S, FR, or FF (Burke et al. Science 174, 1971) and subsequently depleted of their glycogen through repetitive stimulation of an isolated ventral root filament. Cross-sections were stained for glycogen using a periodic acid-Schiff reaction and analyzed using an image processing system which allowed for the measurement of single-fiber optical densities and CSA. This system permitted a more precise identification of those fibers belonging to the motor unit (MU) than did visual inspection alone. The number of fibers belonging to the MU was determined in a number of cross-sections taken along the length of the muscle. CSA was measured for each fiber belonging to the MU. Specific tension (SpT) was calculated as the ratio of P_o : total CSA, where total CSA equals the sum of the areas of all the fibers belonging to the MU.

Type	P_o (g)	Mean CSA (um^2)	IR	Total CSA (cm^2)	SpT_o (kg/cm^2)
S	2.3	1935	80	.0015486	1.49
S	4.7	3007	88	.0026463	1.78
FR	8.6	2234	161	.0035968	2.39
FR	9.6	2629	198	.0048387	1.98
FF	11.5	3239	166	.0053766	2.14
FF	16.0	2953	193	.0056991	2.81
FF	27.5	3693	281	.0103799	2.65
FF	28.5	3287	379	.0124592	2.18

These data suggest that type S units in the TA have a somewhat lower SpT and IR than type FF and FR units, and moreover, a lower SpT than the type S units in the cat soleus Neurosci. Abstr., 25.5, 1984). The FF and FR units appear to have similar SpT and IR, but differ considerably in fiber size. This observation might explain the differences in P_o between FF and FR units.
(Supported by NIH Grant NS 16333.)

64.11 CORRELATION OF ELECTROPHYSIOLOGY AND HISTOCHEMISTRY OF MURINE MUSCLE FIBER TYPES USING HORSERADISH PEROXIDASE. J.A. Florendo*, J.F. Reger*, and P.K. Law (SPON: R.A. Schreiber), Depts. of Anatomy, Neurology, and Physiology/Biophysics, Univ. of Tennessee Ctr. Hlth. Sci., Memphis, TN 38163

Miniature end-plate potentials (MEPPs) were recorded in vivo at 37°C from surface fibers of the fast-twitch extensor digitorum longus (EDL) and the slow-twitch soleus (SOL) muscles of adult Bar Harbor 129 mice. Previous studies demonstrated that the EDL MEPPs had a significantly higher frequency, smaller amplitude, and shorter duration than the SOL MEPPs (Exp. Neurol. 82:404-412, 1983). To test if these electrophysiologic differences were characteristics of the different histochemical fiber types of these muscles, the electrophysiology and histochemistry of individual muscle fibers were compared using horseradish peroxidase (HRP) for identification. MEPPs from individual fibers of EDL and SOL muscles were recorded and measured for frequency, amplitude, and duration. After each recording, the muscle fiber was injected with HRP by depolarization at 2mA for 30 sec for subsequent cytochemical identification. Each whole muscle was then removed and immediately frozen in methyl-butane cooled by liquid nitrogen. Serial sections (10 μm) were stained for HRP, to identify the fiber from which the recording was made, and for succinic dehydrogenase, phosphorylase, and acidic and alkaline myosin ATPase. The MEPP parameters of each fiber were compared with its cytochemical profile. Muscle fibers exhibiting higher frequencies, smaller amplitudes, and shorter durations corresponded with fast glycolytic or type IIB fibers. Fibers demonstrating lower frequencies, greater amplitudes, and longer durations were found to be slow oxidative or type I fibers. And fibers with overlapping characteristics were shown to be fast oxidative glycolytic or type IIA fibers. Therefore, this study demonstrates a direct correlation between MEPP characteristics and histochemistry of fiber types.

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64.12 SEXUAL DIMORPHISM OF MUSCLE FIBER TYPES IN THE LARYNX OF *XENOPUS LAEVIS*. G.E. Gray, D.A. Sassoon, and D.B. Kelley (SPON: W. Walthall) Department of Biological Sciences, Columbia University, New York, NY, 10027.

Sex-specific vocal behaviors of the adult South African clawed frog, *Xenopus laevis*, are sensitive to circulating androgens (Wetzel and Kelley, *Horm. Behav.*, 1983). Males produce mate calls, prolonged bouts of repetitive trills that attract females. Females produce ticking, a brief and intermittent vocalization, signalling sexual unreceptivity. The larynx, which is the vocal organ, is markedly sexually dimorphic in overall size and in muscle mass. Using histochemical methods, we examined adult laryngeal muscle for sex differences in fiber type and studied the effects of circulating androgens.

Laryngeal muscle from sexually mature males and females, one-month castrated males, and one-month testosterone-treated ovariectomized females was cryostat sectioned and histochemically reacted for succinate dehydrogenase (SDH) and ATPase activity . Thigh muscle, which has no apparant dimorphism, was assayed for comparison. Our results show that muscle fiber types differ in the larynx of male and female *X. laevis*. Female laryngeal muscle is composed of a heterogeneous population of fiber types, in which both the SDH and ATPase reactions reveal a population of dark, light, and intermediate-staining fibers. Male laryngeal muscle is composed of a homogeneous population of fibers that stain dark for SDH, intermediate for ATPase at pH 8.5, and light for ATPase at pH 4.5. These results suggest that male laryngeal fibers are of the fast-twitch-oxidative-glycolytic type (terminology of Peters et al., *Biochem.*, 1972). Thigh muscle does not show sexual dimorphism in its fiber type. Its staining pattern for SDH and ATPase is heterogeneous, although SDH activity is lower than in laryngeal muscle of either sex, suggesting an overall greater oxidative capacity in the larynx. In castrated males and testosterone-treated females, the overall pattern of staining was similar to that of intact untreated adults. We therefore are investigating whether regulation of fiber type is due to hormone action at an earlier period of development. The fiber type of the male larynx suggests that it is especially adapted for the demands of mate-calling, which requires fast muscle contraction and high oxidative capacity for prolonged activity.

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64.13 MORPHOLOGY-HISTOCHEMISTRY OF INTRINSIC LINGUAL MUSCLES IN MACACA FASCICULARIS. Roxanne De Paul*, Amy Wood*, James Abbs, & Robert Sufit. (Spon. C. Welt) Speech Motor Control Labs.(Waisman Center) & Dept. of Neurology. Univ. of Wisconsin, Madison, WI 53705-2280.

Despite the importance of primate intrinsic tongue muscle functions in speech and feeding, current data consist primarily of gross morphology; there is little or no information on primate tongue muscle fiber types, their inter- or intramuscular distribution, or the nature of the associated connective tissue. These data are basic to the understanding of tongue motor control and associated CNS organization. Likewise, because these muscles coactively manipulate the three-dimensional shape of the tongue, their mechanical inter-relations are also of importance.

Whole tongues were obtained from 10 M. Fascicularis monkeys and variously blocked to permit serial sectioning in the coronal, parasaggital, and horizontal planes. The tissue was mounted with tragacanth gum, quick frozen at 160 degrees C in liquid nitrogen-cooled isopentane, and sectioned serially (8 u) to permit longitudinal and cross-sectional views of superior longitudinal (SL), inferior longitudinal (IL), transverse (T), and vertical (V) muscles and their inter-relations. Sections were processed using modified Gomori trichrome, NADH-tetrazolium reductase, myosin ATPase, reverse ATPase, and Verhoeff Van Giesen methods.

Morphologically, these tongue muscles were notably different from limb muscles. Fiber inter-digitation among the four muscles was striking. Connective tissue (both collagen and elastin) was seen along the length of most fascicles, providing mechanical interfaces to mucosa, other fascicles of the same muscle, and other intrinsic muscles. As such, these muscles were inseparable mechanically; contraction of one intrinsic muscle would appear to change the orientation of other intrinsic muscles, hence yielding complex interdependent actions.

There were fiber type variations both among and within these intrinsic muscles. In the very tip of the tongue, fibers in the four muscles were almost exclusively type II. Slightly more posteriorly, the strong predominance of type II fibers in SL and IL muscles was maintained while, by contrast, there was an abrupt transition in V and T muscles to about 40% Type I fibers. Most posteriorly, Type I and II fibers were distributed about equally in all muscles. These fiber type variations suggest that the anterior and posterior segments of SL and IL may function separately, i.e., type II fiber contractions underlie the characteristic rapid movements of the tongue tip while the slower, postural actions of the posterior tongue body logically are more dependent upon type I fibers. The potential neural correlates of these intra-muscular variations (e.g., motor and sensory cortical fields, XIIth nucleus MN musculotopy) need to be investigated. (Supported by NIH; NS-13274 & HD-03352; & MDA).

64.14 MUSCLE SPINDLE DISTRIBUTION IN THE INTRINSIC TONGUE MUSCLES OF M. FASCICULARIS: RELATION TO MUSCLE FIBER TYPES. Robert L. Sufit, James H. Abbs, Roxanne De Paul*, & Amy Wood*. Speech Motor Control Labs. (Waisman Center) & Dept. of Neurology. Univ. of Wisconsin, Madison, WI 53705-2280.

As described in a companion paper (De Paul et al., this meeting), the intrinsic tongue muscles of M. Fascicularis manifest a nonuniform distribution of type I and type II muscle fibers; Specifically, the most anterior portions of the superior longitudinal, inferior longitudinal, transverse, and vertical muscles (the tongue tip) are comprised almost exclusively of type II fibers, while the posterior portions of these same muscles have approximately equal numbers of type I and type II fibers.

The present study, conducted on five animals also studied in the companion paper, was aimed at determining if muscle spindles were concentrated in regions comprised of type I fibers. Such spindle concentration has been reported for many limb muscles and appears to be paralleled by preferential stretch reflex activation of muscle fibers in the muscle regions where the spindle receptors are located. This possibility has significance for the intrinsic tongue muscles inasmuch as (1) several investigators have reported an absence of a stretch reflex in the anterior tongue muscles, and (2) anterior and posterior segments of the tongue are involved in quite different motor actions, indicating probable differential control.

The whole tongues from the five M. Fascicularis were variously blocked to permit serial sectioning in coronal, parasaggital, and horizontal planes. Tissue was quick frozen at 160 degrees C in liquid nitrogen-cooled isopentane. The serial sections (8 u) were processed to permit identification of muscle spindles using modified Gomori trichrome and Verhoeff Van Giesen methods. Sectioned tissue from each tongue was examined along several representative 4-5 cm segments, always including the very tip. Because muscle spindles in the tongue are reported to be different from those in limb muscles, several criteria were utilized by two independent observers to ensure positive identification.

Spindles were not numerous. However, they were seen almost exclusively in the posterior portions of the four intrinsic muscles examined. That is, there were no spindles in the tongue tip and most were in the posterior one-half. As such, consistent with limb muscles, spindles were absent in the intrinsic tongue muscle regions comprised mainly of type II fibers and identified readily in regions which had a sizable proportion of type I fibers.

These data reinforce the probable independent activation of the anterior and posterior portions of the intrinsic tongue muscles and suggest that stretch reflexes might be discernible if one were to monitor EMG activity posteriorly. (Research supported by NIH; NS-13274 & HD-03352; & MDA).

64.15 HISTOCHEMISTRY OF GASTROCSOLEUS MUSCLES IN THE TWITCHER MOUSE
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The twitcher mouse (twi) is a recently discovered authentic model of globoid cell leukodystrophy (GLD) and the genetic defect is a deficiency of galactosylcerimadase activity (Kobayashi et al, Brain Res. 1980). The primary pathological lesions involve the meylin of both central and peripheral nervous systems. In peripheral nerve, axons are not involved and remyelination follows demyelination (Duchen et al, Brain, 1980). The disease is progressive and the animals usually die within 8 weeks. In a recent study, A.M. Yeager and associates (Science, 1984) have shown that hematopoietic cell transplantation (HCT) prolonged survival and peripheral nerve remyelination in the twi. Motor conduction velocities remain slow in HCT-treated twi, but the animals remain active (Toyoshima et al, Neurology, 1985). There are limited data on the effects of this disease and prolonged demyelination-remyelination on muscle fibers in hind limbs. Reports in patients with GLD have suggested that congenital muscle fiber type disproportion (CMFTD) occurs and may have a neurogenic mechanism. We have examined soleus, plantaris and gastrocnemius muscles in untreated and HCT-treated twi and controls at 28, 40, 84 and 109 days to determine whether there are any histological-histochemical changes. The muscles were frozen in isopentane and liquid nitrogen, sectioned in a cryostat at 10 µm and reacted for myofilbrillar ATPase at alkaline (pH 9.4) and acid (pH 4.35), NADH diaphorase and modified trichrome using established techniques. Serial cross sections were obtained, photographed and the muscle fibers (mean of 426) typed as I (oxidative), IIA and IIC (oxidative-glycolytic) and IIB (glycolytic). Whole muscles of the twi were smaller than controls. At 34-45 days, the soleus (mean wet weight) was 31% control, the plantaris was 29% control and the gastrocnemius was 25% control. There were no degenerating or regenerating fibers and no target fibers. Muscle fiber atrophy was evident at 40 days and was more prominent in type II fibers than type I. There was no fiber type grouping, group atrophy or CMFTD. Studies in the normal soleus revealed a mean (4 animals) of 33% type I, 65% type IIA and 2% type IIC fibers; no type IIB fibers were observed. In the twi, the mean percent of type I (34%) fibers remained the same, no IIB fibers were seen, somewhat fewer type IIA (59%) fibers and more type IIC (7%) fibers were present. The type IIC fibers were increased at 84 and 109 days only. This study suggests that in the twi, demyelination is not associated with fiber type grouping, but muscle fiber atrophy and some increase in type II fibers occurs.

64.16 INFLUENCE OF NEURAL ACTIVITY ON MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERISTICS OF RAT SKELETAL MUSCLE.
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In this study we have used three models of altered rat hindlimb muscle activity to determine the factors of neural innervation responsible for intact skeletal muscle structure and function. These were: 1) sciatic nerve crush producing denervation with subsequent reinnervation, 2) spinal cord section with resultant paraplegia, and 3) hypokinesia by hindlimb suspension. All of these resulted in disuse of the hindlimb, but with the lesions at different functional levels of the motor system. At times ranging from 0 to 4 weeks the predominately slow (type 1) soleus and the predominately fast (type 2) extensor digitorum longus (EDL) muscles were removed for analysis. Actomyosin ATPase reactions were performed on mid-belly cross-sections of the muscles for evaluation of fiber number, fiber type proportions, and quantitative morphological analysis. These results were correlated with muscle activity as measured by single unit and whole muscle electromyography.

In the soleus, all of these treatments produced an increase in the proportion of type 2 fibers at the expense of type 1 fibers. Total fiber count showed that this was due to a conversion of reaction characteristics rather than to a drop-out of type 1 fibers. Morphological analysis showed a decreased mean diameter and an increased variation in fiber diameter of all fiber types with all treatments. The EDL was less affected by each of these treatments. These similar histochemical effects were associated with very different patterns of electrical activity. Nerve crush abolished neurally-induced electrical activity until innervation was re-established. In contrast, spinal cord section produced little change in muscle activity, although the hindlimbs were not used for ambulation. Hypokinesia resulted in the muscles becoming virtually electrically silent, but without a structural lesion to the neuraxis.

All of these treatments produced qualitatively similar changes in the morphological and histochemical characteristics of the soleus in spite of very different patterns of muscle electrical activity. This suggests that some feature of these treatments other than activity, per se, is responsible for the normal fiber type characteristics. The common feature of these treatments is the prevention of weightbearing and useful ambulation. These data support the hypothesis that the soleus depends not only on neural activity but also on load-bearing for maintenance of normal muscle characteristics.

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REFLEX FUNCTION I

65.1 MECHANICAL ANALYSIS OF "ANTAGONOGENIC" REFLEX ACTION IN DECEREBRATE CATS.
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The mechanical response properties of an intact joint should be determined not only by the inherent mechanical properties of the muscles acting at the joint and autogenetic reflexes acting on these muscles and synergists, but also by the actions of pathways which interconnect the antagonists. For example, one would predict from electrophysiological studies that the force response of a muscle stretched by joint rotation should be enhanced by disinhibition through the reciprocal Ia inhibitory pathway from the released antagonist (Hultborn H, et al.,Acta physiol. scand., 96:368, 1976). I evaluated the actions of such "antagonogenic" pathways by making simultaneous mechanical measurements on pairs of antagonists acting at the cat's ankle. Cats were decerebrated at the premammillary level under halothane. The right foot was disarticulated and the tendons of either soleus and tibialis anterior or soleus and extensor digitorum longus were connected through separate myographs to two length-controlled motors. Ramp-and-hold length changes of 0.25 to 8 mm in amplitude were applied either simultaneously or individually. Force responses were measured at ramp termination (dynamic response) or at the end of the 300 ms hold phase (static response). Excitabilities of the two muscles were adjusted by changing the initial length of the flexor relative to the extensor or by coactivating the muscles with stimulation in the red nucleus using tungsten microelectrodes. Force responses in soleus were reduced by up to 50% when soleus stretch was accompanied by stretch of one of the flexors, so long as the flexor was not slack and gave measureable responses to stretch. The inhibition was present over a wide range of initial forces in both muscles. A powerful inhibition of soleus could also be produced by vibrating the flexor at 150 Hz and 50 µm peak-to-peak amplitude. Increases in the responses of soleus due to simultaneous release of a flexor (normal mechanical coupling) were also observed. These antagonogenic effects were abolished by dorsal root section. Antagonogenic effects on flexors due to stretch or release of soleus were seldom observed.

These results show that antagonogenic pathways are potentially quite powerful and suggest that they act to enhance the mechanical stiffness of soleus but not the stiffnesses of ankle flexors in the decerebrate cat. This pattern could possibly be achieved, according to known connections, by a facilitation of transmission in the flexor-coupled Ia inhibitory interneurons (cf. Hultborn, et al., ibid.). It would be presumed that the extensor-coupled Ia inhibitory interneurons are inhibited or activated so intensely by the brainstem that transmission from afferent sources is occluded.

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65.2 LACK OF STRETCH REFLEX COMPENSATION FOR FATIGUE INDUCED CHANGES IN STIFFNESS OF THE FLEXOR POLLICUS LONGUS.
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The hypothesis that stiffness is regulated by feedback from muscle force and length receptors has been supported experimentally by the observation of compensation for both yielding and inherent stiffness asymmetries, and by the small variability of stiffness over a wide range of initial forces. However, in decerebrate cats these properties are achieved without significant force feedback.

The present experiments in humans were designed to test for force feedback and stiffness regulation in the face of internal disturbances to the hypothesized force feedback loop. Stretch reflexes were compared at the same level of motor neuron pool output, but at different forces, and correspondingly different inherent muscle stiffnesses. This was achieved by fatigue.

Joint position disturbances (4 deg. ramp in 150 ms) were applied at the interphalangeal joint of the thumb while normal adult subjects contracted the flexor pollicus longus (FPL) to produce a flexion torque. The subjects were instructed not to react to the disturbances. Joint position and torque and FPL EMG were measured before, during, and after ramp disturbances in joint angle. Stretch reflexes were studied at several initial torques prior to and about 45-60 min. after fatigue. Fatigue was induced by a series of 50 to 100 intermittent isometric contractions at a torque near the maximal torque employed in the reflex studies.

Stiffness was measured before reflex action (0-40 ms after ramp onset, prereflex intrinsic stiffness), just after short latency reflexes(65-100 ms after ramp onset), and in the steady state (350-650 ms after ramp onset). Prior to fatigue, stiffness increased with initial torque or EMG at all time periods. Fatigue reduced muscle gain (as measured by the slope of the torque-EMG relationship) up to 40% in some subjects without affecting the EMG power density spectrum. Pre-reflex intrinsic stiffness had the same dependence on initial torque as it had prior to fatigue. Stiffness at other times was lower after fatigue whether compared at the same torques or the same EMG's. The degree of stiffness reduction was related to the degree of fatigue.

Since stiffness was lower at the same level of EMG, the muscle force increment and hence the force feedback inhibition should have been reduced, leading to an increased EMG response if the gain of force feedback was significant. In contrast, EMG responses superimposed well when compared at the same initial value, suggesting that the response was uniquely related to the initial value of EMG, and was not influenced by feedback from muscle force receptors. Thus, force feedback appears to play a negligible role in the FPL stretch reflex, and stiffness is not preserved in the face of fatigue.

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65.3 IDENTIFICATION OF NEUROMUSCULAR DYNAMICS OF THE HUMAN ANKLE DURING TIME-VARYING CONTRACTIONS. R.E. Kearney and I.W. Hunter, Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montréal, Québec, Canada, H3G 1Y6.

It is well known that the mechanical and reflex properties of the neuromuscular control system change dramatically with the level of muscular activity. System identification studies from our laboratory have shown that, under stationary conditions, elastic joint stiffness and reflex gain increase proportionaly with increasing mean tonic activity. However, it is not known how these properties behave under time-varying conditions such as frequently occur in functional situations. The objective of the present work was therefore to identify the time course of changes in neuromuscular dynamics throughout a time-varying isometric contraction of the human ankle muscles.

We have used a technique similar to that of Soechting et al (J.Neurophysiol., 46:1226, 1981). We have, however, extended the method by deconvolving the input waveform from the output so that the system impulse response function may be determined without requiring the input waveform to be white.

Subjects were trained to generate 2s long isometric contractions in response to a computer-generated tracking stimulus at 5s intervals. Simultaneous, stochastic perturbations of ankle position were elicited by applying a computer-generated pseudo-random sequence to the input of our ankle actuator. The actuator input was shifted with respect to the tracking stimulus by one sample for each presentation. The voluntary contraction therefore followed the same time course for each response in the ensemble. The ankle perturbation was different for each response; indeed the perturbation sequence across the ensemble was the same as along a realization. Consequently, system identification could be performed across the ensemble, rather than along a particular realization. The major advantage to this was that mean torque was quasi-stationary across the ensemble but time-varying along each realization. This identification was repeated for each point in the response; the resulting ensemble of impulse responses functions characterizes the time-varying system dynamics.

Using ensembles of 200-300 reponses we have identified the dynamic ankle stiffness at intervals of 5ms throughout the contraction. Stiffness impulse response functions identified for times before the contraction started, or after the target level had been reached, were comparable to those obtained using stationary techniques. However, during the rising phase of the contraction joint stiffness was much larger than expected. This increased stiffness was associated with a decrease in the variance accounted for by the impulse response functions. We are currently attempting to determine whether these changes reflect true changes in joint mechanics or arise from difficulties associated with the identification technique.

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65.4 POSITION DEPENDENCE OF STRETCH REFLEX DYNAMICS AT THE HUMAN ANKLE. P.L. Weiss, R.E. Kearney and I.W. Hunter. Biomedical Engineering Unit, McGill University, Montréal, Canada, H3G 1Y6.

Although the ankle joint angle varies substantially during functional activities, little is known about the effect this has on stretch reflexes at the ankle. The purpose of this study was to examine the effect of changes in mean ankle position on the human ankle stretch reflexes during tonically-maintained contractions held over most of the range of motion. The ankle was placed at randomly selected mean positions, target levels of triceps surae (TS) or tibialis anterior (TA) tonic activity were generated, and the ankle was displaced by small amplitude, stochastic perturbations. System identification techniques were used to identify the dynamic relation between positively rectified ankle velocity and TS EMG (TS stretch reflex) and between ankle velocity and TA EMG (TA stretch reflex) at each tonic level/ankle angle combination.

The principal finding was that TS stretch reflexes depended strongly upon the position of the ankle while TA stretch reflexes did not. The TS stretch reflex magnitude increased by as much as a factor of 23 as the ankle was progressively dorsiflexed. In contrast, position-dependent changes in the TA stretch reflex magnitude were relatively small and variable. Such disparate behaviour is consistent with previous results where we demonstrated differences in the shape, linearity, and gain of the TS and TA stretch reflex dynamics (Kearney & Hunter, Experimental Brain Research, 1983; 1984). Moreover, it is not unexpected given the number of physiological, biomechanical, and functional differences between these two agonists.

The question to be answered is what mediates the position-dependent facilitation of the TS stretch reflex? It was not due to changes in the level of skeletal motoneuron excitability since significant variations in the mean EMG were not evident; for a given level of tonic activity, changes in mean agonist EMG were small and not systematically related to either ankle angle or the magnitude of the reflex response. A more probable explanation is that changes in mean ankle position acted by modulating the efficacy of the stochastic ankle perturbation. This could be accomplished in several ways. First, transmission of the afferents mediating the TS stretch reflex could be altered by position-induced discharge of spinal interneurons. Second, the sensitivity of the muscle spindle endings to the dynamic perturbation could be altered by changes in the extent of fusimotor drive. Third, transmission of the dynamic position perturbations to the spindle, and hence the magnitude of the spindle receptor discharge, could be altered by changes in the mechanical properties of either the extrafusal or intrafusal muscle fibers. The relative importance of each of these mechanisms remains to be determined.

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65.5 AMPLITUDE MODULATION OF THE SOLEUS H-REFLEX IN THE HUMAN DURING WALKING AND STANDING. C. Capaday* and R.B. Stein. Department of Physiology, University of Alberta, Edmonton, Canada T6G 2H7.

The role of the stretch reflex (or its electrically elicited counterpart with electrical stimulation, the H-reflex) in the control of movement remains controversial. Stretch and H-reflexes are highly modulated during locomotion of a decerebrate cat on a treadmill (Akazawa et al., J. Physiol. 329: 553, 1982), but have yet to be studied in detail in normal human subjects (Morin et al., Neurosci. Lett. 33: 47, 1982). One problem that is encountered with surface electrodes on human subjects is that during each step the position of the electrodes may change relative to the nerve and hence the effective stimulus intensity may vary.

To overcome this problem a range of stimulus voltages were applied to the tibial nerve and the direct effect of stimulating motoneurons (M-wave) was monitored by electrodes over the soleus muscle, as well as the reflex (H-wave). Intervals between stimuli were varied in a random fashion within the limits of 0.4 and 2.0 s. Computer analysis allowed averaging of the waves at 16 different phases of the step cycle (measured with respect to EMG activity in the tibialis anterior muscle). The value of the H-wave was determined using stimulus voltages which produced comparable M-waves during each phase and hence similar, effective stimulus intensities.

In all subjects tested the amplitude of the H-reflex was strongly modulated during the walking cycle and was highest during the stance phase, when the soleus muscle was active. In many subjects the peak reflex occurred close to the peak in soleus EMG activity, but in others it occurred earlier in the cycle. The form of the reflex variation during the step cycle could also be quite different in form than that of the EMG produced during stepping. The H- and M-waves were also determined using similar stimulus intensities when the same subjects were standing and exerting varying amounts of steady force. For equal stimulus strengths and EMG levels, the H-reflex was much larger, up to 3.5 times, during steadily maintained contractions than during walking.

The large reflexes, when subjects were standing, are consistent with the fine control of position in this task. Similarly, the reflexes during walking are greatest during the stance phase, when they will assist in maintaining the upright position of the body against gravity. The reflexes are smallest during the swing phase when they would be counter-productive and would resist the flexion of the ankle. However, since the reflex amplitude is task dependent (greater in standing than walking at the same EMG and stimulus levels), and is not always closely related to the EMG produced during a given task such as walking, the strong modulation of the H-reflex during walking is not simply a passive consequence of the changes in soleus motoneuron excitability, but must depend on central mechanisms in addition to those that modify firing.

65.6 JENDRASSIK MANEUVER INCREASES SOLEUS H REFLEXES WITHOUT CHANGE IN BACKGROUND EMG. R. Dowman and J. R. Wolpaw. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201 and Depts. of Neurology and Anatomy, Albany Medical College, Albany, NY 12208.

In the Jendrassik maneuver (JM) contraction of remote muscles (e.g. wrist extensors) increases H reflex amplitude (e.g. in the soleus). The present study examined whether change in background soleus EMG is responsible for this phenomenon, and whether it can be obtained at different levels of background soleus EMG.

H reflexes, recorded from the soleus (sol), were elicited by stimulation of the posterior tibial nerve (PT) in ten subjects (8 male, 2 female; 25-40 yrs old). The Jendassik maneuver consisted of squeezing a hand grip. Recording electrodes placed over the extensor digitorum (ED) monitored the level of contraction. The experiment was controlled by computer. Subjects initiated each trial by maintaining sol background EMG within a preset range for a randomly determined 1-2 sec interval. The preset range was 0-70 μV for the low background condition and 70 μV-3.0 mV for the high background condition. All subjects maintained sol background EMG within reasonably stable limits (average values were ± 3.2% for the low background condition, and ± 9.6% for the high background condition), eliminating the need for tighter baseline controls. Once the sol background requirement was met, a 2000 Hz tone was given which lasted 1.5 sec. The PT stimulation was given 300 msec following the onset of the tone. During the control condition (C) subjects held the handgrip but did not squeeze. During the Jendrassik condition (J) subjects squeezed the handgrip as hard as possible as soon as the tone came on. The experimental design consisted of presentation of 4 blocks, with 5 stimuli given during each block. One half the subjects received CJJC and the other half JCCJ. Rectified EMG was quantified during the 1200 msec interval preceding the tone as well as during the 300 msec interval following onset of the tone.

The JM increased H reflex amplitude under both the low background (37%) and the high background (43%) conditions ($p<.001$). An increase in ED EMG began 100-200 msec following onset of the tone. There was no change in sol or tibialis anterior background EMG detected before or following onset of the tone. These results suggest that general changes in sol motoneuron excitability are not responsible for the H reflex increase. This increase may be the result of presynaptic modulation of Ia input.

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65.7 MUSCLE RESPONSES TO PASSIVE KNEE MOVEMENT AT CONSTANT VELOCITY AND THEIR MODULATION BY VARIOUS MANEUVERS IN NORMAL MAN. S. Desjardins*, C.L. Richards* and M. Filion (SPON: A. Parent). Centre de Recherche en Neurobiologie, Fac. Méd., Université Laval, Québec, CANADA G1K 7P4.

We studied the effects of various facilitatory maneuvers on quadricpes (Q) and hamstrings (H) muscle responses to passive flexion and extension movements of the knee joint. The movements were imposed on the subjects' limbs at a constant velocity of 30 deg/s using a KIN-COM dynamometer. Each trial consisted of 5 repetitions of movement from knee flexion to extension to flexion through an arc of 90°. A one second pause separated each change of direction. Torque of limb resistance and EMG signals were recorded during the movements. Recordings were made in different conditions: the subject being instructed to relax or to engage in different maneuvers: Jendrassik Maneuver (JM), reading aloud, counting backwards, lightly swinging the free leg, and chewing gum. Resistance to passive movement was expressed as the area under the average torque/angle curve for each trial.

EMG responses of Q and H were quantified as the area under the curve of rectified and time averaged signals (time constant 0.2 sec). EMG responses were more common in Q than H and occurred often during Q muscle shortening (as the leg was extended). A single factor ANOVA for repeated measures and post-hoc Tukey tests were used to quantify differences in EMG and resistance across the conditions. The standard JM significantly ($P<0.05$) influenced both torque and EMG responses of the Q muscle. When performed during flexion it increased the stretch reflex; during extension it increased the shortening reaction. Reading aloud and counting backwards did not cause significant facilitation. However, the muscular responses during these activities and those during the JM differed significantly from those observed while swinging the free leg. This maneuver inhibited the muscle responses ($P<0.05$).

The present results confirm and extend those of Sharmann and Norton (1977) and Andrews et al. (1973) in that normal subjects exhibit responses during passive muscle shortening and lengthening and that these responses may be facilitated by different maneuvers. The present results moreover show that although remote muscle activity is often effective in facilitating limb muscle responses to passive movement, some types of remote muscle activity (such as leg swinging here) may cause inhibition of responses to passive movement.

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65.8 FREQUENCY FOLLOWING OF THE M2 COMPONENT OF EMG STRETCH RESPONSE. J. S. Thomas. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

Using 10 Hz square wave patterns of torque modulation it is possible to produce profound entrainment of agonist EMG activity by the periodic load signal. Periodic EMG modulation patterns produced in elbow, wrist and finger muscles of human volunteer subjects have been studied in an attempt to evaluate the frequency following capability of the "M2" latency component of the load response seen to a discrete step increase in torque load. As the frequency of torque modulation is increased from ("discrete") 0.3 Hz step increases in torque load to a 2 Hz square wave of periodic torque modulation, M1 and M2 latency peaks of evoked EMG response retain their "discrete" pattern. For periodic load changes between 3 and 8 Hz each load onset occurs during some phase of the response to a previous load change and subjects show an increase in response variance which makes it difficult to judge precise response homologies. Above 9 Hz response variance is minimal and "segmentations" of EMG modulation are seen which are similar to the M1 and M2 phases of the discrete load pattern if compared from the moment of modulated torque load onset in each case. However, above 9 Hz the M2 component to the loading phase of the periodic signal is partially occluded by an M1 latency "off response" to the unloading phase of torque modulation.

If the 10 Hz periodic torque modulation is terminated in the load phase, the masking off response does not occur and both M1 and M2 latency responses are seen to procede from the previous load onset. This effect is seen even if the cycle terminating the periodic torque is unpredictable. Since M2 latencies are homologous with step responses if calculated from the moment of modulated load onset (=maximum of shortening velocity, = onset of the shortening phase of the periodic position excursion) and the movement parameters do not deviate from those of the previous forcing cycle for 1/2 cycle (until the missing unloading phase of the periodic torque signal - i.e., 50 msec at 10 Hz) each phase of load onset must be evoking an M2 latency excitation.

65.9 DEPRESSION OF SHORT LATENCY REFLEXES TO ELECTRICAL STIMULATION AFTER PROLONGED TENDON VIBRATION OF CAT TRICEPS SURAE. L.F. Hayward*, R.P. Nielsen*, C.J. Heckman*, and R.S. Hutton. Department of Kinesiology, University of Washington, Seattle, WA 98195.

Previously, we reported that prolonged vibration of human Achilles tendon produced persistent depression in soleus H-reflexes but had potentiating effects on tendon jerk responses (Heckman et al. Exp. Neurol. 86:576, 1984). However, electrical stimulus intensities set at 1.8 times threshold (XT) elicited post-vibration H-reflex amplitudes comparable to control values suggesting that H-reflex depression at lower multiples of threshold may have been caused by disfacilitation and selective Ib afferent stimulation due to vibration induced elevated Ia electrical thresholds as has been shown in cat (Coppin et al. J. Physiol. 219:18P, 1970). In this study, changes in aggregate triceps surae reflex responses and group I electrical threshold following 20 min tendon vibration (200Hz, $\leq 250\mu$) in cat (N=20) were further examined.

Due to closeness of the afferent and efferent axonal thresholds in the LG and MG muscle nerves, observations of H-reflex EMG recordings, unconfounded by direct M-waves, were rare. Therefore, in most recordings VR_S L_7 and S_1 were sectioned. Following vibration, monosynaptic responses were usually depressed or abolished at 1.2 XT for reflexes (equal to approximately 1.4 XT of Group I volley) but recovered to control amplitudes within 20-35 min. Depression and time to recovery were XT dependent. At >1.5 XT for reflexes, post-vibration potentiated monosynaptic responses were commonly seen, most likely reflecting post-tetanic potentiation of group Ia pathways activated at their new and higher axonal thresholds. Elevated Ia axonal electrical thresholds were observed to recover to pre-vibration values in parallel with post-vibration reflex recovery to control amplitudes at 1.1-1.4 XT for reflexes. Post-vibration Ib axonal electrical thresholds did not change. Findings were in general accord with post-vibration depression seen in human soleus H-reflexes (op. cit.). These findings add further support for proposing that post-vibration depression in human H-reflexes may be caused by disfacilitation and autogenetic inhibition due to increased selectivity of Ib afferent fiber activation.

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65.10 STRETCH REFLEX DYNAMICS IN SPASTIC MUSCLE. D.L. Campbell*, R.K. Powers, and W.Z. Rymer (SPON M. Dal Canto). Rehabilitation Institute of Chicago, IL, 60611.

Previous investigators have reported that stretch reflex activity in spastic muscle is prominent during dynamic stretch, but not during maintained stretch. This feature of reflex behavior has been attributed to an enhanced dynamic sensitivity of primary spindle endings under excessive dynamic fusimotor drive (e.g. Herman,R., Brain., 93:273,1970). In contrast, previous work in our laboratory provides little support for the fusimotor theory, and suggests instead that a decrease in stretch reflex threshold is the primary cause of spasticity (Powers, R. et al., Neurosci. Abstr., 10:329,1984).

In the present study, quantitative measures of the relative sensitivity of spastic muscle to dynamic and maintained stretch were based on the torque and EMG output recorded during different portions of ramp and hold extensions of the forearms of spastic hemiparetic subjects. A torque motor configured as a position servo applied 1 radian angular extensions about the elbow at various ramp velocities. A PDP 11/23 computer controlled the presentation of stretch stimuli and the recording of joint angle, torque and surface EMG from elbow flexor and extensor muscles.

Contrary to previous reports, torque and EMG levels recorded during maintained stretch were an appreciable fraction of those recorded near the peak of the ramp portion of the stretch. With the exception of two of the fourteen subjects studied, high levels of dynamic torque were associated with high levels of static torque, so that the ratio of these two variables remains relatively invariant across a wide range of levels of reflex output. Spastic hypertonia is thus not generally associated with a selective increase in the response to dynamic stretch. The presence, however, of the two exceptional cases suggests that such a selective increase can sometimes occur. In order to more thoroughly evaluate the dynamic behavior of the spastic stretch reflex, we plan to re-test a sample of our spastic subjects as well as normal subjects utilizing alternative stimulus waveforms, such as sinusoids or pseudo-random binary pulses.

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65.11 INHIBITION IN THE SPINAL CORD OF THE NORMAL AND SPASTIC MUTANT MOUSE IN VITRO. M.R. Duchen* and T.J. Biscoe* (SPON: K.W.T.Caddy). Department of Physiology, University College London, Gower St., London WC1E 6BT.

The mouse mutant, spastic, shows a heritable defect in motor function, manifest as tremor and rigidity. Pharmacological agents, such as the relaxant benzodiazepines and pentobarbitone, which enhance the actions of the inhibitory amino-acid gamma-aminobutyric acid, GABA, ameliorate the symptoms (Biscoe & Fry, (1980), B.J. Pharmacol., 75, 23-35), and an increased number of benzodiazepine binding sites has been demonstrated in the spinal cord of affected animals (Biscoe et al., (1984), J.Physiol. 352, 509-516). In addition, neurochemical studies show a deficit in binding sites for strychnine, thought to represent the receptor for the inhibitory amino-acid, glycine (White & Heller, (1982), Nature, 298, 655-657).

The functional properties of reflex pathways and the synaptic physiology of motoneurones have therefore been examined, using a preparation of the mouse spinal cord in vitro. Dorsal root reflexes (DRRs) and dorsal root potentials (DRPs), indices of GABA-mediated primary afferent depolarisation, and dorsal to ventral root reflexes (D-VRRs) of mutant and normal mice have been compared. In both normal and mutant animals, the DRR and DRP were prominent, without any obvious difference between the animals. The D-VRR in the normal mice consisted predominantly of a monosynaptic response, with little polysynaptic activity. In contrast, the D-VRR in the mutant was characterised by a large polysynaptic wave of activity lasting 50 to 100 msec., following a monosynaptic response that was often relatively small in amplitude.

Intracellular recordings from motoneurones of normal mice with electrodes filled with potassium acetate showed that, although polysynaptic epsps were present, only the monosynaptic epsp was likely to generate an action potential. In the mutant, large polysynaptic epsps were characteristic, often generating multiple action potentials. When recordings were made with electrodes filled with potassium chloride, a prominent late depolarising psp, lasting up to 150 msec, was evoked in normal cells by orthodromic stimulation. Spontaneous depolarising psps were also now prominent. Both spontaneous and evoked chloride dependent psps were rarely seen in mutant cells. These events presumably represent psps mediated by either GABA or glycine. The findings in the mutant are thus consistent with the neurochemical findings of a deficit in glycine receptors.

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65.12 ADAPTIVE PLASTICITY IN THE PRIMATE H REFLEX. J. R. Wolpaw, R. Dowman, and E. Vander Schaaf*. Wadsworth Center for Labs and Research, New York State Dept. of Health, Albany, NY 12201; and Depts. of Neurology and Anatomy, Albany Med. Coll., Albany, NY 12208.

The study of memory substrates in higher vertebrates requires a stimulus-response pathway which is defined and accessible, capable of displaying adaptive change, and contains the responsible substrates. Recent studies (J. Neurophysiol. 50:1296-1319, 1983; J. Neurosci. 4:2718-1724, 1984) have shown long-term adaptive plasticity in the simplest, best-defined, and most accessible pathway in the primate CNS, the wholly spinal, largely monosynaptic path underlying the earliest response to sudden muscle stretch, the spinal stretch reflex (SSR). Monkeys can change SSR amplitude when confronted with a task requiring change. Change occurs over months and persists for long periods. Its features strongly suggest the presence of persistent segmental alteration. In an effort to determine whether change in afferent input, i.e. in muscle spindle stretch sensitivity, is responsible for SSR adaptive change, we tried to demonstrate comparable adaptive plasticity in the amplitude of the H reflex (HR), the electrical analog of the SSR.

Monkeys (Macaca nemestrina) with chronic EMG electrodes in calf muscles and nerve cuff electrodes around tibial nerve were trained by computer to maintain a given level of background gastrocnemius EMG activity. At unpredictable times a 1 msec pulse just above M response threshold was given via the cuff. The computer monitored subsequent EMG and calculated the amplitude of the HR, which occurred 12-24 msec after the pulse. Under the control mode, reward followed 200 msec after the pulse. Under the HR↑ or HR↓ mode, reward followed only if HR amplitude was above (HR↑) or below (HR↓) a criterion value. Animals worked 3,000-6,000 trials/day over 3-6 months.

Results from the initial 3 animals indicate animals can change HR amplitude and that HR adaptive plasticity is similar to SSR adaptive plasticity. HR change, increase or decrease, also occurs slowly over weeks and months and is comparable to or greater than SSR change. It appears to persist over gaps in performance.

These results suggest that change in afferent input is probably not the mechanism of SSR adaptive plasticity. The most likely mechanism for SSR and HR adaptive plasticity appears to be change in Ia synaptic function, perhaps due to change in presynaptic inhibition. In addition, the results extend the phenomenon of spinal reflex adaptive plasticity to the lumbar spinal cord. In this location it should be more accessible to acute studies of its neuronal and synaptic substrates in anesthetized animals.
(Supported by NIH NS22189 and by United Cerebral Palsy.)

65.13 POST-TETANIC CHANGES IN THE AFFERENT AND EFFERENT ACTIVITIES IN KITTENS. P. Bawa, Department of Kinesiology, Simon Fraser Univ., Burnaby, B.C. V5A 1S6.

Suprathreshold test stimuli (3-5xTh) were applied to MG + LGS muscle nerves before and after conditioning tetanic stimulation (500/sec, 5-30 sec) of the same nerves in nembutal anaesthetised kittens 1-90 days of age. Resulting compound action potentials of intact L_7DR and monosynaptic reflex in distally cut L_7VR were recorded with bipolar silver electrodes.

Central gain of the reflex was high in newly born kittens and it gradually decreased to zero by 30 days postnatal age. In very young kittens, during tetanic stimulation, afferent peak/peak amplitude decreased and was also depressed post-tetanically. This was possibly due to decreased conduction velocity and greater dispersion in conduction times of different afferents. In older kittens and adult cats, peak/peak amplitude increased during tetanus, possibly due to the increased size of individual spikes of tetanised afferents (Eccles & Krnjevic, J. Physiol. 149: 274, 1959).

Post-tetanic depression and potentiation of monosynaptic reflex were observed in kittens, depression being more dominant in younger kittens and potentiation in older kittens and adult cats. The dynamic balance between depression and potentiation changed gradually with age. Both depression and potentiation decayed with their characteristic time constants. These time constants were long in the newly born kittens and decreased gradually with age. Also, both time constants increased with increased duration of tetanus.

This work was supported by NSERC

66.1 KYNURENIC ACID ANTAGONIZES SYNAPTIC AND AMINO ACID EXCITATION OF HAMSTER SPINAL DORSAL HORN NEURONS IN VITRO. S.P. Schneider and E.R. Perl. Dept. of Physiology, Univ. of North Carolina Sch. of Medicine, Chapel Hill, NC 27514.

Our previous experiments using an in vitro spinal cord slice preparation (Soc. Neurosci. Abstr., 10: 488, 1984) suggested there is a differential sensitivity of dorsal horn neurons to aspartate (ASP) and glutamate (GLU). Neurons especially sensitive to these compounds are preferentially located within the superficial dorsal horn (laminae I and II) and tend to be excited by volleys in slowly-conducting primary afferent fibers. We have now studied the effects of kynurenic acid (KYN), a putative amino acid antagonist, on synaptic and amino acid excitation of neurons in this in vitro spinal cord slice preparation.

Synaptic excitation of some dorsal horn neurons by dorsal root volleys was found to be reversibly antagonized by bath application of 1 mM KYN. Most of these neurons were depolarized and vigorously excited by addition of GLU to the bathing medium; this effect was reduced or blocked by application of KYN. Neurons reacting in this way were usually in laminae I or II. Synaptic activation by volleys in the unmyelinated (C) and slowly-conducting, myelinated (Aδ) fibers was more susceptible to KYN than was activation by volleys in the rapidly conducting, myelinated (Aαβ) fibers.

Synaptic excitation of other neurons in the same preparation was quite resistant to bath application of KYN. KYN-resistant neurons were located throughout the dorsal horn and tended to be quite unresponsive to GLU (no excitatory effects to concentrations exceeding 3 mM). Most KYN- and GLU-resistant neurons were only excited by Aαβ components of primary afferent volleys.

These results are consistent with the concept that some but not all dorsal horn neurons have excitatory receptors for GLU or ASP and that primary sensory and/or spinal cord neurons terminating within the superficial dorsal horn use ASP or GLU as a neurotransmitter.

Supported by NIH grant NS10321 and USPHS fellowship (to S.P.S.) NS07317.

66.2 **MODULATION OF SOMATOSENSORY RESPONSES OF DORSAL HORN NEURONS IN THE FELINE LUMBAR SPINAL CORD BY STIMULATION OF THE VESTIBULAR NUCLEI.** **L. L. Cooper and J. O. Dostrovsky, Dept. of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.**

The somatosensory responses of dorsal horn neurons have been shown to be modulated by stimulation of a number of central sites including components of the motor system such as motor cortex and red nucleus. However, the effect of stimulation of the vestibular nuclei (VN) on dorsal horn (DH) neurons has received little attention, despite the fact that stimulation of the VN has been observed to alter the excitability of primary afferents, in addition to motoneurons. The aim of this investigation was to study the effect of stimulation of the VN on somatosensory responses of DH neurons in the feline lumbar spinal cord.

Experiments were performed on 6 cats anesthetized with either alpha chloralose or sodium pentobarbital and paralyzed with gallamine triethiodide. A tungsten recording microelectrode was introduced into the lumbar enlargement of the spinal cord. Bipolar stimulating electrodes were introduced into the VN ipsilateral and contralateral to the recording site. The effect of stimulating the VN (100 ms trains, 0.1 ms pulse duration, 500 Hz) was assessed on the just suprathreshold response of DH neurons. These responses were evoked by electrical stimulation within the neuron's receptive field 30 ms after the end of the conditioning train. Stimulating sites were verified histologically. Each neuron was categorized on the basis of its response to mechanical and thermal stimulation of its receptive field as a low threshold mechanoreceptive (LTM), a nociceptive specific (NS) or a wide dynamic range (WDR) neuron.

Stimulation of the ipsilateral and/or contralateral VN with currents of 300 μA or less inhibited the evoked responses of 60% of the neurons. The threshold responses of both nociceptive (9/14 WDR and 2/8 NS) and nonnociceptive (10/13 LTM) neurons were inhibited. For those neurons inhibited by stimulation of the ipsilateral and contralateral VN the threshold currents necessary to produce inhibition from each side were approximately equal. Three NS and two LTM neurons were excited by VN stimulation. The excitation occurred during the train and, except for one LTM neuron, was not followed by inhibition. These results show that stimulation of the VN can modulate the somatosensory responses of some DH neurons. The lateral vestibulospinal tract (LVST) is the only descending pathway which originates in the VN and descends to the lumbar spinal cord. As the LVST terminates in lamina VII and some DH neurons have dendrites which extend into lamina VII, the observed effects may have been mediated by the LVST. Alternatively the effects may have been mediated by reticulospinal neurons which are known to be activated from the VN. (Supported by Canadian MRC.)

66.3 SPIKE SHAPE CHARACTERISTICS OF IDENTIFIED CAT DORSAL ROOT GANGLION (DRG) NEURONS. H.R. Koerber, R.D. Rose, M.J. Sedivec & L.M. Mendell. Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

We have carried out intracellular recordings from cell bodies in L7 and S1 DRGs to examine whether shape of spikes recorded from the cell body correlated with the receptor type innervated by the peripheral fiber. Previous work in nodose (Belmonte & Gallego, J. Physiol., 342:603-14) and spinal ganglia (Strauss & Duda 1982 Bratisl. led. Litsy 5:513-640) suggests such an association exists. In Strauss & Duda distinctions were made only between high and low threshold cutaneous mechanoreceptors in the cat. In the present work we have recorded from afferents innervating primary (group Ia) and secondary (group II) muscle spindle receptors, Golgi tendon organs (group Ib), cutaneous type I and II slowly adapting receptors (SA I and SA II), G1, G2 and D-hair receptors, and Aβ and Aδ fiber high threshold mechanoreceptors (HTMRs). These experiments were performed in anesthetized (Nembutal) cats using electrodes filled with 4% Lucifer Yellow in 1-2M LiCl (87% of cells) or 3M KCl (13%). A total of 84 cells with spike amplitude >60 mV was analyzed. We measured the duration of the spike at baseline and found that these averaged the least in rapidly conducting muscle afferents (0.7 ms ±0.0 SE in group Ia, 0.6 ms ±0.0 in Ib). Cell bodies of group II muscle afferents, SA Is, SA IIs, G1 and G2 cutaneous afferents exhibited spike widths with means ranging from 0.8 to 1.2 ms. A small sample of cells with Aβ fibers supplying HTMRs was found to exhibit broader spikes (mean duration 1.6 ms ±0.1). D-hair afferents were characterized by much narrower spikes (mean 1.2 ms ±0.1) than Aδ afferents supplying HTMRs (3.9 ms ±0.9). Thus in both Aβ and Aδ conduction velocity groups, cell bodies supplying HTMRs exhibited broader spikes. This broadness was due in large part to an inflection point or hump on the falling phase which was more prominant in the case of cells with low conduction velocities. A single DRG cell with a peripheral C fiber responded only to high threshold input and had a spike width of 7.1 ms. Four additional C fibers whose receptive fields could not be characterized also had very broad spikes (7-10.4 ms) and humps. These data indicate that factors responsible for spike shape differ in a systematic way among cell bodies supplying different receptor types. We have also observed that slowly conducting afferents generally produce broader spikes than fast conducting afferents. That receptor type must play at least as prominent a role as peripheral conduction velocity is evidenced by the finding that spike shape depended on threshold of cutaneous receptor activation within Aβ and Aδ conduction velocity groups of fibers. Supported by NIMH MH08323 (RDR), NS14899 & NS16996 (LMM).

66.4 COMPARISON OF THE DISTRIBUTION OF MET-ENKEPHALIN, DYNORPHIN, AND NEUROTENSIN IMMUNOREACTIVE NEURONS IN THE DORSAL HORN OF THE CAT AND RAT SPINAL CORDS V. Seybold and K.E. Miller, Dept. of Anatomy, University of Minnesota, Minneapolis, MN 55455.

The dorsal horn of both rat and cat is used as a model for investigating sensory processing in the spinal cord. Enkephalin (ENK), dynorphin (DYN) and neurotensin (NRT) have all been implicated in modulating nociception, and neurons containing these peptides are found throughout the dorsal horn. This study compared the distribution of these three peptides in spinal neurons of comparable segements from rat (L4) and cat (L5).

Data were collected from 6 rats and 4 cats. An intrathecal cannula was implanted in each animal, and two doses of colchicine were administered over a 48 hr period. At the end of the colchicine treatment the animals were perfused with Zamboni fixative. Spinal segments were dissected, placed in 5% sucrose overnight, and sectioned transversely at 50 um. Sections were placed alternately into either met-enkephalin (ENK), dynorphin 1-8 (DYN), or neurotensin (NRT) antisera and processed for PAP immunocytochemistry. Immunoreactive (IR) neurons in lamina I and laminae IV-VII were mapped and counted. Total numbers of IR neurons were estimated and cells/volume (mm^3) calculated. The Mann-Whitney and Kruskal-Wallis tests were used to determine statistical differences between species and among laminae within each species.

Lamina I contained numerous ENK-, DYN-, and NRT-IR neurons of both small (5x10 um) and large (35x40μm) size. In both species, ENK-, DYN-, and NRT-IR cells were comparable in number/segment, but rat contained a higher density of cells ($26.6/mm^3$) than cat ($11.3/mm^3$). Immunopositive cells were also found throughout laminae IV-VII; the region of greatest concentration moved from lateral to medial in a ventral progression through these laminae. These neurons were multipolar and ranged from 10x10 to 40x20 um in size. The rat laminae IV-VII contained a larger number of cells/mm^3 than the cat for each of the three peptides [rat: ENK(200)>DYN(170)>NRT(50), cat: ENK(30)>DYN(20)>NRT(2)]. In cat, NRT-IR neurons comprised a smaller percentage of the total-IR cells as compared to the rat.

The results of this study indicate there are distinct differences in numbers of ENK-, DYN-, and NRT-IR neurons both between species and among laminae for each species. These differences need to be taken into consideration in understanding the functional aspects of these peptidergic neurons in the spinal cord. The connections of these cells were not examined in the present study, however neurons in laminae I and IV-VII are known to project to the brainstem. Therefore, these neurons may be involved in ascending pathways related to sensation. Supported by NS17702.

66.5 SPINAL CORD LAMINA X ULTRASTRUCTURE OF THE CAT
K.E. Miller and V. Seybold, Dept. of Anatomy, University of Minnesota, Minneapolis, MN 55455.

During the past several years an interest has developed in the grey matter region surrounding the central canal. Several reports have noted a large distribution of peptidergic terminals within this region, and one report has shown that neurons within this region respond selectively to nociceptive stimuli (Nahin et al., JCN 220:321,1983). Despite the interest in this area very little is known about its ultrastructure. This study examines the ultrastructure of lamina X in the cat lumbar spinal cord.

Animals used for this study were perfused with 2% formaldehyde, 2.5% glutaraldehyde in 0.1M phosphate buffer. Spinal cord segments L4-6 were dissected and sectioned (200 um) on a vibratome. Sections were dehydrated and embedded in Epon 812. Thin sections were cut, stained with lead citrate and uranyl acetate, and examined with a JOEL 100C electron microscope.

The region 200 um lateral to the central canal could be divided into 3 zones. An ependymal zone, comprised of low columnar to cuboidal ependymal cells, lined the central canal. Beneath these cells was a large subependymal zone consisting of microglial cells and many glial fibrous processes which were arranged parallel to the longitudinal axis of the central canal. A few fibrous astrocytes were present within this layer. This zone was approximately 75 um thick at its widest point lateral to the canal. A dendritic zone occurred lateral to the glial fibrous process zone. The components of the dendritic zone were dendrites, lightly myelinated and unmyelinated axons, and cells. The medial portion of the dendritic zone blended with the lateral edge of the glial zone such that fibrous processes were interspersed with lightly myelinated axons and dendrites. The dendrites ranged from 0.5 to 2 microns in diameter and were surrounded by synaptic terminals. Four types of terminals were identified based upon vesicular constituents: a. small round clear vesicles (40 nm); b. small flattened clear vesicles (60x30 nm); c. small round clear vesicles and large dense core vesicles (110 nm); and d. small round clear vesicles and small dense core vesicles (70 nm). Cells in this dendritic region consisted of astrocytes and neurons (10-25 um in diameter).

The results of this study indicate the region 200 um lateral to the central canal includes a glial zone, which may be analagous to the substantia gelatinosa centralis defined by Rexed, and a dendritic zone, which contained a variety of synaptic contacts. Terminal types with large dense core vesicles suggest a peptidergic input, and ultrastructural localization of neuropeptides in this region is in progress.
Supported by NS 17702.

66.6 IMMUNOCYTOCHEMICAL-IDENTIFICATION OF AXONS CONTAINING CO-EXISTENT SEROTONIN AND SUBSTANCE P IN THE DORSAL HORN AND LAMINA X OF THE CAT LUMBAR SPINAL CORD. T. Tashiro* and M.A. Ruda. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

We have recently directed our attention to identifying axons containing both serotonin (5-HT) and substance P (SP) in the spinal cord. Co-existence of 5-HT and SP in axons of the ventral horn has previously been shown to be very common and to occur in the vast majority of SP axons (Ruda, Soc. Neurosci. Abstr., 1984). The present study examined co-existence of 5-HT and SP in axons of the dorsal horn and lamina X of the cat by an immunologically specific double immunofluorescence method.

5-HT antisera were raised in rabbit and monoclonal SP antiserum was raised in rat. Species non-cross-reacting secondary antisera were tagged with either FITC or TRITC. Double-labeled tissue sections were examined using epifluorescence and filter combinations specific for each fluorochrome. All reagents were tested for crossreactivity and non-specificity.

In the superficial laminae of the dorsal horn, co-existent strands were occasionally detected among the terminal plexi of 5-HT and SP immunoreactive fibers. They were often oriented rostrocaudally. Co-existent strands were more numerous in lamina V where they appeared as short fibers with random orientations. In sagittal sections through lamina V, the double-labeled axons could be seen in the area intervening between the dense periodic patches of SP axons. Co-existent axons were also occasionally found within the dense SP lamina V plexus. In lamina X, the density of co-existent strands was much greater than that in the dorsal horn. In the area immediately adjacent to the central canal, co-existent strands appeared to travel rostrocaudally. The lateral portion of lamina X contained mostly short fibers with random orientations. The co-existent axons appeared as strands of variable length with numerous varicosities. The varicose strands varied in size from very fine axons with thin, elongated varicosities to thicker axons with large bulbous varicosities.

The present study demonstrates that axons containing both 5-HT and SP are found in spinal cord laminae concerned with regulation of somatic and visceral sensory functions in addition to motor function. However, in the dorsal horn, they represent a very minor component of either 5-HT or SP afferent input. Dorsal horn 5-HT afferents likely originate from a subpopulation of brain stem raphe neurons which do not contain SP and thus are distinct from the brain stem neurons which contribute the numerous 5-HT and SP co-existent axons to the ventral horn.

66.7 SOMATOTOPIC ORGANIZATION AND RESPONSE CHARACTERISTICS OF CELLS IN THE CERVICAL SPINAL CORD OF THE CAT. L.S. Sorkin, D.G. Ferrington and W.D. Willis. Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, TX 77550.

Little information is available concerning the characteristics of sensory interneurons located in the cervical spinal cord. In this study, we examined the receptive field properties and somatotopic arrangement of sensory cells in spinal segments C_6 and C_7 of the chloralose anesthetized cat. Extracellular recordings were made from 191 cells using carbon fiber microelectrodes.

Cells were placed into four categories based on their responses to graded mechanical stimulation of the skin and subcutaneous tissue: Low Threshold (LT) cells responded maximally to innocuous levels of cutaneous stimulation, 34.7%; High Threshold (HT) cells responded exclusively to noxious levels of cutaneous stimulation, 20.6%; Wide Dynamic Range (WDR) cells responded to both innocuous and at a higher frequency to noxious levels of cutaneous stimulation, 22.7%; and Deep (D) cells responded to stimulation of subcutaneous tissue, 22.7%. LT cells were the most superficial in the cord; their average depth below the cord surface was 1.92 mm (lamina IV). WDR cells (av. depth 2.09 mm, laminae IV and V) were located below the LT cells and above the D cells (av. depth 2.58 mm, lamina VI) and HT cells (av. depth 2.60 mm, lamina VI), which were progressively deeper in the gray matter. The difference between all groups except the HT and D cells was significant at the $p < 0.01$ (ANOVA) level.

The somatotopic organization of cells with cutaneous input was similar to that found in lumbar cord. A large ventral paw and toe area in the medial portion of C_6 and C_7, as well as a smaller dorsal paw area lateral to it, were bordered by representations of the medial forearm and elbow in rostral C_6 and the lateral arm in caudal C_7. Glabrous skin receptive fields were observed only for cells recorded in medial C_7; however, only 8.4% of cells in medial C_7 with cutaneous receptive fields had glabrous skin input. Deep cells had no obvious somatotopic organization.

In contrast to lumbar cord, the locations of receptive fields of cells studied in the same electrode track was frequently seen to shift, although they usually showed considerable overlap. Although the pattern of somatotopic organization is similar in cervical and lumbar dorsal horn, the variation of receptive fields within vertical columns suggests a difference in the spread of afferent fiber collaterals.

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66.8 MORPHOLOGICAL FEATURES OF IDENTIFIED SPINOTHALAMIC TRACT NEURONS IN THE PRIMATE. C.N. Honda, D.J. Surmeier and W.D. Willis. Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, Texas 77550.

Spinothalamic tract (STT) neurons in the lumbosacral spinal cord of the primate (Macaca fascicularis) were intracellularly stained with horseradish peroxidase (HRP) and examined at the light microscopic level.

A sample of STT neurons projecting to the contralateral ventrobasal thalamus was studied. Each STT neuron was categorized on the basis of responses to graded mechanical cutaneous stimulation into conventional classes: low threshold (LT), high threshold (HT), and wide dynamic range (WDR). Intracellular injections of HRP (10% in Tris/KCL) revealed a population of STT neurons with cell bodies located in laminae IV through VI (mostly lateral V), with perikaryal dimensions ranging from 15x20 µm to 30x50 µm. At the light microscopic level, only a few neurons possessed somatic or dendritic spinous processes.

Proximal dendrites were predominantly oriented in the transverse plane and radiated throughout laminae IV through VI. Distal dendrites were more variable in both their orientation and laminar distribution. Dendritic arbors extended from 500 µm to over 1mm in the rostrocaudal direction. The dendrites of some STT neurons were confined to the lateral regions of the dorsal horn, whereas others distributed over the entire mediolateral extent of the gray matter. Dendrites extended as far dorsally as lamina I and as far ventrally as lamina VII. Most STT neurons possessed dendrites coursing through the reticulated portions of lamina V, and several had dendrites extending into the lateral white matter. In many instances, at least one major dendritic branch extended medially to arborize within the vicinity of the central canal.

Axons of STT neurons consistently crossed the midline within a rostrocaudal distance of 500µm from their cell bodies to travel rostrally in the contralateral ventral funiculus. Local axonal collaterals were not discernable. However, a few STT neurons exhibited one or two axon collaterals that left the vicinity of the cell body without branching, and traveled caudally.

Attempts to determine relationships between morphological features of STT neurons and their functional classification are in progress. Nearly one half of the neurons receiving nociceptive afferent input (WDR and HT cells) have dendritic arbors in the superficial layers of the dorsal horn. The only LT neuron examined possessed dendrites whose dorsal extent was lamina III. Thus, preliminary analyses indicate that the laminar distributions of dendritic arborizations of STT neurons appear to be correlated with conventional functional classification schemes.

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66.9 DORSAL HORN CELLS WITH SIMILAR RECEPTIVE FIELDS DISPLAY A WIDE VARIETY OF DENDRITIC FORMS. M.D. Egger, N.C.G. Freeman*, M.F. Jacquin, E. Proshansky* and K. Semba. Department of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854.

Dorsal horn cells (DHC) in the spinal cord of cats responding to low threshold mechanical stimulation of the plantar cushion (PC) were labelled with intracellular HRP. The 27 DHC recovered had cell bodies in spinal segment L7, in Rexed's laminae III (n = 5), IV (n = 17) and VI (n = 5). Cell bodies of the lamina IV DHC were concentrated at the medial edge of the dorsal horn, but with a few as far lateral as halfway across the dorsal horn. Lamina III cell bodies were in the medial quarter of the dorsal horn, while lamina VI cell bodies were located more laterally, one quarter to one half the distance across the dorsal horn. On sections cut in the transverse plane, cell bodies of lamina III cells appeared oriented with a long dorsoventral (DV) axis and a shorter mediolateral (ML) axis (mean: 28 X 11 μm, n = 4). Cell bodies in lamina IV (mean: 27 X 21 μm, n = 14) and lamina VI (mean: 31 X 29 μm, n = 5) appeared more symmetrical.

Dendritic fields varied greatly, for the most part conforming to patterns corresponding to their location in the dorsal horn (Proshansky & Egger, 1977). The lamina III cells had their greatest dendritic spread in the rostrocaudal (RC) plane. One had RC dendrites extending at least 3360 μm. This cell's dendrites extended 430 μm ML and 490 μm DV. Dorsoventrally, dendrites of the lamina III cells were confined within laminae I-IV. The dendritic spread of the lamina IV cells tended to be more symmetrical (mean: 350 μm (ML); 560 μm (DV); 700 μm (RC) (n = 11)), but there was much variation among these cells, with medially located cells tending to have more narrowly bunched dendrites than those of the more laterally located cells. Dorsoventrally, some lamina IV cells sent dendrites dorsally through lamina I into the white matter, and ventrally to lamina V or VI. Lamina VI cells had long, relatively unbranched dendrites. Dorsoventrally, the dendrites of the lamina VI cells ramified within laminae IV to VII. The dendritic field of one lamina VI cell measured: 850 μm (ML); 1170 μm (DV); 700 μm (RC).

The receptive field (RF) areas of lamina III cells tended to be slightly larger than the surface of the PC, while the mean RF of lamina IV cells was twice that large. Lamina VI cells tended to have the smallest RF, about half that of lamina III cells. In lamina III, ML dendritic spread was positively related to RF area ($r = 0.998$, $p < 0.05$). For lamina IV cells, there was a correlation between ML dendritic spread and ML position of the cell body ($r = 0.79$, $p < 0.01$), but there was no relationship between either of these anatomical variables and RF area. In lamina VI, the ML position of the cell body tended to be related to RF area ($r = 0.82$, $p < 0.10$).

66.10 ANATOMY OF DORSAL HORN CELLS RESPONSIVE TO SLOWLY ADAPTING TYPE I INPUT. P.B. Brown and L.A. Ritz. Physiol. Dept., W. Va. Univ. Med. Center, Morgantown, WV 26505.

Input from slowly adapting type I (SAI) fibers is relayed with high security in the dorsal horn, and ascends in the dorsolateral funiculus (DLF). Spinocervical tract (SCT) cells do not respond to SAI input, but some cells of the postsynaptic dorsal column system do. The morphology of the dorsal horn cells, and of contacts they receive from SAI axons, may help identify the ascending pathway and the mechanism for high synaptic security.

In chloralose-anesthetized cats, two lumbosacral dorsal horn cells which responded to SAI input were iontophoretically injected with horseradish peroxidase. Cell 1's soma was 36 X 48 um, at the lamina III-IV border, in the lateral half of the dorsal horn; its low-threshold excitatory receptive field (LTERF) covered the posterior calf and thigh. The cell had 12 primary dendrites, mostly dorsal and medial, extending from the lamina II-III border into lamina V, 440 um laterally and 280 um medially, 750 um rostrally and 650 um caudally. Dendrites were profusely branched and invested with spines for most of their lengths. Cell 1's axon ascended the DLF, with no collaterals. The soma of cell 2 was 24 X 52 um, in medial lamina V; its LTERF was located on the plantar foot. Cell 2 had 9 primary dendrites radiating in all directions in the transverse plane. The dendrites were less profusely branched than those of cell 1, had no spines, and extended from lamina III to lamina VII, into the dorsal columns and the lateral funiculus, and 350 um rostral and 500 um caudal. The axon of cell 2 gave off fine collaterals as it coursed laterally in the gray matter; these collaterals arborized loosely in laminae V-VII. The axon then projected rostrally in the DLF with no further collaterals.

An SAI axon innervating a Haarscheibe at the proximal end of cell 1's LTERF was injected with HRP: its boutons distributed in a narrow radial sheet which intersected most of the rostrocaudal length of cell 1's dendritic tree. SAI boutons made 9 contacts with 1° through 4° rostrodorsal dendrites, at seven sites.

The two cells studied to date suggest that the ascending path is in the DLF, but not in the SCT, since their dendritic morphologies are different from SCT cells. The basis for high synaptic security may be the multiple contacts between afferent boutons and a cell.

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66.11 EXTENT OF THE ROSTRAL PROJECTION OF SMALL DIAMETER (Aδ & C) PRIMARY AFFERENT FIBERS IN LISSAUER'S TRACT. R.J. Traub, M.J. Sedivec, and L.M. Mendell, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

The rostral projection of large diameter cutaneous primary afferent fibers (Aβ) projecting in the dorsal columns (DC) of monkeys, cats, and rats has been well documented. On the other hand, the rostral projection of small diameter (myelinated and unmyelinated) cutaneous primary afferents (Aδ & C) is not well established. Recent anatomical studies have shown Aδ and C primary afferents comprise approximately 50% of Lissauer's tract (LT) at the lumbosacral level (Chung et al. JCN 184:587 and ibid, 186:451) and Aδ afferents have been shown anatomically to project in LT 3-4 segments rostral of their root entry zone (Culberson and Brown, J. Neurophysiol. 51:516, but see LaMotte JCN 172:529).

We have developed a method of antidromically stimulating the central projections of small diameter (Aδ & C) primary afferent fibers in LT in the cat lumbosacral spinal cord without stimulating large diameter afferents (Aβ) in the DC. Stimulation sites were intersegmental borders and mid segments from L3/L4 to L7/S1. Recordings were made from the central end of cut dorsal rootlets caudal to the stimulating electrode or from 2 locations on the sural nerve simultaneously. This allowed the most accurate measurement of conduction velocity. Histological procedures verified the position of the stimulating electrode. The results indicate that some Aδ afferents which enter the spinal cord via the S1 dorsal root project rostrally at least as far as rostral L4 (minimum of 4 segments). However, C fibers which arrive over the S1 dorsal root project to the L6/L7 border but not to mid L6.

In order to determine the modality of the Aδ afferents in LT (D-hair or high threshold mechanoreceptors (HTMR)) we made single unit recordings in dorsal rootlets, DRG cells, or LT. Peripheral stimulation was from the sural and tibial nerves. Antidromic stimulation was from LT and the DC at various rostrocaudal levels. All dorsal roots except L7 and S1 were cut acutely. The results show a minimum of 34% of the recorded afferents have a rostral projection of at least 2-3 segments in LT. These afferents could not be activated from the DC at the same rostrocaudal level. None of the afferents that projected in LT responded to low threshold natural stimulation. Rostral projections of D-hair afferents were found only in the DC. Afferents with a rostral projection of at least 2 segments in LT had a faster peripheral conduction velocity (27.7±2.0 m/s; n=23) than those with a shorter rostral projection (21.3±1.4 m/s; n=36) ($p<.01$).

These results show a projection of Aδ HTMRs (but not Aδ D-hairs) and C primary afferents into LT. The Aδ can project rostrally up to 4 segments but the C afferents project rostrally no more than 1 segment. Supported by Program Project PO1 NS14899 and the Helen and Harry Aibinder Scholarship fund to RJT.

66.12 EVIDENCE FOR THE EXISTENCE OF RELATIVELY INEFFECTIVE PROJECTIONS FROM THE SURAL NERVE TO DORSAL HORN NEURONS USING INTRACELLULAR METHODS. L. M. Pubols, H. Hirata, and M. E. Fogelsong*. Neurological Sciences Institute and Department of Neurosurgery, Good Samaritan Hospital and Medical Center, Portland, OR 97209

It was previously reported (L. Pubols, Soc. Neurosci. Abstr. 9:260, 1983) that electrical stimulation of the sural nerve (SN) demonstrates an excitatory input from that nerve to dorsal horn neurons that is not predictable on the basis of receptive field location. Impulses were elicited by nerve stimulation in some cells whose natural receptive fields (RFs) fell outside the region of skin innervated by the sural nerve (SN region). The purpose of the present investigation was to extend those findings by using intracellular methods to reveal any SN projections too weak to elicit impulses during extracellular recording. Micropipettes were used to record the extracellular and intracellular responses of L_6 and L_7 dorsal horn neurons to electrical stimulation of A^{δ} fibers in the SN and to noxious and non-noxious mechanical stimulation of the skin in adult cats anesthetized with sodium pentobarbital. Twenty-eight cells were studied in detail. Nine cells responded extracellularly with impulses to SN stimulation. In 3 of these 9 the RF fell outside the SN region. Of the remaining 19 units, 7 showed one or more of the following signs of subliminal excitatory input from the SN during intracellular recording: 1) EPSP's to SN stimulation, 2) impulses to SN stimulation during depolarization of the cell induced by intracellular current pulses, 3) impulses to SN stimulation when the excitability of the cell had been increased due to the injury caused by impalement. Three of these cells had an RF in the SN region, and 4 had an RF outside it.

Surprisingly, some cells whose RF's overlapped the SN region had no detectable excitatory responses to SN stimulation. Two of the 12 cells that had no excitatory responses to SN stimulation had such fields. This may be attributable to overlap of innervation of the SN region by other nerves. Alternatively, it may indicate that natural stimuli are, under some circumstances, more effective in demonstrating projections than are single electric shocks to nerves.

In conclusion, the present study has shown that subliminal, as well as supraliminal, excitatory effects to SN electrical stimulation are detectable in many, but not all, dorsal horn cells whose natural receptive fields are outside the SN skin region. It has been suggested (P.D. Wall, Phil. Trans. B, 278:361, 1977) that under some conditions, especially following partial denervation, projections detectable in this manner may be capable of mediating responses to natural stimuli, thus altering the modality and/or receptive field properties of dorsal horn neurons. (Support: NIH:NS19523)

66.13 PROJECTIONS OF FIBERS FROM SLOWLY-ADAPTING TYPE I RECEPTORS TO CAT SACROCAUDAL SPINAL CORD. L. A. Ritz and P. B. Brown. Dept. of Physiology, West Virginia Univ. Medical Center, Morgantown, W.V. 26506.

Recently we examined the somatotopic organization of cat spinal cord at caudal and thoracic levels, where the dorsal horns are fused (continuous across the midline). We observed that: 1) dorsal skin is represented laterally in the fused dorsal horns and ventral skin medially; 2) some dorsal horn neuron receptive fields (RFs) span the dorsal or ventral midline (i.e., the RFs are bilateral); 3) dorsal root fibers, as determined by Fink-Heimer degeneration techniques, project to the medial and lateral portions of the contralateral dorsal horn. We wish to determine, using intracellular staining techniques: (a) whether axons with crossed collaterals have uncrossed collaterals as well; and (b) whether the crossed projections are somatotopically appropriate.

In chloralose-anesthetized cats, primary afferent fibers from slowly-adapting type I (SAI) receptors were impaled within the dorsal columns and iontophoretically injected with horseradish peroxidase. Stained neuronal elements were visualized, in serial transverse sections, at the light microscopic level.

We have successfully recovered five individually-stained SAI fibers. Preliminary observations indicate that the five fibers have some or all of the following morphological features: 1) one to three collaterals, branching off the parent axon, within the same section; 2) collaterals which course rostrally as they approach the dorsal horn; 3) collaterals which curve around the medial aspect of the dorsal horn; 4) collaterals within the dorsal horn which give rise to several thick or thin branches; 5) for four units with dorsal RFs, a termination zone in lateral dorsal horn; for the one unit with a ventrolateral RF, a termination zone in medial dorsal horn; 6) for the four units with dorsal RFs, multiple branches of the same collateral which cross the dorsal gray commissure, within the fiber bundle at the lamina IV/V border, and project to the contralateral lateral dorsal horn; 7) crossing fibers, from the same parent axon, at several rostrocaudal levels.

These preliminary findings indicate that (a) an axon projecting to sacrocaudal cord can have both uncrossed and crossed collaterals; and (b) crossed projections appear to be somatotopically appropriate.

This research was supported by USPHS grant NS12061.

66.14 **PROPERTIES OF SPINAL CORD INTERNEURONS WHICH PROCESS INPUTS FROM THE FEMORAL VEIN.** B. J. Yates, F. J. Thompson and J. P. Mickle. Depts. of Neuroscience and Neurosurgery, Univ. of Florida Col. of Med., Gainesville, FL 32610.

Both anatomical and electrophysiological studies have suggested that veins of the feline hindlimb, including the femoral vein, have sensory innervation. Femoral venous afferents (FVA) are activated when intravenous pressures exceed 2-3 mmHg (Thompson et al., 1983); input along these afferents enters the L6 spinal cord (Thompson and Yates, 1985) and activates interneurons found in Rexed's laminae III-V and VII. Many of these interneurons comprise reflex pathways to skeletal muscle motoneurons (Yates and Thompson, 1985). The purpose of the present study was to determine the response properties of spinal interneurons following stimulation of the femoral vein and to show the patterns of convergence of inputs from muscle and skin on the FVA-activated interneurons.

Studies were conducted on decerebrate-spinal cats. Penetrations were made using 15-30 MΩ electrodes filled with 3 M KCl. Responses were recorded from units located 700-1500 μ beneath the L6 cord dorsum. High frequency stimulation was used to show that recordings from primary afferents were not included in the data; an attempt was made to activate most deeper units by antidromic stimulation of the L6 ventral root to determine if they were motoneurons. The responses of interneurons were recorded following electrical activation of FVA, cutaneous and muscle afferents.

65% of the tested interneurons responded to stimulation of the femoral vein with a burst of action potentials; 32% responded with a combination of one or more bursts of action periods and a period during which the firing rate was reduced below the spontaneous rate. The excitability of interneurons was altered for a long duration following vein stimulation ($\bar{X}$ = 58 msec, S = 85).

93% of the tested FVA-activated interneurons could also be driven by stimulation of the tibial nerve; 71% could also be driven by sural nerve stimulation; 67% could be driven by stimulation of both nerves. Effects of inputs from muscle and skin did not simply mirror the effects of inputs from the femoral vein; complex integration of inputs appeared to occur. Inputs along several muscle afferents were shown to affect the excitability of a single FVA-activated interneuron. Such inputs arose from muscles with vastly different locations in the hindlimb. Cutaneous and muscle afferents which carried inputs which affected the interneurons included A-αβ fibers.

Experiments utilizing HRP-filled pipettes are currently underway to determine the locations and morphologies of FVA-activated interneurons.

66.15 THE LATERAL NEUROPIL OF THE MECHANORECEPTORS WITHIN THE SPINAL CORD OF SNAKES. D.M. Schroeder, Medical Sciences Program, Indiana University School of Medicine, Bloomington, Indiana 47405.

Segmentally within the ventrolateral edge of the spinal cord of snakes are large neurons known as marginal cells. They are located primarily within the intravertebral areas and are closely associated with the denticulate ligament which is immediately adjacent to it. The ligament changes in character at that particular point from being filled with collagen to one without collagen. A number of large neurons are aligned along the width of this part of the ligament and their short but branching dendrites extend into the lateral neuropil that is located between their cell bodies and the edge of the spinal cord and ligament. Evidence suggests that this area of the spinal cord has mechanoreceptive functions, responding to bending of the spinal cord. The ultrastructure of this area was examined, concentrating especially on the lateral neuropil. The prominent features of this lateral neuropil are tubular processes oriented parallel to the length of the spinal cord and to the denticulate ligament. They consist of two sizes: a larger one accompanied by a number of smaller ones. The larger tubular processes vary in diameter from 0.75 μm to approximately 1.0 μm; they appear to be at least as long as the main receptor area, approximately 150 μm. Contents of these tubular processes include: microtubules and neurofilaments; small clear vesicles are found in small patches and if located near the edge, are associated with a dense area of the membrane; scattered large, clear vesicles; the few mitochondria are very elongated. The origin of these processes has not been determined; fibrous glial cells, located at the periphery of the mechanoreceptor area, give rise to elongated processes but it is still not clear whether all of these tubular processes are similar and originate from the same cell type. All the small tubular structures have approximately the same diameter, 0.12 um, and appear to contain only neurofilaments. They are found in groups and are always near one of the larger tubules. Most of these small tubules eventually leave the plane of section but some are at least 4 μm long. They originate from the dendrites of the large neurons. These dendritic processes are filled with mitochondria, often very tightly packed; multivesicular bodies, microtubules and neurofilaments are also present. Along the sides of the dendrites are usually synaptic boutons; these boutons as well as others distributed throughout the neuropil contain clear round vesicles. A number of small tubules emerge from the ends of the dendrites in a rostral and caudal direction and join a larger tubular process, passing by the dendrite. Assorted glial processes are distributed throughout the rest of the neuropil and along the edge of the spinal cord.

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67.1 PATTERNS OF GENE EXPRESSION IN CHICK RETINA.
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We are interested in obtaining molecular markers for individual cell types in the retina. For this purpose, we have constructed a library of cDNA clones from chick retina. In-situ hybridization data will be presented on the distribution of various cloned mRNAs in the retina and in other parts of the nervous system.

67.2 SIALIC ACID-RICH LAYER IN DEVELOPING CHICK RETINA FORMS CENTRAL-PERIPHERAL GRADIENT. S.E. Raiguel* (SPON: R.E. Marc). Dept. of Sensory Sci., Univ. Texas G.S.B.S., Houston, TX. 77030

Recent reports appearing in the literature have indicated that the binding of the sialic-acid sensitive agglutinin from the slug, Limax flavus is specific, stable, and suitable for histologic use when conjugated to an appropriate chromogen (Schulte, et al, 1984). In view of the role which sialation has been proposed to play in the development of the avian visual system (Edelman, 1983), this lectin appeared to be an ideal tool for visualizing the changes in the distribution of sialic acid which have been shown to accompany development (Schlosshauer, et al, 1984). Slug lectin (LFA) was obtained from Calbiochem (San Diego, CA.), conjugated to FITC in our laboratory, and applied to deparaffinized sections of alcohol/acetic acid-fixed chick eyes taken at 8, 12, and 18 days of embryonic development. At eight days of incubation, fluorescence microscopy revealed a crescent of sialic acid-like activity lying in the ganglion cell layer of the inner retina. Initially, this crescent comprised the central one-third of the retina, and was about 20 micrometers thick at the center, becoming gradually thinner peripherally. As development proceeded, this band of fluorescence increased in extent and thickness, until by day 18, the inner plexiform and ganglion ganglion cell layers of the entire circumference of the neural retina were included. In older embryos, sublaminations within this band were evident. Older specimens also displayed bright bands of fluorescence in the photoreceptor outer segments and in the outer limiting membrane. Fluorescence could be completely eliminated by carrying out lectin incubations in the presence of 100 mM N-acetyl neuraminic acid (Sigma type IV), or by pretreatment of the sections with sialidase. LFA staining therefore appears to be specific for sialic acid in this context.

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2. Edelman, G.M. (1983) Science 219:450-457
3. Schlosshauer, B., U. Schwarz, and U. Rutishauser (1984) Nature 310:141-143

67.3 EXHUBERANCY OF SYNAPTIC RIBBON FORMATION IN THE OUTER PLEXIFORM LAYER OF THE CAT RETINA. David H. Rapaport. School of Anatomy, University of New South Wales, Kensington, NSW 2033 AUSTRALIA.

Several regressive events such as cell death and synapse elimination occur during development of the nervous system. Some of these processes have recently been investigated in the mammalian retina which is a good developmental model due to the limited number of cell types, and their lamination. Since many photoreceptor synapses in the adult are characterized by presynaptic ribbons, study of ribbon formation should reflect photoreceptor synaptogenesis.

Investigation concentrated on central retina, since this is where many developmental events in the cat retina begin. Fetuses of known gestational age, or postnatal kittens were processed for electronmicroscopy. The eyes were removed, and the retina dissected. A wedge of retina was taken from the region of the area centralis, and ultrathin sections (0.09um thick) were cut from the apex. Photomontages of the outer plexifom layer (OPL) were made, and the area of the sample measured with a computer graphics tablet. At E(embryonic)59 the OPL is present over the central 3% of retina. At this age synaptogenesis can be recognized by the presence of ribbons in photoreceptor terminals; often they are associated with two bipolar postsynaptic elements, forming a dyad. At E62, triads with ribbons and vesicles could be observed. The density was 0.33 ribbons/um^3, but this value increased rapidly to a value of 0.63 ribbons/um^3 at P(postnatal)3, and remained at this level over P5, P7, P9, P10, and P11. Sometime after P11 ribbon density begins to decrease: 0.42 ribbons/um^3 at P17, 0.36 ribbons/um^3 at P30 and 0.28 ribbons/um^3 at P42. For almost a year there appears to be a gradual decrease in ribbon density to reach the adult value of 0.13-0.16 ribbons/um^3 (two animals). Repeated measures from different sections of the same retina gave similar densities, indicating that variations in the values obtained between subjects were not due to errors of measurement, but to differences in age, location of the sample, etc. Over the period studied the length of the synaptic ribbons increased slowly from $\bar{X}$=0.22um soon after birth to $\bar{X}$=0.38um at P42 and eventually to $\bar{X}$=0.47um in the adult.

These data demonstrate an exuberancy of synaptic ribbon development in the OPL of the cat retina during the last embryonic and first postnatal weeks of life, with a 3-4 fold reduction in ribbon density during the second week. Several mechanisms could contribute to this decrease: coalescence of ribbons, loss of ribbons through cell death or synapse elimination, and the increase in the volume of the OPL as the animal grows. It is of interest that the rapid decrease in synaptic ribbon density begins shortly after eye opening, suggesting that visual experience may be important in triggering the decrease.

67.4 STRETCH OF THE RETINA CONTRIBUTES TO THE DENDRITIC FIELD AREA OF GANGLION CELLS IN THE BLACK MOOR GOLDFISH. Peter F. Hitchcock. The University of Michigan, Ann Arbor, MI 48109.

As the goldfish ages, its retina grows by the addition of new neurons at the margin, and a balloon-like expansion of the existing retina. With the expansion of the retina, the ganglion cell dendritic fields also expand (Hitchcock and Easter, abstract, Soc. for Neuroscience, 10:465, 1984). It was hypothesized that this increase in the size of the dendritic fields was due to towing of the dendrites by the expanding retina, analogous to the enlargement of a drawing on an expanding balloon.

To test this hypothesis, ganglion cells in a strain of goldfish, commonly known as the black moor, have been studied. This animal has a developmental defect such that the vitreal chamber is grossly enlarged, relative to the normal goldfish, which results in a concomitant gross expansion of the retina. The magnitude of this expansion, however, is highly variable. The prediction is, assuming that the growth of the black moor retina is otherwise normal, that the ganglion cell dendritic fields will be larger in the retinae that have undergone the most expansion.

Ganglion cells were stained by retrogradely transported HRP applied to intraorbital cuts of the optic nerve, and viewed in the wholemount. Dendritic field areas were determined for a subtype of large cells by tracing individual dendrites at 1250X magnification and marking the location of the distal tips on a 150X camera-lucida drawing. The enclosed areas were then measured using a digitizing tablet. Three pairs of retinae were studied. Each consisted of a 'large' and 'small' retina from animals that were matched for lens diameter (2.5, 2.6, and 3.2 mm, respectively), a predictor of retinal area and number of ganglion cells in the normal goldfish.

Within each pair, the dendritic fields of the cells in the central region of the 'large' retina were significantly larger than those in the central region of the 'small' retina with a similar lens diameter (Mann-Whitney, $p<.05$; n=20, 20, and 14, respectively). The average difference in area of the 'large' and 'small' retinae was 35%. The average difference in the dendritic field areas in the 'large' and 'small' retinae was 22%. These data suggest that the towing of the dendrites by the expanding retina is a contributing mechanism for establishing the area of the ganglion cell dendritic field.

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67.5 RETINAL CROWDING REDUCES THE SIZE OF DENDRITIC FIELDS OF ALPHA GANGLION CELLS. M. A. Kirby and L. M. Chalupa
Department of Psychology, University of California, Davis 95616.

It has been suggested that the dendritic field size of individual retinal ganglion cells is regulated early in development by interactions among neighboring ganglion cells of the same functional type (Wässle et al., 1981). If this is the case, a manipulation such as in utero uniocular enucleation, which results in a greater than normal density of ganglion cells in the remaining eye (Chalupa, et al., 1984), should produce ganglion cells with smaller than normal dendritic fields.

To assess this possibility, we compared dendritic field diameters of alpha ganglion cells in normal retinae to those of adult animals that had one eye removed either prenatally (on embryonic days 42 and 51) or 6 days after birth. Ganglion cells were labeled by central injections of HRP, retinae were reacted using a modified Hanker-Yates procedure, and then whole-mounted. Cells were sampled from the temporal retina along a corridor established by a line drawn through the optic disk and the area centralis. Alpha cells were differentiated into "ON" or "OFF" types based on their level of stratification within the inner plexiform layer (Peichl and Wässle, 1981), and drawn under oil using a X40 Zeiss, Planapochromat objective. Dendritic field sizes were estimated from these drawings by calculating the mean of the two longest orthagonal diameters.

A plot of dendritic field diameters as a function of retinal eccentricity revealed that the alpha cells of the normal retinae (N=108), as well as those from the postnatally enucleated animal (N=24), were comparable to the Golgi material of Boycott and Wässle (1974). In contrast, in the remaining retina of prenatally enucleated cats (N=144), the diameters of dendritic fields of "ON" and "OFF" alpha cells were significantly less extensive. The magnitude of this effect varied as a function of retinal eccentricity, being most pronounced within 5 mm of area centralis where the differences averaged approximately 100 microns, about 25% of the mean normal size. A similar, but less pronounced difference, was also observed for somal diameters. The most straightforward interpretation of these findings is that increased cell density exacerbates the dendro-dendritic interactions normally present during early development.

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67.6 CHANGES IN THE STRUCTURE AND FUNCTION OF RETINAL GANGLION CELLS DURING METAMORPHOSIS IN *RANA PIPIENS*. B. Frank*, and J.G. Hollyfield* (SPON: S.J. Fliesler). Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Developmental changes in the visual system of *R. pipiens* are required to mediate the adaptive behavior to the changing external environment during metamorphosis. Prey catching is not seen in tadpoles; we have determined that this response is first observed at Taylor & Kollros stage 24, indicating the neural substrate for this behavior is present by this time. As a first step, we have followed the neuronal changes in the retina during the metamorphic period. Ganglion cells (GC's) were visualized in tadpoles and adults by backfilling with HRP (5-25%) into either the optic nerve or tectum using pressure or electrophoretic injection. These backfills produced extensive Golgi-like profiles of dendritic arbors; often dendritic spines could be seen. The GC's were classified according to soma size, dendritic branching patterns, and lamina of arborization in the inner plexiform layer (IPL). The origins of the identified cell types were studied by injecting ^{3}H-thymidine into tadpoles at various stages, allowing the tadpoles to mature and survive 2-3 weeks postmetamorphosis, then backfilling the GC's with HRP to visualize cell types in relationship to their neuronal birthdays.

In the mature frogs, there are at least 7 distinct types of GC's based on the above morphological criteria. The structure-function relations for the GC types were shown by making discrete HRP injections into single sublamina of the optic tectum or caudal thalamus and observing the classes of GC's which were backfilled.

Tadpoles also have at least 7 distinct GC types which all broadly correspond to those of the small frog. However, the smallest GC's often have either no dendritic process or only a rudimentary process; this would account for earlier reports of the absence of small cells seen in Golgi preparations of tadpole retina. Another interesting aspect which grossly differs between tadpole and adults, is the presence of a population of displaced ganglion cells (DGC's) in the tadpole; this DGC array is virtually absent in the adult (only 5 DGC's observed out of over 10,000 HRP filled cells examined). To analyze the fate of these DGC's, a stage series of 10 micron sectioned retinas was examined for pyknotic nuclei or migrating cells in the IPL. Migrating cells were seen to peak at stage 24; all of the migrating cell bodies in the IPL could be backfilled with HRP, indicating that the DGC's of the tadpole migrate to the GCL during metamorphic climax.

67.7 Optic Fibre Arrangements on the Retinal Surface of an Animal with an Ordered Optic Nerve and an Animal with a Disorderly Optic Nerve. J.L.James* and S.M.Bunt, (SPON:R.M.Gaze). Department of Anatomy, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, U K.

All vertebrate classes examined have optic nerves with an orderly arrangement of optic fibres within them. The only exceptions are the mammals where there seems to be considerable variation between species. Man is presumed to have an orderly optic nerve while all other mammalian species examined as adults show varying degrees of disorder in their optic nerve pattern. It has been suggested that the arrangement of fibres as they grow along the optic nerve and tracts may contribute to the formation of the orderly central optic projections in lower vertebrates. Why is it that the mammals do not appear to have orderly optic fibre pathways?

We have investigated the pattern of optic fibre growth across the retina of the goldfish (which has an orderly optic nerve) and the hooded rat (which has a disorderly optic nerve in the adult). 12 Goldfish received partial optic nerve sections at the dorsal or ventral pole of the optic nerve cross section close behind the eye. HRP was applied to the cut in a gelfoam pledget. The procedure was repeated with 12 Long Evans hooded rats. After time for HRP transport the animals were perfused, the retinae dissected out and processed as wholemounts with DAB/Ni/Co. The pattern of backfilled retinal ganglion cells was drawn. Pie slices of the retinae were then selected, dehydrated and processed for electron microscopy.

Examination of the electron micrographs showed that, in the goldfish, if peripheral retinal ganglion cells were filled by a dorsal optic nerve fill, only the superficial fibres of the intraretinal fibre bundles were filled with HRP. When central ganglion cells were filled by a central HRP application only deep fibres were filled in the retinal fibre layer. In contrast, in the rat it was difficult to get a fill of only peripheral or central ganglion cells. Only HRP applications made very close to the back of the eye formed pie slices of filled ganglion cells in the retinal wholemount. The electron microscopy showed filled fibres distributed throughout the retinal ganglion cell fibre layer, filled fibres mixed with unfilled fibres.

One possible explanation of this contrast between the mammals and the lower vertebrates is that they possess numerous ganglion cell types and the growth of the different types occurs at different rates and in varying patterns across the retinal surface. In contrast the growth of lower vertebrate retinae is orderly and circumferential.

67.8 ELIMINATION OF THE TRANSIENT IPSILATERAL RETINOTECTAL PROJECTION IN THE DEVELOPING CHICK. Steven C. McLoon. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Previous studies have described a transient ipsilateral retinotectal projection present in the developing chick. Anterograde labeling studies show that the disappearance of this projection coincides with the degeneration of nearly half of the cells in the retinal ganglion cell layer. In order to determine whether cell death selectively eliminates the ipsilateral retinotectal projection, retinal ganglion cells giving rise to the ipsilateral projection were retrogradely labeled early in development, and the retinas were examined after the period of cell death. If cell death eliminated the projection, no labeled ganglion cells should remain. On the ninth day of incubation (E9) chick embryos received an injection of fast blue along the rostral and inferior margins of one tectum. At the peak of the ipsilateral projection, E11, half the embryos were fixed, and their retinas were whole mounted on glass slides. The remaining embryos were processed on E18, well after the disappearance of the ipsilateral projection. Retinas contralateral to the injected tectum were examined by fluorescence microscopy to determine the extent of the injection. Only animals with labeled ganglion cells across the entire extent of the contralateral retina were included in the analysis. The labeled ganglion cells were counted in the retinas ipsilateral to the injected tectum from these animals. Approximately half the number of labeled ganglion cells remained in the ipsilateral retinas of the E18 embryos as compared to the E11 embryos. Examination of sections of the ipsilateral retinas from the E18 embryos showed that the labeled cells were confined to the ganglion cell layer and displaced ganglion cell layer. These results suggest that cell death does not selectively eliminate those ganglion cells which project ipsilaterally during early development since the proportion of those lost is similar to that of all ganglion cells lost during the period of cell death. The remaining ipsilaterally projecting ganglion cells must retract their aberrant axons.

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67.9 PROTEIN SYNTHESIS IN THE RETINA AND RAPID PROTEIN TRANSPORT IN THE DEVELOPING HAMSTER VISUAL PATHWAY. K.L. Moya, S. Jhaveri*, G.E. Schneider and L.I. Benowitz. Dept. of Psychology and the Whitaker College, M.I.T., Cambridge, MA 02139 and Dept. of Psychiatry, Harvard Med. Sch., McLean Hosp., Belmont, MA 02178.

The pattern of proteins expressed in the retina has been shown to change during development in higher vertebrates and during regeneration in lower vertebrates. We examined developmental changes of proteins synthesized in the retina and rapidly transported to the lateral geniculate (LGB) and superior colliculus (SC). ^{35}S-methionine was injected into the right eye of hamsters on postnatal days 2 (P2), 6, 17 and in mature animals. After 4 hours to allow retinal proteins to be transported to the SC, the retina, left LGB, left SC (LSC) and right SC (RSC) were analyzed by 2-D gel electrophoresis and visualized by fluorography.

Several major changes occur in the profile of proteins synthesized in the retina during the first 3 weeks of postnatal life. At P2 and P6, α and β tubulin were heavily labelled whereas by P17 α tubulin had disappeared and β tubulin markedly decreased until it was undetectable in the adult retina. A β tubulin associated protein, M.W. 52Kd, pI 5.4, a constituent of SER and RER but not of plasma membrane (Strocchi et al., J. Neurochem. 37: 1295, 1981) did not change markedly. A 28Kd protein present in trace amounts at P2, progressively increased until P17 and in the adult was the most heavily labelled spot on the fluorograms. This latter finding is in accordance with observations by Bock et al. (Neurosci. Abstr. 10: 1030, 1984).

Analysis of proteins in the contralateral LGB and SC showed that the 28Kd protein observed in the retina was rapidly transported and that its presence in the LGD and SC paralleled its synthesis in the retina, being barely detectable at P2 and P6 and becoming prominent at P17 and adulthood. A rapidly transported protein of 50Kd, pI 5.0 was labelled at P2, somewhat diminished at P6 and continued to decrease until it could not be detected in adult animals. Amino acid injected into the eye readily diffused into the circulation and was carried throughout the brain at P2 and P6. Axonally transported proteins were therefore distinguished from those locally labelled by examining the differences between the pattern of labelled proteins in the LSC which received the major projection from the injected eye and the RSC.

Correlated with the changes in retina and in rapidly transported proteins are striking developmental transformations in optic tract axons as they arborize in their target structures, as well as changes in their ability to grow after damage or in response to nearby denervation. (Supported by NIH EY00126, EY02621, EY05504, EY05690.)

67.10 ANALYSIS OF IMMATURE RETINAL GRAFTS MADE TO ADULT HOSTS BY LIGHT AND ELECTRON MICROSCOPY. J. R. Blair* and J. E. Turner (SPON: M. Levitt), Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

A retinal grafting model utilizing neonatal rats as donors and adult rats (225 g male Sprague Dawley) as hosts has been used in our lab to study the development of immature retinal grafts in situ and the ability of these grafts to initiate repair of the lesioned adult retina. Donor tissue is taken from neonatal rats (1-2 days post-partum) of the same strain as hosts. Twenty-three animals received lesions and grafts bilaterally with a graft survival rate of >80%. Eyes were removed for analysis by light, scanning, and transmission electron microscopy 4 weeks after receiving grafts. Analysis at the light level revealed that not only had the vast majority of grafts survived, but that they continued to develop in an organotypical manner reminiscent of intact retinas of littermates. The majority of layers present in the intact retina were present in the grafts including the ganglion cell (GCL), inner plexiform (IPL), inner nuclear (INL), outer plexiform (OPL), and outer nuclear layers (ONL). In addition there was evidence for the presence of an optic fiber layer (OFL), outer limiting membrane (OLM), and developing inner receptor segments. In areas of close apposition of the graft and host retina, the plexiform regions of the two merged. This integrating phenomonon between graft and host was enhanced in areas where the host's ILM had been disrupted such as the host lesion site. The absence of an ILM on the vitreal surface allowed direct observation of the graft GCL by SEM revealing ganglion cells of different size classes as well as smaller cells corresponding to displaced amacrine and glial cells. Open areas in the GCL allowed visualization of the dense meshwork of cellular processes and synaptic terminals of the IPL. Ganglion cell process collected on the graft surface to form large fasicles, which left the graft to course along the dense OFL of the host retina for up to 2 mm. Close examination of a fasicle showed individual fibers leaving the fasicle as it crossed the host retina. These fibers penetrated the OFL and were lost in the deeper layers of the host retina. The presence in the graft of ribbon synapses, conventional synapses, inner segment formation, and ultrastructural characteristics of the major retinal cell types has been confirmed by TEM. This work shows that mammalian neonatal retina grafted to the lesioned adult retina will continue normal development and initiate repair of the lesioned host retina by integration to an as yet undetermined extent. The phenomena noted should be further enhanced by utilizing less mature donor tissue. Supported by grant EY04377 from the National Eye Institute awarded to J.E.T.

67.11 CELL SURFACE CHANGES IN THE DEVELOPING OPTIC NERVE OF MICE. R.D. Lund, V.H. Perry, and C.C. Lagenaur. Department of Anatomy and Cell Biology and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

In previous work (Lagenaur et al., Soc. Neurosci. Abst. 10 759, '84), a monoclonal antibody to a cell surface glycoprotein of mouse CNS neurons, designated M6, was isolated and found to inhibit neurite outgrowth in vitro. We have studied the distribution of this antigen in the developing eye and optic nerve, in part to see whether there would be changes correlated with known maturational processes and partly as a baseline for studies using the mouse neuron marker after transplantation of mouse retina to rat brains (Chang et al., Soc. Neurosci. Abst., 1985).

Anti-M6 staining of retinas and nerves was performed on sections lightly fixed with paraformaldehyde. In adults, while the synaptic and optic fiber layers of the retina are heavily stained, the optic nerve and tract are completely unstained. At birth by contrast, there is heavy staining of all parts of the optic pathway. By 4 days postnatal (PN), individual bundles of fibers lose their staining as they leave the optic fiber layer to enter the optic nerve head. By day 6, there is a pronounced diminution of staining in the proximal part of the optic nerve which persists until between 14 and 18 days PN. At this stage, anti-M6 staining is lost from the rest of the nerve, leaving only the part of the axon in the retinal optic fiber layer stained.

It is suggested that M6 may be an important constituent of the cell membrane involved in maintaining interaxonal adhesion necessary for axonal extension and fasciculation. The loss of staining coincides with the cessation of optic axon growth and with the early stages of myelination when oligodendrocyte processes interpolate between individual axons breaking up the bundles of unmyelinated fibers seen at early postnatal times. The fact that only that part of the optic axon exposed to oligodendrocytes loses its staining with maturation suggests that the loss of M6 from the cell surface is a specific local response to the presence of this glial cell class.
Supported by NIH grant EY 05308 and a NATO travel fellowship.

67.12 DEVELOPMENT OF RETINOTECTAL PROJECTIONS FROM RETINAE TRANSPLANTED TO THE CEREBRAL AQUEDUCT (SPON: W. Cameron) M.H. Hankin* and R.D. Lund, Department of Anatomy and Cell Biology, School of Medicine, and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Previous studies suggest that retinal fibers prefer to grow on, or near, surfaces of the brain (Lund et al., 1982; Silver and Rutishauser, 1984). In contrast, cortical fibers seem to have a propensity for growth through a neuropil. We wondered whether the outgrowth of retinal fibers, when presented with a choice of unusual surfaces, would be consistent with these results. To this end, retinae from embryonic day (E) 13.5 mice (C57BL/6J) were transplanted into the cerebral aqueduct of newborn rats (Sprague-Dawley) whose right eye had been removed. We wished to know how soon axons would grow out of the transplants, whether they would follow the aqueduct surface, injection path, or some other cue (e.g. radial glia), and whether they would preferentially innervate the denervated tectum?

Survival times of 2-60 days were used: 80-90% of the transplants survived. Animals were perfusion fixed with 4% paraformaldehyde and the brains placed in 30% sucrose in phosphate buffered saline (PBS) overnight. Frozen sections were processed with (1) silver stain, and (2) immunohistochemically using a monoclonal antibody (anti-M6) directed against a 35 kD mouse neuronal cell surface glycoprotein (courtesy Dr. Carl Lagenaur, University of Pittsburgh).

After short survival (<1wk) silver and anti-M6 staining fail to reveal obvious fibers emanating from transplanted retinae entering either side of the tectum, although fiber bundles are seen within the transplant. By 11d postnatal, axons can be seen with anti-M6 and silver staining leaving the transplants and running in fascicles towards the superior colliculus. With longer survival (>4wks), a substantial terminal field is seen in the superior colliculus on the side contralateral to the missing eye. Axon bundles were never seen coursing along the injection pathway or along the surface of the aqueduct itself.

This study shows that transplanted retinal axons can grow through brain structures, rather than across a surface, to attain a target region. The dynamics of this process and the substrates for the growing axons are presently under investigation.
Supported by NIH grants EY05308 and EY05823.

67.13 MOUSE TRANSPLANTS INTO RAT STUDIED WITH MOUSE SPECIFIC ANTI-M6 IMMUNOCYTOCHEMICAL STAINING. F.-L.F. Chang, R.D. Lund, M.H. Hankin, and C.F. Lagenaur. Dept. of Anatomy and Cell Biology, and Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Neurons taken from embryonic animals survive transplantation to host brain where they frequently become incorporated in host tissue and interconnect with it. However, neither radioactive tracers nor HRP are ideal for studying such transplant - host interactions because of the problem of labeling the entire transplant without tracer leaking to the surrounding host. To overcome this difficulty, we have used an antibody specific to a mouse CNS neuronal surface glycoprotein (M6) to stain transplanted mouse neurons and their projections in host rat brains.

Embryonic day 13 mouse cortical or retinal tissue was transplanted to newborn rat cortex or adjacent to the superior colliculus respectively. After one month of survival, host rats were perfused with 4% paraformaldehyde; frozen cut or vibratome sections were incubated in primary antibody (anti M6) overnight, and reacted with the secondary antibody - the anti-rat IgG conjugated with HRP. Finally, tissue sections were processed with diaminobenzidine (DAB) as the chromogen and intensified with OsO_4. For electron microscopy (EM), vibratome sections were further postfixed in 1% OsO_4 for 1 hour, dehydrated, and embedded in Epon. Thin sections were cut, and examined without lead and uranium double staining.

Mouse cortical and retinal transplants stained darkly over a pale background of non-antigenic rat tissue. The staining of the transplants occurs in a manner identical to the staining in an adult mouse, with reaction product on the membrane surfaces of cell bodies, dendrites and unmyelinated axons. Within host rats, axon plexuses of transplant projections were stained; under the EM, we observed groups of stained unmyelinated fibers in the unstained rat neuropil. Areas of terminal regions of transplant projections were also stained as demonstrated by the labeling in host tectal surface for retinal transplants or local forebrain projections for cortical transplants. Myelinated axons, however, failed to stain, even when in EM sections they were cut open by vibratome sectioning to expose the axonal membrane to antibody.

In summary, our results demonstrate that mouse CNS transplants survive in host rats and send projections into host tissue. The mouse specific antibody permits identification not only of the transplanted cell bodies but also of their terminal axon distribution in the host. This allows the pattern of transplant connectivity to be studied at both the light and electron microscopic levels without the usual invasive tracer techniques. Supported by NIH EY05283.

67.14 Superior Colliculus Transplants: Pharmacological studies in Rats. G.T. Golden, T.N. Ferraro, R.G. Fariello, G.G. Smith*. (Spon: R.N. Harner). Neurology and Research, VAMC Coatesville PA 19320 and Thomas Jeff. Med. College, Phila. PA 19107.

Embryonic presumptive superior colliculus (Tectum) from E15-17 rat embryos was transplanted adjacent to the midbrain of intact 1 day old Long Evans hooded rats. Three to nine months later transplant animals were 1) sacrified by decapitation for HPLC amino acid analysis, or 2) prepared for in vivo microiontophoretic studies, of transplant responsiveness to application of putative amino acid neurotransmitters.

For HPLC analysis, whole brains were dissected on ice and the transplant (TP), host visual cortex (VC), host superior colliculus (SC) and host inferior colliculus (IC) were frozen on ice within 5 minutes. An ultrasensitive HPLC triple-column ion-exchange flurometric amino acid analysis technique, (Ferraro and Hare, Analyt. Biochem. 143, 82-94, 1984) was used to measure over 30 amino acids and related primary amino compounds in transplant and host brain regions. HPLC analysis of normal rat VC, SC and IC demonstrated that these areas have distinctively different amino acid profiles. VC has the highest levels of taurine, glutamate, tryptophan, ethanolamine, ammonia and methyllysine while being lowest in glycine, GABA, B-alanine, lysine, homocarnosine and arginine. IC was highest of the three areas in glycine, lysine, homocarnosine and arginine, while SC was highest in GABA and B-alanine and lowest in methyllysine.

In normal rat superior colliculus tissue, GABA, glutamate, glutamine, glycine, aspartate and taurine were present in μm concentrations and together comprised over 75% of all compounds quantified. Similar to normal superior colliclus, HPLC amino acid analysis revealed that GABA, glutamate, glutamine, glycine, aspartate and taurine were the major components of the superior colliculus transplants, together comprising over 75% of all compounds. Although GABA levels in transplants were higher than any other putative amino acid transmitter, transplant GABA reached only 67% of normal SC levels. Aspartate levels in transplants were 72% of normal SC while transplant levels of glycine, glutamate and taurine were at or above normal SC levels.

Microiontophoretic studies tested responsiveness of transplant neurons to iontophoretic application of GABA/bicuculline, glutamate, and acetylcholine/atropine sulfate. These studies revealed that 57% of all transplant neurons tested responded to application of glutamate while 62% of all transplant neurons responded to GABA, 50% of these GABA units were also responsive to bicuculline, a GABA antagonist.

Transplant units were not responsive to acetylcholine/atropine. The significance of these findings will be discussed. Supported by VA funds.

67.15 TRANSNEURONAL TRANSPORT OF WGA-HRP IN THE XENOPUS VISUAL SYSTEM. S. M. Royer and P. Grant. Department of Biology, University of Oregon, Eugene, Oregon 97403.

Recently, transneuronal transport of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) has been demonstrated in the visual systems of the developing chick and adult mammals (Itaya and Van Hoesen, 1981, Brain Res. 236:199; Gerfen et al., 1982, Exp. Brain Res. 48:443). These observations suggested that WGA-HRP might be a useful tool for investigating the time course and pattern of innervation of the visual neuropils during development of Xenopus laevis.

Xenopus tadpoles and juveniles were injected intravitreally with 0.7-1.0% WGA-HRP (Sigma). After survival times of 1-14 days, the brains were fixed in phosphate-buffered glutaraldehyde, sectioned at 50 microns with a Lancer Vibratome, and reacted for HRP with TMB, followed by stabilization of the reaction product by post-reaction with DAB and cobalt (Perney et al., 1983, Soc. Neurosci. Abst. 9:300). The sections were mounted on slides and examined under bright and dark field optics.

WGA-HRP was taken up by intact ganglion cells and transported anterogradely in the optic nerve and tract. In stage 54-56 tadpoles, label appeared in the contralateral optic tract, diencephalic neuropils, tectal optic fiber neuropil, and basal optic tract one day after injection. After two days, we found heavier labelling in the primary visual neuropils and also transneuronal labelling of perikarya in the thalamic nuclei and deeper tectal layers, as well as in ependymal cells. Both labelling of the primary visual neuropils and transneuronal labelling increased in extent and intensity with longer survival periods. After five days transneuronal labelling was also observed in the nuclei isthmi bilaterally. The time course of WGA-HRP transport in juveniles both to the primary visual neuropils and transneuronally was somewhat slower than in tadpoles. The pattern of WGA-HRP labelling in juveniles, while generally similar to the tadpole, reflected the more advanced development of the visual system at the later stage. In the juvenile, the ipsilateral retinal projection was labelled, and there was increased development and differentiation of the thalamic neuropils and nuclei. In no case, in either tadpoles or juveniles, was there any evidence of transneuronal transport of WGA-HRP to cells in the ipsilateral optic tectum. In both juveniles and tadpoles, WGA-HRP labelling progressed over time along a rostral-to-caudal gradient in the diencephalon and tectum and along a superficial-to-deep gradient within the tectal layers.

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67.16 DEVELOPMENT OF ANTIGEN EXPRESSION IN A POSSIBLE Y-CELL PATHWAY THROUGH THE CAT LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX. M.G. MacAvoy*, S. Hockfield and M. Sur (SPON: M.F. Eckenhoff). Section of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT and Cold Spring Harbor Labs., Cold Spring Harbor, NY.

The monoclonal antibody CAT-301 recognizes a surface antigen on large cells in laminae A, A1 and the dorsal C-laminae in the cat lateral geniculate nucleus (LGN). Antibody positive cells lie near interlaminar zones, have class I morphology, and project to area 18 (as well as area 17) of visual cortex, suggesting that the antibody specifically recognizes Y-cells in the LGN (Sur et al., Neurosci. Abst., 10:297, 1984). Furthermore, monocular lid suture and dark-rearing, two visual deprivation procedures that lead to a loss of Y-cells recorded physiologically in the LGN, also lead to a reduction of antibody positive cells in the LGN (Hockfield et al., ARVO 287, 1985).

We have examined the postnatal development of antigen expression in the cat LGN, and extended our study to include an analysis of cortical areas 17, 18, 19 and the lateral suprasylvian (LS) cortex. In the LGN, there is little or no antibody staining prior to 8 weeks of age. Antigen expression develops rapidly after 8 weeks, so that by 12 weeks at least half the adult complement of LGN cells are antibody positive.

In the visual cortex of adult cats, CAT-301 stains both pyramidal and non-pyramidal cells. In areas 17 and 18, the most dense label appears in laminae 3 and 4a. Lamina 5, as well as laminae 2 and 6, are moderately labelled. Lamina 4b and lamina 1 are completely free of label. Antibody staining is lighter in area 19 and the LS cortex, but similar in laminar pattern to that in areas 17 and 18 except for lack of label in layer 2.

The development of CAT-301 labelling is essentially similar across the different visual areas, but shows distinct differences in onset and maturation for different cortical laminae. In areas 17 and 18, for example, layers 3 and 4a show antibody labelling earliest, from about 2 weeks of age, to reach nearly adult numbers of labelled cells by 12 weeks. Layers 2, 5 and 6 are almost devoid of label until 8 weeks. Labelled cells in layers 5 and 6 increase rapidly thereafter. Cells in layer 2 are the slowest to develop the adult pattern and intensity of label.

The time course of development of antibody labelling in the LGN is consistent with the time course of development of Y-cell responses in the LGN. The laminar pattern of CAT-301 labelling in area 17 in adult cats shows features consistent with demarcation of a possible Y-cell pathway through the cortex. The laminar differences in development of antigen expression suggest differences in maturation of cells in different laminae of visual cortex, and may imply differences in development of cortical influences over areas and structures receiving input from cortex via the Y-cell pathway. Supported by BNS 8419240 and EY 05241.

68.1 DIFFERENT STRUCTURES IN CAT VISUAL CORTEX REVEALED BY MONOCLONAL ANTIBODIES. Yasuyoshi Arimatsu*, Janice R. Naegele, David Hicks*, and Colin J. Barnstable; Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021

Functionally distinct layers and cell classes have previously been described in the visual cortex with anatomical and physiological techniques. To investigate visual cortical organization at the molecular level, we used area 17 or dorsal lateral geniculate nucleus of the cat to generate monoclonal antibodies which label subsets of layers or subpopulations of neurons within area 17.

Balb/c mice were hyperimmunized with fixed or fresh homogenized tissue injected subcutaneously in Freund's adjuvants. For the final immunization, mice were given an intravenous injection of homogenized tissue in isotonic saline. Four days later, spleen cells were removed and fused with P3-NS1/1-Ag4-1 plasmacytoma cells using 50% polyethylene glycol as the fusing agent. Hybrid cultures were selected in HAT medium and culture supernatants were screened by an immunofluorescence assay on cryostat sections of fixed cat visual cortex. Positive cultures were cloned by plating at limiting dilution and grown in bulk.

Antibodies VC 1.1 and LG 1.1 were classified as IgM's and localized on structures associated with the surfaces of cell bodies and proximal dendrites. Labeled neurons were located primarily in layer 4 but a few were scattered throughout other layers as well. These antibodies exhibited similar cell surface labeling in rat tissue. VC 1.1 stained a primary band of approximately 55-60 KD on immunoblots of rat cortical cell membranes. Preliminary electron microscopic analysis suggests that the determinant resides within presynaptic structures.

Antibodies VC 2.1 and LG 2.1 reacted with layer 1 and layers 4-6 of visual cortex in a manner which suggested the pattern of termination of geniculate afferents. Layers 2+3 were unstained, however the border between layers 3 and 4 was marked by a striking, scalloped pattern of staining. Layers 4 and 5 were labeled continuously and within layer 6, irregular bands of tissue were stained.

Antibody VC 3.1 strongly labeled neuronal cytoplasm in layer 2+3 and weakly labeled some neurons in deeper layers. Layer 2+3 labeling appeared bright against a general labeling of neuropil which was present in all layers.

These antibodies demonstrate some molecular differences between cortical neurons and in addition suggest molecular similarities between some but not all cortical layers.

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68.2 MUSCARINIC RECEPTORS IN VISUAL CORTEX OF CAT, MACAQUE AND HUMAN. D. Parkinson and N.W. Daw. Department of Cell Biology and Physiology and McDonnell Center for Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

Pharmacological studies have suggested that there may be two types of muscarinic cholinergic receptors. While antagonists such as atropine, N-methylscopolamine and quinuclidine benzilate show high and similar affinities for both muscarinic receptors, pirenzepine binds with different affinities to the two sites.

Membranes prepared from primary visual cortex of adult cats bind ^{3}H-N-methylscopolamine (^{3}H-NMS) with high affinity. Scatchard plots of ^{3}H-NMS binding were linear with a Kd of 0.15 nM and maximum binding of 717 pmoles/mg protein. A Hill plot of the data gave a slope which was not significantly different from unity. Binding of ^{3}H-NMS (0.2 nM) was progressively inhibited by increasing concentrations of pirenzepine (1 nM-5 μM). Non-linear Scatchard plots were obtained which could be resolved into high (Kd = 12 nm) and low affinity (Kd = 245 nM) components with equal numbers of binding sites in each. These results suggest that there are two types of muscarinic receptors in cat visual cortex.

Receptor autoradiography was used to identify the anatomical location of these two receptors in coronal sections of cat visual cortex. For comparison, sections from visual cortex of macaque and human were also examined. Sections were incubated in the presence of 1 nM ^{3}H-NMS alone, with pirenzepine (1 and 3 μM) to distinguish receptor types or atropine (1 μM) to define nonspecific binding. ^{3}H-NMS binding in cat visual cortex was found in upper and lower cortical layers but essentially absent from middle layers (layer 4). There was no difference in the pattern of labelling in extrastriate visual cortex. The two concentrations of pirenzepine progressively inhibited ^{3}H-NMS binding but with no effect on the relative labelling pattern. In macaque, ^{3}H-NMS binding differed between primary and extrastriate visual cortex. In area 17, labelling was present in upper and lower layers to equal intensities while only part of layer IV was unlabelled. A thin layer of label could also be seen around the layer IV/V boundary. Area 18 was diffusely labelled, more heavily in upper layers. In the presence of pirenzepine, higher labelling was seen in area 17 than area 18. ^{3}H-NMS binding in human visual cortex was different from that seen in macaque. Heaviest labelling was seen in outer layers. Layer IV was unlabelled except for a faint band in the middle and a thin highly labelled band in lower layer IV. The latter was restricted to area 17. Lower layers were less heavily labelled.

These results show that muscarinic receptors in visual cortex of cat, macaque and human show different anatomical localizations. In addition, the two receptor subtypes that can be differentiated with pirenzepine appear to have very similar distributions.

68.3 GABA MARKERS IN DEVELOPING VISUAL CORTEX: IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC STUDIES IN THE PERINATAL CAT. C.A. Stark* and T.P. Hicks, Dept. of Medical Physiology, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada T2N 4N1

The traditional view of the development of cortical inhibitory processes has been that these are manifested relatively late in ontogeny, perhaps several weeks postnatally. More recent microiontophretic experiments (Wolf, Hicks and Albus, unpublished) have revealed a surprisingly robust inhibitory role for GABA in animals as young as the second week of age, operating in both spatial and temporal domains. For such synaptic processes to be operative, GABA must be present in cortex, it must be released following appropriate activation of the presynaptic neurones, and there must be a mechanism for its inactivation subsequent to its release. The release criterion has been demonstrated: cortical cup and push-pull cannula experiments have shown that GABA is released into artificial CSF during local electrical stimulation of primary visual cortex (Hicks, 1985). The present communication reports results of experiments addressing the other two criteria: presence (Immunocytochemistry), and re-uptake (autoradiography) of GABA.

Animals ranging in age from 5 days prenatal to 28 days postnatal (the majority being from the first week postnatal) have been used for immunocytochemistry of GABA and autoradiography of ^{3}H-GABA. In general, immunoreactive GABA and ^{3}H-GABA uptake increased with age. Prenatal uptake was observed of ^{3}H-GABA, even in the presence of saturating concentrations of β-alanine and amino-oxyacetic acid. In the autoradiographic studies, between birth and day 1, ^{3}H-GABA uptake rose in layer III and fell in layers I and VI; between days 1 and 4, uptake diminished slightly in layers III and IV and by day 8, uptake in layers I and II had increased to moderate levels. Immunocytochemical studies of GABA have confirmed and extended these results, documenting the ontogeny of GABA-containing neurones in perinatal cortex.

The present results provide supportive evidence for the unexpected finding that GABA mediated synaptic transmission appears to be extensive and operational at a very early stage in development in sensory cortex.

This work was supported by the MRC of Canada and the Alberta Heritage Foundation for Medical Research.

Hicks, T.P. (1985) Some functional and neurochemical determinants of GABA mechanisms in neonatal and adult visual cortex of cats. Invest. Ophthal. Vis. Sci. 26C:266.

68.4 THE EFFECT OF ENVIRONMENTAL COMPLEXITY ON THE NUMERICAL DENSITY OF NEURONS AND ON THE SIZE OF THEIR NUCLEI IN THE VISUAL CORTEX OF CAT. C. Beaulieu and M. Colonnier, Department of Anatomy, Laval University, Quebec.

In a recent study of cat visual cortex, statistically significant differences in the numerical density (Nv) of neurons were found between individual cats. It was suggested that these differences may be due to the relative richness of the (unknown and probably varied) rearing conditions of the animals used in that study. In order to verify this possibility, 2 groups of 6 animals were raised either in isolation (impoveriched condition, IC) or in a colony (enriched condition, EC) from the time of weaning to the age of 8 months. They were paired by litter and by sex. The weight, length and width of the brains were measured, and the number of neurons per mm^3 of striate cortex was estimated using a method of size-frequency distribution.

The weight, length and width of the brains were found to be greater by about 5-7% ($p < 0.05$) in the EC cats. The neuronal Nv is 18% less in EC than in IC cats (47,000 and 57,000 neurons per mm^3; $p < 0.001$). This percentage difference is maintained in most laminae. The average cortical thickness is about 4% greater in EC cats, but this slight difference is not statistically significant. The number of neurons under 1 mm^2 of cortical surface (Nc) is 15% smaller in EC cats (82,000 as compared to 96,600 in IC cats; $p < 0.05$). The area of the nuclei of neurons is 10% larger in the EC cats for the total cortical depth ($p < 0.01$). This was also significant in all layers except layer I.

The lower Nv of neurons in the EC cortex is probably not due to a decrease in the number of neurons but rather to an increase in the mass of the neuropil. The latter would result from an increase in size of the dendritic trees of EC neurons. This is consistent with the demonstrated increase in size of neuronal nuclei (which would somehow reflect the larger mass of the neuronal cytoplasm) and with the larger weight and size of the brain. The increase in thickness does not fully account for the lowered Nv. The surface of the area 17 must also be increased. If this interpretation is correct the number or width of ocular dominance columns should also be increased in area 17 of EC cats. These findings demonstrate that the richness of the environment can lead to interindividual differences of the type seen in our previous study. (MRC grant MT-3735 and FRSQ training grant to C.B.).

68.5 THE DIFFERENTIAL EFFECT OF IMPOVERISHED AND ENRICHED ENVIRONMENTS ON THE NUMBER OF "ROUND ASYMMETRICAL" AND "FLAT SYMMETRICAL" SYNAPSES IN THE VISUAL CORTEX OF CAT. M. Colonnier and C. Beaulieu, Department of Anatomy, Laval University, Quebec.

In a recent study of cat visual cortex, it was shown that there are interindividual differences in the numerical density (Nv) of symmetrical synapses associated with flat vesicles (FS synapses), but not of asymmetrical synapses associated with round vesicles (RA synapses). Since many of the properties of visual cortex neurons which are affected by the environment are GABA-dependent, it was suggested that the selective interindividual differences of FS synapses might be due to environmental factors. In order to verify this possibility we have estimated the Nv of both types of synapses in 2 groups of 6 cats raised either in isolation (impoverished condition, IC) or in a colony (enriched condition, EC) from the time of weaning to the age of 8 months. The animals were paired by litter and by sex.

Paradoxically, the Nv of all synapses is 12% smaller in the enriched condition ($p < 0.001$). This change is almost entirely due to changes in FS synapses. The average number of RA synapses is only 2% less in the EC cats and this difference is not significant ($p > 0.1$). The Nv of FS synapses, on the other hand is 45% smaller in EC cortex ($p < 0.001$). The coefficient of variation of FS synapses which in the previous study (of cats with an unknown rearing history) was on the order of 30% has been reduced to 5% in IC and to 11% in EC cats. The synapse to neuron ratio of both types of synapses shows an inverse trend. There are about 15% more RA synapses and 36% fewer FS synapses per neuron in EC cats.

The differences in the number of synapses in the two groups of animals as well as the reduction in the coefficient of variation in each of the two groups demonstrates that the richness of the environment can lead to interindividual differences of the type seen in our previous study. We suggest that the great difference seen in the Nv of FS synapses may be related to the fact that many of the neuronal properties affected by rearing conditions are GABA-dependent. If this interpretation is correct, we further suggest that during the critical period, these properties would be determined not by the addition but by the pruning of FS synapses. (MRC grant MT-3735 and FRSQ training grant to C.B.).

68.6 THE EFFECTS OF BICUCULLINE ON THE INTRACORTICAL VISUAL EVOKED RESPONSE IN THE MONKEY. M.A. Kraut*, J.C. Arezzo and H.G. Vaughan, Jr. Depts. of Neuroscience and Neurology, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

The laminar analysis of flash visual evoked potentials (VEP), current source density (CSD) and multiple unit activity (MUA), suggests that inhibition plays a substantial role in intracortical processing of visual information (Kraut et al., Neurosci. Abstr. 9, 1983). Immunocytochemical techniques have demonstrated a high concentration of presumably inhibitory GABAergic neurons in the thalamo-recipient laminae of primate visual cortex. We have utilized bicuculline, a GABA antagonist, to investigate the role of GABA dependent processes in the generation of the VEP.

Data were collected from 20 electrode penetrations in 2 unanesthetized monkeys (M. fascicularis) before and after the intracortical infusion of approximately 1 ul of 1.0 mM bicuculline methiodide at the level of lamina IV. VEP, CSD and MUA were analyzed from only those passes unaccompanied by either seizure discharge or alterations in the epidural VEP waveform recorded at a horizontal distance approximately 0.5 cm from the site of infusion.

Bicuculline had a pronounced effect on the latency and amplitude of all major VEP components. The earliest component affected, N40, is generated in lamina IVCb and partially reflects the initial postsynaptic activation of cortical stellate cells. The onset latency of the MUA and current sink associated with this component was increased by 3 to 5 msecs. A second effect of bicuculline was to extend the laminar distribution of excitatory MUA. Prior to infusion, increases in MUA to binocular stimulation were confined to the parvocellular thalamo-recipient laminae (IVA, IVCb) while after infusion, increases were consistently recorded throughout lamina IV and in the lower portions of lamina III. In contrast, bicuculline did not alter the character or spatial extent of ocular dominance regions. The observed bicuculline effects were fully reversible within 2 hours of infusion.

Our findings illustrate the importance of GABA and thus of inhibition in the genesis of the VEP. The timing of the effects suggest that inhibitory processes may modulate the sensitivity of several cortical neurons, including the initial thalamo-recipient cells. The intra-columnar distribution of flash evoked activity appears mediated by physiological processes, whereas the differentiation of ocular dominance regions is largely determined by the anatomical segregation of afferent terminals within the thalamo-recipient laminae.

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68.7 SPECIFIC PATTERNS OF INTERLAMINAR AXONAL PROJECTIONS MADE BY NEURONS WITH SMOOTH OR SPARSELY SPINED DENDRITES IN MONKEY STRIATE VISUAL CORTEX. J.S. Lund. Center for Neuroscience, University of Pittsburgh, School of Medicine, Room 336C Scaife Hall, Pittsburgh, PA 15261.

Interlaminar projections of local circuit (LC) neurons with somata in divisions of lamina 4 have been studied in Golgi preparations of infant and adult macaque monkey striate cortex. In the infant material it is clear that the majority, if not all LC neurons, have both local axon arbors, in the same lamina as their dendrites, and axon trunks that travel vertically between pia and white matter to provide arbors to other layers. The patterns of interlaminar projections appear specific for each neuron variety and also reflect the laminar locus of the LC neuron's cell body and dendritic field. The LC neuron axon arbors do not necessarily mimic the interlaminar relays of spine bearing neurons with the same laminar location. While in the adult the LC neuron axon trunks passing between layers often fail to impregnate in Golgi preparations (due probably to myelination) those neurons successfully impregnated indicate that infant axon arbor patterns are preserved into the mature visual cortex.

From 4Cβ, for example, at least four different LC neuron interlaminar projection patterns have been identified which arise from morphologically distinct LC neurons. These projections target respectively (i) lamina 6, (ii) lamina 3B, (iii) lamina 4A, or (iv) laminae 5A and 4Cα. The spine bearing stellate neurons of the same layer primarily target laminae 4A-3B, but contribute less prominant collaterals to laminae 6, 5A, 4Cα as well as 4Cβ locally. Comparing the LC and spiny neuron projections, it seems that the axons of LC neurons each appear to target only one or two of the laminae served by spiny neuron collaterals.

The presence of these LC neuron interlaminar projections and the knowledge that thalamic axons entering the divisions of lamina 4 terminate on both spine bearing (probably excitatory) neurons and LC neurons (many of which contain the inhibitory neurotransmitter GABA), together suggest that the relay of information from thalamic input layers to efferent neuron groups in striate cortex does not occur in a simple excitatory fashion. Rather, the anatomical evidence indicates that thalamic inputs activate excitatory, inhibitory and modulatory neurons which not only interact within the input laminae, but also they pass on their activity directly to other layers in a pattern unique to each type of neuron. It is therefore proposed that the LC neuron varieties are each specialized to accomplish different roles outside their immediate lamina location. The functional weighting of each of these relays will need to be determined physiologically. Supported by EY05282.

68.8 SOMATOSTATIN-LIKE AND VASOACTIVE INTESTINAL POLYPEPTIDE-LIKE IMMUNOREACTIVITY IN THE VISUAL CORTEX OF THE ECHOLOCATING BAT, MYOTIS LUCIFUGUS. L.K. Laemle and J.R. Cotter. Depts. of Anatomy UMDNJ-New Jersey Med. Schl., Newark, NJ 07103 and SUNY, Buffalo, NY 14214.

An ever increasing number of putative neurotransmitters and neuromodulators is being identified in the mammalian and non-mammalian visual system. Although studies are under way in several laboratories to try to ascertain their roles in vision, their functions for the most part remain unknown. Somatostatin (SRIF) and vasoactive intestinal polypeptide (VIP) are among those neuropeptides which have been identified in cell bodies and fibers in the visual cortex and retina of several species, and whose functional significance remains unclear.

Myotis lucifugus is a species with an all rod retina, in which discrete cycles of visual activity can be isolated. The visual cortex of Myotis is comprised of five layers; a molecular layer, and four layers of cells. The superficial cell layer (layer II/III) is thin and contains densely packed perikarya. The intermediate cell layer (layer IV) is thicker, and cells are less densely packed. Layer V is represented by a discontinuous layer of scattered triangular cells; the deep cell layer (layer VI) is thicker, and cells are closely packed.

We have demonstrated SRIF- and VIP-like immunoreactivity in fibers and perikarya in the visual cortex of three euthermic animals, and compared the distribution of the two peptides. Animals were sacrificed by administration of an overdose of anesthesia; brains were fixed in Bouins solution, embedded in paraffin, and sectioned at 12u in the coronal (2 animals) or saggital (1 animal) plane. SRIF and VIP were localized light microscopically using the appropriate antisera and the PAP method of Sternberger. Staining was judged specific by the absence of DAB reaction product following adsorption of the primary antiserum with synthetic peptide and/or replacement of the primary antiserum with normal preimmune rabbit serum. SRIF-like immunoreactivity was present in all cortical layers. Fine immunoreactive terminals were most numerous in layer II. Larger diameter, branching fibers were observed in layers I and II. Immunoreactive perikarya were morphologically uniform, and were located predominantly in the deep cell layer (layer VI). VIP-immunoreactive fibers, terminals, and perikarya were more numerous than those which were immunoreactive for SRIF. VIP-positive fibers coursed through all cortical layers; terminal plexuses were concentrated in the superficial cell layer. VIP-positive perikarya were observed in both superficial and deep cell layers and were morphologically more diverse than SRIF-positive perikarya. (Supported by NIH grant EY04204 to LKL).

68.9 Segregation of On-center and Off-center Afferents in the Tree Shrew Striate Cortex. D. Raczkowski and D. Fitzpatrick. Dept. of Anatomy, Duke University Medical Center, Durham, N. C. 27710

In the lateral geniculate nucleus of the tree shrew, on-center and off-center neurons are located within different laminae (Conway and Schiller, 1983, J. Neurophysiol. 50: 1330-1342). Neuroanatomical studies and microelectrode recordings have demonstrated that these laminae project to separate layers within the striate cortex (Conley, Fitzpatrick and Diamond, 1984, J. Neurosci., 4: 171-197; Norton, Kretz and Rager, 1983, Invest. Ophthal. Suppl. 24: 265). The aim of the present experiment was to determine how individual on-center and off-center geniculate afferents terminate within the visual cortex. To achieve this aim, we injected horseradish peroxidase into single, physiologically identified axons in the optic radiations.

To date, this study of the tree shrew's visual cortex has revealed that individual on-center and off-center geniculate axons do terminate in separate tiers in layer IV. On-center axons supply a dense arborization to layer IVa and provide only a sparse input to layer IVb. In contrast, off-center axons terminate predominantly in layer IVb. The terminal fields of some axons occupy almost the entire width of layer IVa or IVb, while the terminal fields of others are more restricted. Some of the axons that arborize mainly in either layer IVa or IVb also give rise to collaterals that terminate in layer III; no collaterals were seen in layer VI.

These results provide anatomical evidence for the existence of 'on' and 'off' parallel channels in the tree shrew geniculostriate pathway. The functional significance of preserving the segregation of these pathways into layer IV of the striate cortex is not clear. However, as a consequence of this circuitry, some neurons in the tree shrew striate cortex may be influenced exclusively by either the 'on' or 'off' channel.
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68.10 PHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF CORTICOTECTAL CELLS IN THE HOODED RAT. B.R. Schofield, L.E. Hallman* and C.-S. Lin. Dept. Anatomy, Duke Univ. Med. Cntr., Durham, NC 27710

Corticotectal (CT) cells in area 17 of the hooded rat were identified by antidromic activation from the superior colliculus. Quantitative receptive field analysis was conducted using computer-controlled stimuli. Identified CT cells were then impaled and injected with horseradish peroxidase. Our goal was to examine the relationship between the morphology and physiology of CT cells.

Most CT cells respond to visual stimuli at all orientations; however, quantitative analysis reveals that some cells are orientation selective (criteria as in Schiller et al., '76). Similarly, most CT cells are not selective for direction of stimulus movement, although a few directionally-selective CT cells have been found.

The responses of CT cells to stimuli of different velocities suggest that subtypes may exist. Some CT cells respond to a wide range of velocities (e.g. 10-120°/sec); some cells exhibit a high-pass response (e.g. respond to 60-100°/sec, but not to 20°/sec); and others show a low-pass response (e.g. respond to 10-20°/sec, but not to 40-80°/sec).

CT cells labelled with horseradish peroxidase share several morphological traits. All are layer V pyramidal cells. The basal dendrites are distributed throughout the depth of layer V, and often extend into layers IV and VI. The horizontal extent of these dendrites can measure up to 300um in the medial-lateral and anterior-posterior axes.

The apical dendrites of CT cells arborize in layer I in a terminal tuft that can spread over a much wider area than the basal dendritic tree (up to 800um). These processes are thin and spiny and travel in the upper portion of layer I. The apical dendrite also gives rise to several oblique dendrites that extend with little or no branching into layers II/III and IV. The axons of CT cells have numerous intracortical collaterals, most of which arborize within the region occupied by the basal dendrites. Axonal swellings, presumably presynaptic boutons, can be found in layers IV, V and VI. A few collaterals can be traced for longer distances in layer V (200-300um); in one case a collateral could be followed for 700um, extending almost to the lateral border of area 17. In contrast to the local network, the long horizontal collaterals show very little branching.

We conclude that CT cells in the hooded rat are similar to those in other animals. Our current efforts are directed toward: 1) more detailed analyses of velocity tuning of CT cells; 2) comparisons of CT cells with non-CT cells. Supported by NIH EY05490, EY05777, GM07046, and FFS PD84-066.

68.11 TOPOGRAPHY OF AFFERENT PROJECTIONS TO MONKEY STRIATE CORTEX D. J. Perkel*, J. Bullier* and H. Kennedy (SPON: European Neuroscience Association) INSERM U. 94 16, av. Doyen Lépine 69500 Bron FRANCE

It is generally assumed that the projections which link the multitude of retinotopically organized visual structures connect regions representing the same portion of the visual field. One might expect, then, to find a spatial separation in one structure of neurons which project to separate regions of another structure. To test this hypothesis, we have made paired injections of 2 different fluorescent retrograde tracers, fast blue (FB) and diamidino yellow (DY) in separate regions of macaque monkey striate cortex (area 17 or V1), and examined the degree of intermingling of the populations of labelled neurons in the structures afferent to V1.

In 6 animals one injection was made parallel to and 1-2 mm away from the V1/V2 border. In 4 of these, a second injection was made parallel to the first and at a distance of 2-3 mm, and in 2 monkeys the injections were separated by 7 and 17 mm. In all cases, 2 separate populations of labelled neurons were found in the lateral geniculate nucleus (LGN) and in the lateral and inferior pulvinar. Double-labelled (DL) neurons were observed extremely rarely in these structures. In contrast, the claustrum contained almost completely intermingled populations following injections separated by 2-3 mm, and showed diminished overlap for greater injection separations.

Labelled cells were also found in extrastriate cortex. On two-dimensional reconstructions of the cortical surface, 5 patches of labelling could be distinguished for each tracer, including the readily identified areas V2 and MT. Labelled cells in V2 were found in supragranular and infragranular layers, with a greater density and lateral extent in the latter. Following injections separated by 2-3 mm, the 2 populations were partially overlapping, and DL neurons, concentrated in the zones of overlap, made up 5-10% of the labelled cells within these zones. With greater distances between the injections, there was complete separation of the V2 populations, and no DL neurons were found. In area MT, there was essentially complete intermingling of the 2 populations, except in the cases with 7 mm and 17 mm separations. DL cells constituted 5 - 10% of labelled MT neurons in the regions of overlap when the injections were separated by 2-3 mm, but this fraction dropped to nearly 0% with larger injection separations. Intermediate between areas V2 and MT, the 3 patches of labelled cells on the anterior bank of the lunate sulcus and on the prelunate gyrus contained partially overlapping FB- and DY-labelled cell populations after injections separated by 2-3 mm. The intermingling was greatly reduced following the injections separated by 7 mm, and the populations were separate in the 17 mm case. In these areas, as elsewhere, DL cells were confined to the regions of intermingling and formed 5-10% of the labelled neurons in those regions.

The spatial intermingling of neurons in the claustrum and extrastriate cortex which project to separate regions of V1 may be related to the larger receptive field size and scatter found in these structures, in contrast to the separation found in the thalamus, where receptive fields are smaller and less scattered. This suggests that the topography of connections between two visual structures is related to the characteristics of the visual field representation in the afferent and target structures.

68.12 ILLUSORY CONTOURS CAPTURE STEREOPSIS AND APPARENT MOTION V. S. Ramachandran* and P. Cavanagh* (SPON: R. M. Boynton), Dept. of Psychology, UCSD, La Jolla, CA 92093.

We began with a square matrix of four circular 1° wide black discs from which we removed right angle sectors. This created the impression of a white "subjective" square with its four corners occluding the discs ("illusory contours"). The display in the other eye was identical except for a horizontal disparity introduced between the edges of the cut sectors so that an illusory white square floated out in front of the paper. We superimposed a "template" of this stereogram on a vertical square-wave grating (6 cycles/ degree) and found that the illusory square pulled the corresponding lines of grating forward even though the grating was at zero disparity. This illusion ("stereo-capture") was strong enough to overcome the physical continuity of the lines so that apparent breaks appeared on the lines along the upper and lower borders of the subjective square. The illusion could be seen in 160 msec flashes. Since the disparity of the cut sectors was at least 3 times the grating periodicity the observation implies that the disparity signal derived from the subjective surface was strongly influencing the processing of the lines of wallpaper (grating). This is consistent with a novel effect observed recently by Mitchison and McKee. They found that disparity of the end points of horizontal dot rows presented binocularly could influence the matching of repetitive ambiguous elements in the middle.

We also found that capture disappeared if: 1) Disparities were uncrossed instead of crossed (presumably because of conflicting "occlusion" cues); 2) Real black-white line segments were used instead of subjective contours; 3) The template was superimposed on horizontal gratings (the grating remained flush with the background); 4) The disparity of the subjective square was not an exact multiple of the grating spacing; 5) Curved or tilted subjective surfaces were used. These observations imply that capture results not just from attribution but possibly from synergy (e.g. positive feedback gain amplification) between cells in V1 that respond to disparities conveyed by the wallpaper and those in V2 that respond to subjective surfaces.

Analogous effects were observed in apparent motion ("motion capture"). By removing sectors from discs in an appropriately timed sequence we could generate apparent motion of an illusory square. When a template of this display was superimposed on periodic patterns (e.g. grids of dots or gratings) the dots or lines appeared to move vividly with the square even though they were physically stationary. We conclude that segmentation of the visual scene into surfaces and objects can profoundly influence the early visual processing of stereopsis and apparent motion.

68.13 LOSS OF VISUAL SENSATION IN THE MONOCULAR GANZFELD FOLLOWS RULES COMPARABLE TO BINOCULAR RIVALRY. S.J. Bolanowski, Jr., O.S. King* and R.W. Doty. Center for Brain Research, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642.

Monocular presentation of a contourless visual field (Ganzfeld), or stabilized retinal image, produces dramatic vanishings (blankout) and sporadic returns of visual sensation despite continuing stimulation. When equiluminant Ganzfelden are presented binocularly, blankout does not occur, indicating that the locus of the effect must be central and not retinal. These facts and the observations that even during blankout a) the monocular crescent is commonly perceptually visible, b) pupillary dilatation accompanies the blankout, and c) pressure block of the eye in darkness prevents the blankout, all suggest that the phenomenon is related to binocular rivalry (Bolanowski and Doty, Neurosci. Abst., 8:675, 1982). Blankout is thus probably a result of the eye in darkness becoming dominant, suppressing the eye viewing the Ganzfeld. If true, then there should be an elevation of visual threshold in the suppressed eye, comparable to that known to occur in rivalry. To test this, we installed a fiber optic bundle subtending 2.3° on the Ganzfeld surface and measured increment detection thresholds to 10-msec test-probe flashes under three conditions: 1) during binocular viewing of equiluminant Ganzfelden, 2) to the eye viewing the Ganzfeld monocularly prior to blankout, and 3) to that eye during periods of blankout. The test flash was initiated by the observer and its intensity randomly controlled by the experimenter. For a criterion of 50% detection, subjects required a test probe intensity ca 0.4 log units greater for 3) compared to 2); and ca 0.5 log units for 3) versus 1). Such a result is wholly concordant with the supposition that the blankout is a form of binocular rivalry, the eye in darkness suppressing input from the eye viewing the Ganzfeld. Most remarkable was the discovery that this suppression ensued immediately and endured for ca 2-6 sec when the 10-msec flash was presented to the eye in darkness, i.e., blankout in the eye viewing the Ganzfeld was inevitably induced. This ability to control precisely the time of onset of blankout opens up a number of experimental possibilities, including its behavioral and electrophysiological analysis in alert macaques. (Supported by NIH Grant EY04354).

68.14 ESTIMATING THE PROPERTIES OF VISUAL SPATIAL INFORMATION PROCESSORS USING AN EVOKED POTENTIAL INTERACTION PARADIGM. David Ryan-Jones* & M.A. Berkley, (SPON: J. Tunkl), Florida State Univ., Tallahassee, FL 32306.

Current theories of vision suggest that different stimulus features are processed by different neural populations. A variety of psychophysical paradigms have been employed to test this idea and define the characteristics of putative processors. We have applied this strategem in an evoked potential (EP) paradigm to estimate the characteristics of the neural substrate of linear contour and vernier acuity processors. The following assumptions were made: 1) the neural responses (EP) generated by two independent neural populations should show additivity if the responses occur close enough in time; 2) non-independent processors should show little additivity under the same conditions; and 3) spatial overlap of neural populations should show partial additivity.

With these assumptions, evoked potentials were recorded to two briefly presented (100 msec) linear contours (line 2° x .01°) at various onset asynchronies (SOA) where the line targets were: 1) orthogonal to each other; 2) parallel to each other; and 3) when one target was a briefly presented (100 msec) vernier offset and the other was either an 100 msec presentation of an orthogonal line passing through the offset; or a 100 msec presentation of a pair of parallel lines straddling the offset target. The recorded responses were quantitatively compared to the computer-combined sum of the EPs recorded to each stimulus separately.

The results show that for the orthogonal line targets, there was complete summation between the responses elicited by each line target, e.g., recorded EPs and computer-simulated EPs were essentially identical in shape and amplitude at all SOAs. Complete occlusion was observed in the parallel line condition up to SOAs of 100 ms, e.g., recorded amplitude to the parallel line stimulus pair more closely resembled the response elicited by a single line stimulus than the computer-simulated sum. This occlusion effect was seen strongly at spatial separation distances up to 30' and less strongly for separations up to 1°. In the vernier offset-line conditions, the EP recorded to the combined stimuli showed significant summation when an SOA appropriate to compensate for the latency differences between the offset-elicited and line-elicited EPs was employed. Additivity was observed in both vernier offset-line configurations but was greatest when the line passed through the offset.

The results demonstrate that the EP technique can be employed to evaluate the spatial characteristics of neural processors and support the view that: 1) orthogonal contours are processed by separate neural populations; 2) parallel contours are processed by overlapping pools of neural elements, the degree of overlap extending up to at least 1° of separation; and 3) linear contours and vernier offsets appear to be processed by separate neural populations which may share some common elements. (Supported by EY00953)

68.15 THE CHARACTERISTICS AND TIME COURSE OF ADAPTATION TO GRATINGS AS ESTIMATED FROM EVOKED POTENTIALS. Alan Ho* & M.A. Berkley, Florida State Univ., Tallahassee, FL 32306.

The threshold contrast for detecting a test grating increases exponentially as a function of the time a moderate contrast adaptation grating is viewed when the spatial frequency and orientation of the adaptation and test grating are similar.[1] The rate at which sensitivity returns to pre-adaptation levels is also exponential. Changes in apparent contrast during prolonged viewing of a suprathreshold grating have similar temporal properties.[2] Psychophysical studies have reported time constants of 10-20 secs for these changes while electrophysiological and behavioral cat studies of adaptation - recovery have reported much shorter values.[3,4] It has been suggested that the longer psychophysical estimates are due to the use of paradigms which have significant response-time delays.[5]

To obtain time-course estimates not limited by response variables, we have recorded visual evoked potentials (EP) in humans during prolonged viewing of counterphase (8 Hz) gratings and during a recovery period in which the grating target was intermittently viewed. Samples (2 sec) of the EPs elicited in response to 0.6 contrast sinusoidal gratings were recorded at 13 intervals during 45 secs of adaptation and at 5 intervals during subsequent 50 secs of recovery. During adaptation, the target was continuously present while during recovery, the grating appeared only during the recording periods, the intervening intervals displaying a zero contrast target of an equal average luminance. A recording session consisted of 16 adaptation-recovery cycles with a 100 sec rest period between each cycle.

Plots of the first two harmonics (16 & 32 Hz) of the EPs against time showed that the amplitude of these components declined exponentially during adaptation and then increased exponentially during recovery. Time constants derived from these data ranged between 2-7 secs, varying with spatial frequency, and were significantly shorter than psychophysical estimates supporting the view that the latter measures may have been limited by procedural constraints. An unexpected finding was that the maximal amplitude EP often occurred 3-6 secs after the beginning of adaptation. Increases in sensitivity after the start of adaptation have not been reported in the psychophysical literature, but delayed peaks have been observed in single cell adaptation studies.[3] The delayed peak response could be eliminated or made to occur earlier by using adaptation contrasts less than 0.1. A multichannel, mutual inhibition model appears to adequately describe the results.

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(Supported by EY00953-14).

68.16 INDIVIDUAL DIFFERENCES IN SPATIAL FREQUENCY VISUAL PROCESSING. G. Schechter* and E. Callaway. Langley Porter Psychiatric Institute, University of California, San Francisco, California 94143.

Stimulus spatial frequency is a critical variable in visual information processing. Current efforts to explain the role of spatial frequency channels in vision link them to attentional mechanisms. The human visual system can selectively attend to stimuli of specific spatial frequencies contained in a complex visual scene. While studying spatial frequency-attention interactions, we observed wide variability in how individuals responded to low and high spatial frequency components of a complex visual display.

Reaction times (RTs) to stimuli composed of large and small letters, corresponding to low and high spatial frequencies, were compared under separate instructions to attend to designated large letters, small letters, or both. Under instructions to attend equally to both size letters, some subjects had faster RTs to large targets compared to small, whereas others had faster RTs to small targets compared to large. Subjects quantitatively divided into 2 groups on these contrasting response patterns were compared across the 3 attention conditions. There was a significant group by stimulus size by attention interaction. The distinct intersubject differences observed in the equal attention condition also emerged in the selective attention conditions. The largest group difference occurred in response to small targets when attended.

The transient-sustained dichotomy describes visual processing systems differentially sensitive to low and high spatial frequencies, respectively, and has been demonstrated structurally, functionally and behaviorally. If the transient-sustained concept is invoked, intersubject differences might be interpreted as individual variation in the dynamic balance between the two systems. Subjects who naturally responded faster to large targets (i.e., were more sensitive to low spatial frequencies) might have a transient processing system that inhibits the sustained. Other subjects who responded faster or equally to small compared to large targets (i.e., were more sensitive to high spatial frequencies) may have a more active sustained system that inhibits the transient one.

Differences between normal individuals in response to low and high spatial frequency stimuli were demonstrated. Extreme imbalances between transient and sustained processing in pathological states require confirmation.

68.17 CORTICAL DYNAMICS OF DEPTH, BRIGHTNESS, COLOR, AND FORM PERCEPTION. S. Grossberg* and E. Mingolla. Center for Adaptive Systems, Boston Univ., Boston, MA 02215.

A real-time visual processing theory is used to unify the explanation of a wide variety of perceptual phenomena involving depth, brightness, color, and form perception. The theory is also used to analyse and predict phenomena concerning the architecture of striate and prestriate visual cortex. This analysis concerns the processing rules whereby a Boundary Contour System (BCS) and a Feature Contour System (FCS) (Grossberg and Mingolla, Psych. Rev., 1984, 92, 173) differentially react to scenic properties before interacting to generate a percept of color-and-form-in-depth. Predicted BCS properties have been supported by experiments of von der Heydt, Baumgartner, and Peterhans (Science, 1984, 224, 1260) on monkey visual cortex. These properties also provide computer simulations of perceptual grouping and segmentation phenomena, including the grouping of textured images, randomly defined images, and images built up from periodic scenic elements. The BCS provides a unified set of rules for analysis of edges, textures, and surfaces (shape-from-shading). The complex cells of the BCS pool wavelength-sensitive LGN inputs into a broad-band boundary signal (cf. Thorell et al., Vision Res., 1984, 24, 751), whereas the double-opponent cells of the FCS preserve the separation of LGN wavelength-sensitive properties. Multiple spatial scales within these systems are used to parse two-dimensional scenes into a representation of color-and-form-in-depth that is suggested to occur in V4. Binocular disparity-sensitive BCS interactions give rise to signals, called filling-in generators and filling-in barriers, which control the brightness and color signals leading to visible percepts. Computer simulations of brightness data led to this filling-in model (Cohen and Grossberg, Percep. Psychophys., 1984, 36, 428) whose mixed electrotonic and chemical transmitter interactions are formally the same as those reported among the H1 horizontal cells of the turtle retina (Piccolino et al., J. Neurosci., 1984, 4, 2477). The cortical filling-in process may thus be an evolutionary homolog of a retinal process. Applications are made to such additional perceptual phenomena as: tissue contrast, binocular rivalry, transparency, McCullough effect, metacontrast, and hyperacuity.

68.18 SPATIAL CONTRAST SENSITIVITY IN IMPAIRED LEARNING AND RECOGNITION OF FACES. M. Rizzo,* J.J. Corbett,* H.S. Thompson,* A.R. Damasio, (SPON: G.W. Van Hoesen). Div. of Behavioral Neurology and Neuro-ophthalmology Clinic, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Recent work suggests that the recognition and learning of complex images may rely on spatial filtering characteristics of the human visual system. When high or low spatial frequencies are filtered from pictures of faces, their recognition by normal subjects is impaired. Prosopagnosia, an impairment of the recognition and learning of human faces, might thus result from impaired perception in spatial frequency channels carrying information crucial to these processes. We studied two stable prosopagnosic subjects to investigate the hypothesis that an impairment of spatial contrast sensitivity (SCS) at specific frequencies might be sufficient to explain prosopagnosia.

Both subjects #1 and #2 had sustained bilateral lesions in the occipito-temporal region, shown by computerized tomography and magnetic resonance. Neither subject could learn new faces no matter how lengthy and repeated the exposure. In addition, subject #1 could not recognize the faces of persons she knew before her illness. Both subjects had normal intellect and language, visual acuity of 20/20 OU and no evidence of retinopathy or opacity of the ocular media. Neither had alexia, visual disorientation, ocular apraxia, optic ataxia, or neglect. Electro-oculographic study showed normal scanning of facial features in both; each subject could make subtle discriminations between faces they were not required to learn or recognize.

SCS was tested with the Cadwell CTS-5000 system utilizing the ascending method of limits for presentation of vertical sinusoidal gratings to both eyes. Compared to controls, subject #1 had a relative reduction of SCS at high frequencies but retained adequate SCS in this range to permit accurate processing of high frequency information in room light, for printed materials. Subject #2 had normal SCS at all frequencies.

The findings show that prosopagnosia can be seen with intact SCS, as demonstrated in #2. In addition, SCS at low and intermediate frequencies was entirely normal in #1. The method used here was sensitive enough to detect her relative SCS impairment at high frequencies, but it is unlikely that this defect caused her agnosia since she had sufficient high frequency function to process accurately the high frequency information which composes line drawings. These results are consistent with the notion that recognition and learning of faces depends on the full range of spatial frequencies. The presence of adequate visual sensory ability and normal oculomotor scanning of facial features in these subjects also supports the hypothesis that prosopagnosia is a result of defective visually triggered memory, rather than the consequence of a primary perceptual defect.

68.19 SPATIO-TEMPORAL SEPARATION OF STRIATE FROM EXTRASTRIATE CORTICAL ACTIVITY IN HUMANS. R. Srebro. Depts. Ophthalmology and Physiology, Univ. of Texas, Southwestern Medical School, Dallas,TX 75235

A Laplacian analysis of the visually evoked potential field on the scalp (see Srebro, R.J. Physiol 360, 233, 1985 for details of method) allowed separation of striate from extrastriate cortical activity in both time (following stimulus) and space (cortical mantle). Eight subjects were shown stimuli consisting of 2 degree lower hemifield half-disks filled with 14 minute black and white checks as pattern pulses (16 2/3msecs) against a 12 by 18 degree uniformly illuminated background. In each of 3 recording sessions, 12 scalp electrodes were placed using a regular 2cm tessellation from which were derived 4 Laplacian estimators with 1/2 overlap. A region of scalp from 1cm left to 5cm right of Oz and from 3cm below to 5cm above Oz (approximately 35cm^2 of cortical mantle) was ultimately characterized by 10 Laplacian estimators for each subject. Polarity inversions suggested the existence of at least 2 sources of cortical activity separated in both time and space. The set of 10 Laplacian estimators, $|F_i(t)|$, where i indexes spatial location, was modeled by:

$$F_i(t) = a_i g(t) + b_i g(t+\tau)$$

with, $$g(t) = (n(t/c)^{n-1} - (t/c)^n)\exp(-t/c)$$

i.e. by 2 identical biphasic components which sum linearly to produce all of the 10 Laplacian estimator waveforms. A nonlinear least squares fit established the values of n, c, and τ for each subject. This simple model accounted for the waveforms of the Laplacian estimators very well. The values of τ, the delay between the 2 components, ranged from 10 to 30 msecs for the eight subjects studied. There was always considerable temporal overlap between the 2 components. The set of weighting coefficients, $|a_i, b_i|$, was used to construct maps showing the cortical topographies of the 2 components for each subject. The earlier component was strongest near the midline and spread 2 to 3cm laterally. The later component was strongest 3 or 4cm lateral to the midline. In several subjects there was some spatial overlap between the 2 components, probably reflecting the limit of the spatial resolution of the method. These results suggest sequential activation of striate and extrastriate cortex.

69.1 LOCAL NEURONAL CIRCUITS IN THE LATERAL GENICULATE BODY OF THE ALBINO RAT. S. Reinis and D.S. Weiss*, Department of Psychology, University of Waterloo, Waterloo, Ont., Canada N2L 3G1

Multiple unit responses were recorded from spontaneously firing neuronal populations located in the lateral geniculate body of albino rats anaesthetized with Ketamine and Rompun using a single tungsten microelectrode. The positions of the detected spikes in the sweep were stored as well as their amplitudes. During the evaluation of the data, the spikes were divided into eight amplitude classes, from 0 to 7, according to the spike voltage. The lowest, 0 class, represented the noise of the equipment, and was not used for any further calculations. An algorithm was developed to compute the interactions of neuronal groups characterized by a particular amplitude. The algorithm first computed auto- and cross- correlograms between and within the seven classes of spike amplitudes. The most common interspike interval (the peak value of each histogram) was selected from these histograms and was then used for the identification and storage of all the pairs of spikes in the original record which were characterized by that particular interval between them. It is reasonable to expect that the most common interspike interval represents a real, causal interaction between the activities of two neurons. In order to recognize more "real" interneuronal interactions, where firing of one neuron (or group of neurons) is causally related to the firing of another group, and separate them from the "false" interactions which depend on the method of the calculation of correlation histograms, the second spikes related to the most common interspike intervals were then erased from the original record, and the correlation histograms were calculated again. Now, the second most common interspike interval was identified and the relevant spike pairs categorized and stored. In this way, the algorithm proceeded stepwise until the frequencies of the interspike intervals did not differ from data generated randomly. The number of cells involved in the population firing could also be estimated from these calculations. Finally, a model of the sequence of neuronal interactions was constructed, described, and visualized on the screen of the computer. The results show that even in the lateral geniculate body, without any visual stimulation, the nerve cells interact in a complex manner, and form functional systems which may probably be related to a logical analysis of the sensory input. A quantitative expansion of these computer programs may provide a detailed model of the functioning of large neuronal systems.

69.2 DISTINCT METABOLIC SUBDIVISIONS OF THE RAT LATERAL GENICULATE NUCLEUS REVEALED BY CYTOCHROME OXIDASE HISTOCHEMISTRY. P.W. Land. Center for Neuroscience and Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The metabolic requirements of individual laminae in the cat and monkey dorsal lateral geniculate nucleus (LGNd) are extremely sensitive to the integrity of retinal input from the appropriate eye. Thus, monocular enucleation, monocular lid suture and tetrodotoxin induced blockage of retinal ganglion cell activity all result in a marked and persistent reduction in activity of the mitochondrial enzyme cytochrome oxidase (CO) in the corresponding geniculate laminae.

CO histochemistry was used in the present study to examine patterns of metabolic activity in the LGNd of Long-Evans black-hooded rats. Sections through the LGNd of normal adult rats and rats that had one eye removed for 60 days beginning either on the day of birth or at 30 days of age were reacted for CO according to the method of Wong-Riley (Brain Res. 171:11, 1979). Some of these animals also received an intraocular injection of horseradish peroxidase (HRP) and in these cases adjacent series of sections were reacted for CO and HRP.

In rats, there are no clear cytoarchitectonic subdivisions of the LGNd corresponding to the segregated retinal input. Nevertheless, in normal animals the CO reaction reveals distinct metabolic subdivisions within the nucleus that coincide with the distribution of crossed and uncrossed retinal ganglion cell axons. Thus, regions of the LGNd receiving a projection from the ipsilateral eye are considerably more reactive than those receiving a contralateral projection. This pattern is enhanced in the LGNd ipsilateral to the intact eye of animals enucleated at 30 days of age and is obscured in the contralateral nucleus. In animals that had one eye removed on the day of birth, and that have abnormal patterns of retinal axon termination, neither geniculate shows this characteristic pattern of CO reactivity.

Taken together, these results indicate a somewhat different contribution of crossed vs. uncrossed retinal input to the overall metabolic activity of the rodent LGNd. They also suggest that there may be functional differences among cells in different portions of the LGNd despite the absence of overt cellular laminae.

69.3 PARABIGEMINOGENICULATE PATHWAYS IN MAMMALS. T. Hashikawa*[1], J.T. Weber[2], D.P. Van Lieshout*[3] and J.K. Harting[3]*. (Spon.: W.T. Welker). Depts. of Anatomy, Iwate Medical University[1], Morioka, Japan, Tulane Medical School[2], New Orleans, 37240, and the University of Wisconsin[3], Madison, WI 53706.

We have used anterograde and retrograde tracing methods to demonstrate the presence and distribution of parabigeminogeniculate pathways in the opossum, rat, grey squirrel, gopher, tree shrew, cat, Galago and Saimiri. While interesting variations occur in the laterality (i.e., in opossum, rat, grey squirrel, gopher and tree shrew the projection is primarily crossed; in the cat it is bilateral, while in Galago and Saimiri the pathway is primarily uncrossed) and distribution of parabigeminogeniculate axons across these eight mammals, a connectional theme revealed by our data is that in all of the species examined, parabigeminal axons distribute most densely to geniculate zones which are targeted by tectogeniculate axons. In those species possessing laminated lateral geniculate nuclei, the overlap of tectal and parabigeminal axons occurs within small-celled layers and interlaminar zones; neurons in such regions project to supragranular layers of area 17 (i.e., layer 3 and interlaminar zones in tree shrew; layers C_3 and C_2 in cat; layers 4 and 5 and interlaminar zones in Galago; "S" layers and interlaminar zones in Saimiri). Since cells within the superficial layers of the superior colliculus provide the primary input to the parabigeminal nucleus, it follows that small-celled geniculate layers and interlaminar zones are heavily influenced by collicular and "collicular-like" information via two subcortical circuits (tectogeniculate and tecto-parabigeminogeniculate). (See Sherk, '78, '79 for review).

In addition to ending quite extensively within tectally innervated regions of the lateral geniculate nucleus, the present data reveal that in most of the species studied, parabigeminal axons also terminate within other layers (magno- and parvocellular). These findings suggest that parabigeminal information not only has access to supragranular layers of area 17 via the small-celled geniculocortical system (as does tectal information), but in addition can reach sublaminae of layer IV and layer VI via channels arising from neurons within the magno- and parvocellular layers. When the total distribution of geniculate afferents arising from the superior colliculus and the parabigeminal nucleus is considered, it seems likely that colliculo-geniculate interactions play a major role in geniculocortical functions(s).

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69.4 SUBCORTICAL PROJECTIONS TO THE LATERAL GENICULATE NUCLEUS IN GALAGO AND TREE SHREW. D. Fitzpatrick, G. Luppino*, D. Schmechel*, and I.T. Diamond. Depts. of Anatomy and Psychology, Duke University, Durham, NC 27706.

As a first step in understanding the way in which subcortical inputs may influence the pattern of activity in retino-geniculocortical pathways, we are studying the origin, pattern of termination and neurotransmitter characteristics of subcortical projections to the lateral geniculate nucleus (GL) in the prosimian primate Galago senegalensis and in the tree shrew, Tupaia belangeri.

Injections of HRP into GL in both species produce labeled neurons in the reticular nucleus of the thalamus, the superficial layers of the superior colliculus (SC), the pretectum (PT), the parabigeminal nucleus (PBG), and various subdivisions of the pontine and midbrain reticular formation (locus coeruleus, dorsal raphe, and the region surrounding the brachium conjunctivum). Labeled neurons in most of these structures are located either entirely or predominantly ipsilateral to the injection site in GL except for PBG in the tree shrew that appears to be almost entirely contralateral.

Anterograde transport of WGA-HRP from these subcortical structures reveals different patterns of termination within GL. In Galago, SC projections terminate mainly in layers 4 and 5 (the intercalated layers) and in the interlaminar zones (Fitzpatrick et al., 1980). By contrast, PT terminals are most dense in the parvocellular layers, less dense in the magnocellular layers and sparse within the intercalated layers.

In the tree shrew, SC projections terminate most densely in layers 3 and 6 and in the interlaminar zones surrounding layer 5 (Fitzpatrick et al., 1980). The projection from PT appears to consist of two components: one which originates in the anterior pretectal nucleus and terminates in all geniculate layers and one which originates in more caudal portions of PT and terminates in the same regions as SC. The pattern of projection from PBG is distinct from both tectal and pretectal patterns: labeled terminals are found in layers 1, 3, 5 and 6, and in the interlaminar zones adjacent to layer 5, but not within layers 2 and 4.

Finally, the neurochemical characteristics of some of these subcortical projections have been examined with antisera to neurotransmitter-specific markers. For example, we have found a large number of choline acetyltransferase (ChAT) positive neurons in the region of the brachium conjunctivum and almost every neuron in PBG appears to be ChAT positive. Furthermore, GL in both species is distinguished from other thalamic nuclei by a dense pattern of ChAT positive endings. The exact contribution of these two potential subcortical sources to the pattern of ChAT positive endings within GL remains to be determined. (Supported by NSF BNS 8411964, NIH NS19206, NIMH MH04849 and NSF BNS 8209081.)

69.5 DOES THE ASCENDING CHOLINERGIC PROJECTION EXCITE LATERAL GENICULATE NEURONS WHILE INHIBIT THALAMIC RETICULAR NEURONS?

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The ascending reticular activating system has long been supposed to be cholinergic in nature. However, the distribution of cholinergic neurons in the brain has been clarified only recently by means of choline acetyltransferase immunohistochemistry. In the rat cholinergic neurons projecting to the thalamus aggregate largely in the latero-dorsal tegmental nucleus (LDT) of the rostral pons. In the present study the effects of LDT stimulation on single neuronal activities in the dorsal lateral geniculate nucleus (LGd) and in the thalamic reticular nucleus (TR) were studied in rats anesthetized with urethane. The position of stimulating electrode was checked after each experiment on histological sections processed with NADPH-diaphorase histochemistry, which is a reliable method to selectively stain cholinergic neurons in the brainstem.

The firing rate of almost all LGd relay neurons increased upon repetitive stimulation (200 Hz) of LDT, which was weaker than a threshold to desynchronize cortical EEG recorded simultaneously. The effect usually appeared within several hundred msec after the onset of stimulation, and began to fade within a few seconds in spite of ongoing stimulation. The LDT-induced excitation were blocked by scopolamine applied ionophoretically or intravenously. Acetylcholine applied ionophoretically induced a strong increase in discharge, and sometimes led to depolarization block.

Under urethane anesthesia TR neurons usually fired with bursts of several spikes. LDT stimulation stopped the burst activity and induced tonic discharges. The stopping of bursts was brought about by LDT stimulation weak enough to fail to produce desynchronization of cortical EEG. A little stronger LDT stimulation led to EEG desynchronization with tonic discharges of TR cells. Cooling of the cortex blocked the induction of tonic discharges. Ionophoresed acetylcholine also stopped the burst activity and induced tonic discharges of TR neurons.

On the basis of a recent report that, when the resting potential is relatively hyperpolarized, thalamic neurons, in general, generate a burst of spikes superposed on a low-threshold small Ca spike, which is inactivated by depolarization (Jahnsen and Llinás, J. Physiol. 349:205-226,1984), we interprete the present results to indicate that cholinergic stimulation depolarized TR neurons directly and indirectly through neocortical activation. The interpretation challenges the classical concept that the ascending reticular activating system inhibits TR activity, and the inhibition of TR activity disinhibits LGd relay neurons. The ascending cholinergic input from LDT can directly excite LGd relay neurons and also TR neurons.

69.6 EFFECTS OF NICOTINE ON GLUCOSE UTILIZATION IN THE RAT BRAIN VISUAL SYSTEM: M. Dam*, R. Connolly*, G. Wilkerson* and E.D. London (SPON: L. Sharpe).
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Several lines of evidence have demonstrated that acetylcholine has a neurotransmitter role in the visual system (Fibiger, H.C., Brain Res. Rev., 4:327, 1982). Furthermore, ligand binding and metabolic studies indicate that at least some effects of acetylcholine and cholinomimetics in the visual system are mediated through nicotinic receptors (Dam, M. and London, E.D., Eur. J. Pharmacol., 87:137, 1983; London, E.D. et al., Neurosci. Lett., 53:179, 1985).

We extended these earlier findings by examining the effects of D,L-nicotine on local cerebral glucose utilization (LCGU), as measured by the 2-deoxy-D-[1-^{14}C]glucose ([^{14}C]DG]) method in rat brain regions which are components of, or are related to the visual system (Sokoloff, L. et al., J. Neurochem., 28:897, 1977).

Sixteen 3 month old male Fischer-344 rats were prepared with indwelling femoral arterial and venous catheters. At 3 to 4 hr after the withdrawal of halothane anethesia and 2 min before the intravenous injection of [^{14}C]DG (125 μ Ci/kg), three groups were injected subcutaneously with different dosages of D,L-nicotine bitartrate (0.05, 0.1 or 0.3 mg/kg, free base) in 0.9% NaCl. Control animals received a subcutaneous injection of saline 2 min before [^{14}C]DG.

Nicotine stimulated LCGU, in a dose related fashion, in several regions which are part of the visual system. The greatest effects were observed in the superficial layers of the superior colliculus, dorsal and ventral portions of the medial terminal nucleus of the accessory optic system (AOS), inferior fasciculus of AOS, and nucleus of the optic tract (NOT). Significant increases in LCGU also were observed in the dorsal and lateral terminal nuclei of AOS, anterior pretectal area, olivary pretectal and postpretectal nuclei, parabigeminal nucleus, brachium of the superior colliculus, and the lateral posterior thalamic nucleus. These results support and further extend the concept of a cholinergic nicotinic component of the rat visual system. Previous reports demonstrated that the pretectal nuclei, nuclei of AOS, superior colliculus, and lateral posterior thalamic nucleus are involved in mediating visual attention, oculomotor control, stability of retinal images, and visual discriminative abilities (Sprangue, J.M., Invest. Opthalmol., 11:473, 1972, Hughes H.C., J. Comp. Neurol., 175:311, 1977; Blanks R.H.I., Exp. Brain Res. 48:228, 1982). The present findings show that, nicotine stimulates these visual areas and may influence visual-motor function.

69.7 SEROTONERGIC INHIBITION OF THE DORSAL LATERAL GENICULATE NUCLEUS BY THE DORSAL RAPHE. G.A.Marks, S.G.Speciale, K.Cobbey*, and H.P.Roffwarg. Department of Psychiatry, University of Texas Health Science Center, Dallas, Texas 75235.

Serotonergic innervation of the Lateral Geniculate Nucleus (LGN) by the Dorsal Raphe Nucleus (DR) has been demonstrated through a variety of techniques in many different laboratories. The microiontophoretic application of serotonin (5HT) to dorsal LGN (dLGN) relay cells has a potent inhibitory action on both spontaneous and evoked activity. Although reports have indicated both inhibition and facilitation with DR stimulation, Yoshida et al (Br.Res.290) demonstrated that methysergide antagonizes the inhibitory effect of DR conditioning stimulation on dLGN. We are investigating this problem by means of local neurotoxin injections. Preliminary data support the findings of Yoshida et al insofar as our data supports the inhibitory nature of the serotonergic DR-dLGN projection.

Long-Evans hooded rats are implanted with bipolar stimulating electrodes in the DR and optic tract (OT). Cannula-guidetube assemblies with recording electrodes are implanted in each dLGN. Animals are allowed at least one week recovery from surgery. Under light chloral hydrate anesthesia single square wave pulses to the OT are utilized to elicit pre- and postsynaptic field potentials (FPs) in the dLGN. Conditioning stimulation (three pulse train,300Hz) is delivered to the DR, utilizing various c-t intervals. Twenty alternating trials with and without DR preconditioning (every 10 seconds) are carried out in each c-t interval. Only animals in which DR conditioning produces significant inhibition in the amplitude of postsynaptic FPs are studied further. Subjects are pretreated with desmethylimipramine (25mg/kg) ip. One hour later, the dLGN is injected via the cannula with either 5'7-dihydroxytryptamine (5'7-DHT), ascorbate (0.1%) in n. saline (0.2 ul, 8ug) or an equimolar solution of creatinine sulfate and vehicle (0.2 ul). Six days later, the animal is retested under identical conditions. Following retest, the animal is sacrificed for routine histology. Additionally, 300u sections are taken through the dLGN. By the micropunch technique, tissue surrounding the recording electrode is removed and both 5HT and 5HIAA content are determined by HPLC-ED.

In every animal in which conditioning stimulation produced an inhibition of the postsynaptic dLGN FPs, the conditioning electrode was found to impinge upon the DR. In all cases in which 5'7-DHT injections depleted the dLGN of 5HT and 5HIAA, a disinhibition was demonstrated at retest, whereas vehicle injections did not affect the amplitude of the inhibited FPs. These data support the interpretation that serotonergic innervation of dLGN by the DR is inhibitory.

69.8 PERIGENICULATE INPUT TO THE CAT'S LATERAL GENICULATE NUCLEUS: A LIGHT AND ELECTRON MICROSCOPIC STUDY OF SINGLE, HRP-FILLED CELLS.

J. B. Cucchiaro, D. J. Uhlrich, J. E. Hamos and S. M. Sherman, Dept. Neurobiology and Behavior, S.U.N.Y., Stony Brook, NY 11794.

Cells of the cat's perigeniculate nucleus (PGN) receive excitatory input from axon collaterals of relay cells in the lateral geniculate nucleus (LGN). PGN cells, in turn, provide inhibitory feedback to these relay cells. In order to describe the morphology of PGN inputs to the LGN, we recorded intracellularly from PGN cells with HRP-filled micropipettes, characterized their response properties, iontophoresed HRP into individual cells to fill their somata, dendrites and axon arbors, and then studied their morphology with the light and electron microscopes.

PGN cells have large, ill-defined, usually binocular receptive fields and they respond to optic chiasm stimulation at latencies of 2.2-4.0 msec. The labelled PGN cells have large somata, widespread dendritic arbors that extend >1 mm parallel to the geniculate laminae, and axons that arborize locally in the PGN and also project ventrally into the geniculate A-laminae. One PGN cell has been studied with the electron microscope. This cell has four main axon branches that converge onto a limited portion of lamina A (roughly 0.5 mm^3), and one of these branches extends into lamina A1. The innervation approximately follows a projection line thereby demonstrating a fair degree of retinotopic fidelity. Within laminae A and A1, the PGN axon is fine and beaded. Ultrastructural analysis of part of the arbor in lamina A shows that the labelled axon is myelinated only in the PGN and loses its myelin sheath near the dorsal border of lamina A. Within lamina A the axon makes en passant synaptic contacts at swellings which are 0.3-1.0 μm in diameter (mean = 0.7 μm). These synaptic contacts are symmetric, showing little or no postsynaptic density. The HRP labelling partially obscures the vesicle shape, but based on the small size of these labelled terminals, their symmetric synaptic contacts, and their synaptic relationships (see below), we conclude that these correspond to the F1 profiles described by Guillery (Z.Zellforsch. 96:1-38,1969). The synaptic contacts studied thus far from the PGN cell are made exclusively in interstitial, non-glomerular zones of lamina A onto small caliber dendrites, 0.5-2.0 μm in diameter (mean = 1.25 μm). These and other observations indicate that the synaptic relationships of these PGN axon terminals in the LGN are distinct from those of geniculate local circuit neurons (Hamos et al., Neurosci.Abstr. 10:57,1984), thereby suggesting the the two types of inhibitory innervation serve different functions in visual processing.

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69.9 TERMINAL PATTERNS OF THE BRAINSTEM PROJECTION TO THE LATERAL GENICULATE NUCLEUS IN THE CAT: AN ANTEROGRADE TRACER STUDY USING PHASEOLUS VULGARIS LEUCCOAGLUTININ (PHA-L). D.J. Uhlrich, J.B. Cucchiaro and S.M. Sherman. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Electrical stimulation of the brainstem disinhibits relay cells in the cat's lateral geniculate nucleus (LGN) and thereby improves synaptic transmission to the visual cortex. This indicates that the brainstem may play an important role in the gating of visual information. We have studied the anatomical substrate for this brainstem control. While retrograde tracer studies have demonstrated direct projections from the brainstem to the general area of the LGN and the adjacent perigeniculate nucleus (PGN), the morphology of the axon terminal fields within these regions has not been described. In order to visualize these terminal arbors, we used the newly developed technique of anterograde transport of the lectin, PHA-L (Gerfen and Sawchenko, Brain Res. 290: 219-238, 1984). We made multiple injections of PHA-L deep in the midbrain, dorsal to the brachium conjunctivum, in a series of cats. The target for these injections was an area that we and others find to be rich in retrogradely labelled cells following tracer injections in the LGN/PGN complex. Following an 8-10 day postoperative survival, we processed the ipsilateral LGN to show the distribution of PHA-L labeling. We observe Golgi-like filling of terminal processes in several thalamic nuclei, including all laminae of the LGN, the pulvinar, and the PGN.

The labeled axons have boutons distributed en passant or appended to the axon by short stalks; these boutons are presumed to be synaptic terminals. Some terminal arbors consist of extremely fine axons with boutons only 0.2-0.6 μm in diameter, while others are considerably larger in caliber with boutons 1.0-2.5 μm in diameter. Because we saved every section of the LGN, we could serially reconstruct individual axons. This revealed wide morphological variation. Some parent axons enter the LGN and form localized (400-700μm wide) terminal arbors restricted primarily within a single lamina. Other axons branch in a more diffuse fashion and freely cross laminar borders to innervate all regions of the LGN. We also observe single axons that innervate localized areas in more than one visual thalamic nucleus, such as both lamina A in the LGN and a restricted region in the pulvinar. This work demonstrates that the brainstem projections to the LGN can be quite specific and may serve to coordinate thalamocortical transmission in different visual thalamic nuclei.

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69.10 ULTRASTRUCTURE OF AN X-CELL WITH ATYPICAL DENDRITIC MORPHOLOGY. Susan C. Van Horn*, James E. Hamos, and S. Murray Sherman. Dept. Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

Although Golgi studies of cells in the A-laminae of the cat's dorsal lateral geniculate nucleus have revealed considerable morphological variability at the light microscopic level, only two physiological classes of neuron, X- and Y-cells, exist there. Recent studies relating structure to function via the technique of intracellular labeling with horseradish peroxidase (HRP) indicate that much of this heterogeneity can be ascribed to the X-cells. In order to determine if this heterogeneity has ultrastructural correlates, we have engaged in a long-term electron microscopic study of HRP-labeled X-cells. We previously described ultrastructural features for more common morphological types of relay X-cell (Wilson et al., Proc. Roy. Soc. Lond. B, 221:411, 1984), and we report here on such features for a less common type.

The HRP-labeled cell under study is an on-center relay X-cell located in lamina A with the majority of its dendrites running perpendicular to the laminar borders. Numerous beaded or varicose dendrites, emanating from a 15-μm-diameter soma, characterize this cell. In addition, a few spine-like appendages extend from proximal dendrites. We used the electron microscope to serially reconstruct portions of the labeled neuron and its inputs. The reconstructions suggest a synaptic circuitry similar to that of the more common types of relay X-cell. The cell's retinal inputs were all found within 100μm of the soma in contrast to the presumed cortical inputs that were located on more distal dendrites. The proximally located dendritic appendages were sites of synaptic triadic arrangements involving retinal and inhibitory inputs. Although it has been suggested that beaded dendrites such as those of the HRP-labeled X-cell are presynaptic to dendrites of other geniculate neurons (Famiglietti, Brain Res. 20:181, 1970), we found no evidence of clusters of synaptic vesicles in the beaded dendrites of our cell. The varicosities instead are prominent foci of synaptic inputs. However, we found fine, HRP-labeled processes that occasionally swell into terminals. These terminals are 1.5-2.5μm in diameter, contain round vesicles, and make asymmetrical synaptic contacts. We believe that the processes are collaterals originating from the labeled neuron's axon, which subsequently becomes myelinated and enters the optic radiation.

The labeled X-cell's synaptic circuitry thus seems to be remarkably similar to that of other relay X-cells but differ from that of local circuit neuron's which also have X-cell physiological properties (Hamos et al., Neurosci. Abstr., 10: 57, 1984).

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69.11 PASSIVE ELECTRICAL PROPERTIES OF X- AND Y- CELLS IN THE CAT'S LATERAL GENICULATE NUCLEUS. Stewart A. Bloomfield, James E. Hamos, and S. Murray Sherman. Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794.

We studied the passive electrical parameters of geniculate X- and Y-cells in the cat by modeling them as equivalent cylinders according to Rall's formulation. Intracellular recordings were made from neurons characterized physiologically in vivo. Voltage responses to constant current pulses were analyzed to determine each cell's input resistance (R_N), membrane time constant (τ_m), and electrotonic length (L_N). Some neurons were also injected with horseradish peroxidase to determine their soma-dendritic membrane surface areas (A_N). For those cells in which we measured both A_N and L_N, estimates were made of their specific membrane resistance (R_m) and capacitance (C_m). The resting membrane potentials (V_m) of the neurons studied ranged from -51 to -74 mV and did not differ significantly for X- and Y-cells (mean for both classes, -60 mV). We found no obvious relationship between V_m and R_N for these cells, and their current-voltage relationships were linear within a range of at least ±20 mV of V_m.

We found statistically significant differences between X- and Y-cells for R_N (X=22.6 MΩ, Y=15.5 MΩ, $p<.001$), τ_m (X=10.4 msec, Y=8.3 msec, $p<.001$), and L_N (X=1.09, Y=0.95, $p<0.05$). The difference in τ_m reflects a larger R_m for X- than for Y-cells (X=3565 Ω-cm^2, Y=2900 Ω-cm^2). Moreover, X-cells also displayed a much greater range in R_m values (2708-4406 Ω-cm^2) than did Y-cells (2442-3362 Ω-cm^2). This suggests intrinsic differences in membrane properties between X- and Y-cells, with more variability among the former neuronal class. Interestingly, X-cells also display greater variability of both soma-dendritic morphology and the patterns of synaptic innervation than do Y-cells. Finally, the derived values of L_N indicate that both X- and Y-cells possess remarkable "electrical" compactness. That is, synaptic potentials arising at the most distal dendritic sites would be attenuated by only about 33% when recorded at the soma in Y-cells and by roughly 40% in X-cells. There is thus no reason to expect a dramatic difference in synaptic efficacy between peripherally located excitatory inputs, arising primarily from visual cortex, and the proximally located excitatory innervation from retina.

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69.12 QUANTITATIVE ANALYSIS OF RETINAL Y-CELL TERMINATIONS IN THE CAT LATERAL GENICULATE NUCLEUS:A COMPARISON WITH X-CELL TERMINATIONS. M. Esguerra*, P.E. Garraghty and M. Sur (SPON: A. Arnsten). Sect. of Neuroanatomy, Yale School of Medicine, New Haven, CT 06510.

We have analyzed the geniculate terminations of single, physiologically characterized retinal Y-cell axons injected intracellularly with horseradish peroxidase. Terminal bouton numbers, volumes and densities, as well as sublaminar bouton distributions, of 11 Y-cell axons were measured. Statistical intra- and inter-group comparisons involved the Mann-Whitney U test and the Cox-Stuart test for trends.

Contralaterally projecting Y-cell axons, terminating in lamina A and the dorsal C laminae, and ipsilaterally projecting Y-cell axons, terminating in lamina A1, had totals ranging from 434 to 2188 boutons (mean=1207). Within the A-laminae, ipsilateral axons in lamina A1 had more boutons but similar terminal volumes compared to the lamina A terminations of contralateral axons. Both contralaterally and ipsilaterally projecting axons had greater numbers of boutons in the ventral half of lamina A or A1, reflecting a progressive increase in boutons from dorsal to ventral in each of the A-laminae.

ON and OFF center axons did not differ in bouton number or sublaminar bouton distribution within laminae A and A1. Axons with receptive fields central in the visual field tended to have higher bouton numbers and higher bouton densities than those located peripherally. Finally, a comparison of conduction velocities indicated that faster Y-cell axons had more boutons and higher densities than slower axons.

Several features stand out when we compare Y-cell terminations with those of X-cells described previously (Kritzer and Sur, Neurosci. Abst. 10:56, 1984). A positive relationship between conduction velocity and bouton number also exists for X-cells, as well as for the pooled X- and Y-cell data, suggesting that bouton number increases with fiber diameter across both cell classes. X-cells terminate in lamina A or A1 with fewer boutons, smaller terminal volumes, but higher bouton densities than do Y-cells. In contrast to Y-cells, X-cell axons have higher numbers of boutons in the dorsal half of lamina A or A1, suggesting that the retinogeniculate projection biases activity toward X-cells dorsally and Y-cells ventrally within lamina A or A1 (cf. Mitzdorf and Singer, J. Neurophysiol. 40:1227, 1977). Further, bouton densities of X-cells located centrally are lower than those of X-cells located peripherally. Since Y-cell terminations show the opposite trend, the retinogeniculate projection must also partially compensate for the imbalance between X- and Y-cells that occurs with eccentricity in the retina.

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69.13 SEGREGATION OF 'ON' AND 'OFF' UNITS IN CAT DORSAL LATERAL GENICULATE NUCLEUS. A. Naporn, N. Berman, and B. R. Payne. Dept of Anatomy, Boston University School of Medicine, Boston, MA, 02118 (A.N. & B.R.P.) and Dept of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA, 19129 (N.B.).

Neurons in the cat's dorsal lateral geniculate nucleus (dLGN) respond optimally to either the brightening (ON) or dimming (OFF) of their receptive field centres. In the multilaminated dLGN of some species such as macaque, tree shrew, mink, and ferret, these neurons are segregated into ON or OFF laminae. In the multilaminated dLGN of the cat, however, the ON and OFF neurons are believed to be intermingled within each lamina. We decided to determine if this is the case or if they are segregated into domains other than in the laminar dimension.

Electrode penetrations were made into laminae A and A1 of the dLGN in anaesthetised adult cats. Using retinotopic maps of the dLGN as a guide (1), recordings were made from sequential units located along projection lines (2). The responses of single units to light or dark stimuli presented to their receptive field centres were recorded extracellularly and response histograms generated.

We reasoned that if ON and OFF units are intermingled then for any given unit the following unit along a track has an equal probability of displaying the same or opposite centre response. This model would yield a ratio of close to 1:1, same:opposite. Contrary to this expectation, we obtained a ratio of 10:1 for those units located along projection lines (n=360). These data show that ON and OFF units are segregated into domains which are organised perpendicular to laminar borders.

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69.14 FIELD AND MULTIUNIT ANALYSIS OF THE FLASH-EVOKED RESPONSE IN THE MONKEY LATERAL GENICULATE NUCLEUS (LGN). Charles E. Schroeder*, Craig E. Tenke*, Joseph C. Arezzo and Herbert G. Vaughan, Jr. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Previous studies in the monkey striate cortex indicate that: 1) The binocular flash VEP is primarily generated in the principal parvocellular thalamorecipient lamina (4cB), with little contribution from 4cA, the principal target of LGN magnocellular afferents; 2) The field potentials generated in 4cB contain oscillations of a characteristic frequency and duration (160 Hz./approx. 50 ms); 3) Regardless of ocular dominance determined for a particular site, contralateral stimulation elicits earlier cortical activation than ipsilateral stimulation. In order to elucidate the role of the subcortical afferent pathway in this pattern of results, the present study examined the laminar profile of flash-evoked activity in the LGN of the unanesthetized monkey (M. fascicularis).

Field potentials and multiunit activity (MUA) were recorded simultaneously in penetrations through the LGN using a 16 channel multicontact electrode with contact impedances of approximately 300 K ohm and spacings of either 75 or 150 microns. This array permitted recording of activity in parvo- and magnocellular laminae concurrently.

Flash stimuli elicited robust field responses and MUA in magno- and parvocellular laminae, which differed along several dimensions. Parvocellular MUA bursts were less than half the duration and one quarter the amplitude of those in magnocellular regions. The parvocellular field and MUA responses displayed oscillations at 160 Hz and approximately 40 ms duration corresponding to features observed in 4cB. Parvocellular oscillations were twice the frequency of those seen in magnocellular laminae. As in striate cortex, contralaterally driven activity preceded ipsilateral by approximately 2-4 ms. Parvocellular responses were more attenuated by decreased stimulus intensity and interstimulus interval than magnocellular responses. In parvocellular laminae, we also observed clear evidence for interocular inhibition, coincident with excitatory activity in adjacent laminae. In magnocellular laminae MUA responses were substantially less robust to binocular as contrasted to monocular stimulation. This effect was enhanced by decreasing ISI.

These results support our conclusion that the waveform of the cortical VEP to binocular flash predominantly reflects activation of parvocellular terminal zones. The failure of binocular flash to activate the magnocellular thalamorecipient zone may stem from the operation of powerful inhibitory processes evident at the LGN.

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69.15 MORPHOLOGY OF TECTAL EFFERENTS TO DIENCEPHALON VISUALIZED WITH THE AID OF ANTEROGRADE TRANSPORT OF PHA-L. S. Jhaveri*, D.P.M. Northmore+ and G.E. Schneider. Dept. of Psychology and the Whitaker College, M.I.T., Cambridge, MA 02139. +Inst. for Neuroscience, Univ. of Delaware, Newark, DE 19716.

Anterograde transport of kidney-bean lectin (PHA-L) results in a complete, Golgi-like filling of axons and their terminal arbors (Gerfen & Sawchenko, Brain Res., 290: 219, 1984). We have used this procedure to study the morphology of diencephalic axon terminations originating from cells in the upper layers of the superior colliculus (SC) of adult hamsters.

Injections of PHA-L were made iontophoretically (positive current, 4 μA for 15 minutes, pulsed) into the SC. After 5-7 days, brains were processed according to the immunohistochemical procedure of Gerfen and Sawchenko. Injection sites were usually restricted to less than 1/4 of the SC and confined in depth to the superficial gray and optic layers with some encroachment into the intermediate gray. Diencephalic efferents were traced to the lateral posterior nucleus (LP) bilaterally and to the ipsilateral dorsal (LGd) and ventral (LGv) nuclei of the lateral geniculate body.

In the LP, fine labelled axons form a dense terminal meshwork. Although varicosities of various sizes are scattered along the axons, the larger ones appear to be organized into terminal clusters. Occasionally some clusters overlie a cell soma (counterstained with neutral red), but usually are found in the neuropil or closely adjacent to a cell soma.

In the LGd, two patterns of termination are observed. Near the optic tract (OT), short axon collaterals immediately ramify to form a pericellular plexus bearing numerous small varicosities. Further medially in the nucleus, axons are smooth and only occasionally exhibit large (up to 4μm) boutons, frequently overlying neuronal perikarya. These axons have a morphology distinctly different from that seen nearer the OT.

In the LGv (external lamina), a complex terminal field is formed, by fine caliber axons studded with numerous boutons of a very uniform morphology (<2μm, spherical). The boutons occur all over the neuropil, not being confined to perikaryal domains. Single axon arbors are elongated, extending about 30μm mediolaterally, and up to 100μm in the dorsoventral axis, and are indicative of a highly topographic projection.

Thus, tectal efferents to various diencephalic nuclei have very different morphological characteristics. Whether these efferents arise from the same or different subsets of collicular neurons remains to be elucidated. (Supported by NIH grants EY05504, EY00126, EY02621, EY02697 and Univ. of Delaware Research Foundation.)

69.16 PROJECTIONS FROM THE SUPERIOR COLLICULUS TO THE PULVINAR NUCLEUS IN THE TREE SHREW. R.G. Carey, G. Luppino*, D. Fitzpatrick and I.T. Diamond. Div. Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013 and Depts. Psychology and Anatomy, Duke University, Durham, NC 27706.

We have reinvestigated the tecto-pulvinar pathway in the tree shrew by studying the pattern of labeled terminals in the pulvinar nucleus following small injections of WGA-HRP into the superficial layers of the superior colliculus. Injections into the superior colliculus produce two distinct patterns of labeled terminals within the pulvinar nucleus. In the portion of the pulvinar nucleus adjacent to the lateral geniculate nucleus, labeled terminals are distributed in bands, the locus of which is dependent on the position of the injection site in the superior colliculus. In contrast, the caudal and medial portions of the pulvinar, both ipsilateral and contralateral to the injection, are filled with labeled terminals regardless of the position of the injection site within the tectum. These findings suggest that there are at least two subdivisions within the pulvinar: one which appears to maintain in a precise way, the retinotopic map of the superior colliculus and one which does not. Further support for the presence of these two subdivisions comes from the pattern of labeled terminals found in the pulvinar following intraocular injections of WGA-HRP; labeled terminals (presumed to be the result of transneuronal transport from the superior colliculus) are found only in the non-topographically organized zone. In addition, the non-topographically organized zone is distinguished from the topographically organized zone by the presence of strong acetylcholinesterase staining. Finally, preliminary results suggest that these subdivisions have different targets within the cortex and thus may be part of two separate parallel tecto-pulvinar pathways to extrastriate cortex.

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69.17 STIMULUS SIGNIFICANCE AFFECTS PULVINAR VISUAL AND VISUOMOTOR PROPERTIES. D. Burman and L.A. Benevento. Dept. of Anatomy, Univ. of Illinois at Chicago, P.O. Box 6998, Chicago, IL 60680.

Lateral pulvinar single-unit activity was recorded in awake macaques performing a task that tested if a unit would respond differently to a stationary peripheral stimulus according to its significance for intended eye movements. More specifically, would a unit respond one way to a stimulus that served as a saccadic target, and respond another way to a stimulus that signified a later saccade to a stimulus in the opposite direction? In this task, the animal first fixated upon a 1.5^{o} circle (S-0) which appeared at t=0, where "t" is the time elapsed in seconds following S-0 onset. Fixation was maintained upon S-0 until it changed to a 1.5^{o} square, which served as the cue to saccade to a new location. At t=2, the first peripheral stimulus (S-1) appeared at one of eight possible locations, 3.5-10^{o} peripheral to S-0. S-1 was either a 1.5^{o} circle or square. The significance of S-1's shape was as follows. If S-1 was a circle, the animal was cued (by the change in S-0) at t=4 to saccade to S-1 without hesitation. If S-1 was a square, a second peripheral stimulus (S-2, a 1.5^{o} circle) appeared in the opposite direction at t=4, and the monkey had to maintain fixation until t=6, when the animal was cued by the change in S-0 to saccade to S-2. Thus, if S-1 was a circle, the animal had to saccade to it at t=4, but if S-1 was a square, the animal had to saccade in the opposite direction at t=6. Some units responded strongly to the S-1 circle, but gave little or no response to the S-1 square. Since the size and location of S-1 was identical regardless of whether it was a circle or a square, this difference in response must have been due to the significance of S-1. A strong tonic inhibitory response was seen 2000 ms before the saccade, at the onset of S-1 when it was a circle. When S-1 was a square, the tonic inhibitory response occurred only after S-2 onset (again about 2000 ms before the saccade). In either case, there was a strong phasic excitation following the saccade. After saccading to the new target, the animal had to maintain fixation for 2 more seconds before she was rewarded for 1 second. The tonic inhibitory response was seen to reoccur during the reward period, despite the fact that there was no change in visual stimuli at this time. Although not designed for this, the reward could have effectively been a non-visual cue signalling the end of the trial, at which time the stimulus would go off and her eyes moved at will. Since 1) an inhibitory response was seen 500-2000 ms before as saccade regardless of whether the saccade was indicated by S-1, S-2, or the reward, and 2) a phasic excitatory response was seen following a saccade to a new target, we suggest that these neurons may prepare (with inhibition) for a saccade to a target of interest, and signal completion of the saccade (with excitation). (Supported by BRSG 8309 and Sigma Xi.)

69.18 TWO SUBREGIONS WITHIN THE STRIATE-RECIPIENT AREA OF THE CAT'S LATERAL POSTERIOR COMPLEX. Bruce P. Abramson and Leo M. Chalupa Physiology Graduate Group and Department of Psychology, University of California, Davis, CA 95616.

The lateral posterior (LP) complex of the cat's thalamus is thought to contain two visual areas: the principal tectorecipient zone (LPm) and the striate-recipient area (LPl). Here, we report that one of these areas, LPl, can be further subdivided on the basis of its connectivity pattern and functional organization.

Deposits of WGA-HRP into the superficial layers of the superior colliculus revealed anterograde label, not only in LPm, but also in LPl. However, the tectal projection to LPl appeared restricted to the lateral portion of this area, adjacent to the border with the pulvinar. The cells of origin of this projection were studied following electrophoretic deposits of the tracer into this lateral region of LPl. All labeled neurons in the superior colliculus were localized to the superficial layers, primarily SGS 2 and 3. Such deposits also revealed labeled cells in the reticular nucleus of the thalamus, the hypothalamus, the cerebellum, as well as in several cortical visual areas, including areas 17 and 18. By comparison, deposits of WGA-HRP that were confined to the medial portion of the LPl (that is, adjacent to the principal tectorecipient zone) resulted in labeled cells in visual cortex and in the reticular nucleus. These observations suggest that the striate-recipient zone of the cat's LP nucleus is comprised of two subregions: a lateral region receiving tectal as well as hypothalamic and cerebellar projections, and a medial region which appears devoid of these inputs.

The visual response properties of single neurons in the medial LPl also differ from those in the lateral LPl. Most cells in the medial portion of LPl respond securely to specific visual stimuli, possess relatively small receptive fields, and are commonly selective for stimulus orientation and/or direction of movement. In the lateral region of LPl, fewer neurons can be activated reliably by visual stimuli, receptive fields are very large, and cells rarely exhibit response specificity for orientation or direction. At present, however, it is unclear whether or not these two subregions of LPl contain separate representations of the visual field.

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69.19 SUBCORTICAL CONNECTIONS OF SUBDIVISIONS OF VISUAL CORTEX IN TREE SHREWS. M. A. Sesma, L. E. Baker, and J. H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Subcortical connections of four subdivisions of visual cortex of tree shrews were determined by placing small injections of WGA-HRP or ^{3}H-proline into area 17 (2 cases), area 18 (3 cases), and two subdivisions of visual cortex previously defined by cortical connection patterns of area 17 and area 18, the temporal dorsal area (TD) (3 cases) and the occipitoparietal area (OP) (2 cases). All cases were sectioned coronally. TMB and standard autoradiographic methods were used to visualize the tracers. Processed sections or adjacent sections were counterstained for nissl, cytochrome oxidase and/or myelin to determine the architectonic borders of thalamic subdivisions.

The connections of area 17 were similar to those demonstrated in other reports. Area 17 had reciprocal connections with the dorsal lateral geniculate (DGL) and the adjacent pulvinar (PUL). Projections were found to the ventral lateral geniculate nucleus (VGL), the reticular nucleus of the thalamus (R), the superficial layers of the superior colliculus (SC), the pretectum (Pt) and the dorsolateral pons. None of the extrastriate areas had connections with the DGL or VGL. Like area 17, area 18, TD, and OP are also interconnected with the PUL, project to R, the pons and the caudate nucleus. Projections to SC from TD and OP are to layer IV, below the stratum opticum, while area 18 projections were to the lower part of the SGS. Injections in area 18, TD and OP also labeled cells and terminals of the lateral nuclear complex including the ventral lateral (VL) and lateral (L) regions. TD and OP also had reciprocal connections with the interlaminar nuclei, received projections from locus coeruleus and had projections to the Pt. Injections in TD and OP resulted in label in the zona incerta and red nucleus. OP had reciprocal connections with the lateral dorsal nucleus and the mediodorsal nucleus. TD had connections with the subthalamic nucleus and the reticular field of Forel in the hypothalamus. Though all three extrastriate subdivisions had some connections in common, the pattern of subcortical connections was distinct for each area. Area 18 had discrete foci of labeled cells and terminals in PUL, VL, and L and projected to the SGS of SC; TD injections resulted in broader foci of label in the PUL; OP injections labeled the lateral thalamus (VL/L) most densely. Thus, subcortical patterns of connections provide further support for the validity of subdivisions of visual cortex suggested by patterns of cortical connections.

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70.1 THE OPTIC NERVE OF THE LEAST SHREW, *Cryptotis parva*. L.C. Towns, O.B. Mock* and R.M. Matushin*. Department of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

These studies of the least shrew optic nerve were undertaken because both the shrew's small size (4-6 g) and its phylogenetic placement as a common eutherian progenitor make it a useful animal in which to study fundamental organization of the visual system. The optic nerve is 3.3 mm in length with a mean shortest axis diameter of 70 μm. In twelve optic nerves, electronmicroscopic examinations of sections taken 1-2 mm behind the globe indicate that the nerve contains a mean of 2550 myelinated axons. There are approximately one-third as many unmyelinated axons as myelinated. The shortest axis diameter of myelinated axons (including the myelin) ranges from 0.13 μm to 2.17 μm. The frequency distribution of the shortest axis diameters is unimodal and positively skewed with a mode of 0.76 μm. Preliminary data indicate that mean myelin thickness increases with axon diameter up to axon diameters of approximately 0.64 μm. Axons of larger diameter have relatively constant mean myelin thickness. The unmyelinated axons range from 0.13 μm to 1.91 μm and are unimodally distributed with a mode of 0.38 μm. At the level of the optic nerve sampled, the small size of the optic nerve in the least shrew offers an opportunity to view the entire nerve at relatively high magnification. Additionally, the shrew ages rapidly and exhibits a spontaneous maturity onset diabetes. This animal, thus, also offers a model for studies of age-related and diabetes induced changes in the mammalian optic nerve and retina.

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70.2 RETINAL GANGLION CELLS LABELED AFTER INJECTION OF HRP INTO THE GROUND SQUIRREL VENTRAL LATERAL GENICULATE. N. Lugo-Garcia and E. Kicliter. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00901.

In carnivores and primates functional types of retinal ganglion cells are associated with ganglion cells of particular morphological properties. We have been investigating the possibility that this principle may also be applicable to ground squirrels. As part of our studies we are investigating what morphological types of ganglion cells project to several different retinal projection targets. In the present experiments we have determined the sizes of somata of ganglion cells which project to the ventral lateral geniculate nucleus (LGv).

We made iontophoretic injections of horseradish peroxidase (HRP, Sigma type VI, 10% in 0.01 M NaCl) into either the LGv (N=2) or optic tract (N=3) of thirteen-lined ground squirrels. A pressure injection of HRP (50% in 0.9% saline) was also made in the optic nerve of one animal. After three day survival periods the retinas of these animals were reacted according to the Hanker-Yates method (Histochem. J. 9: 789-792, 1977). Profiles of labeled ganglion cells were drawn with a camera lucida and the equivalent circle diameter of each profile was calculated with a Zeiss ZIDAS image analysis system. Histograms illustrating the relation between cell size and frequency were constructed from 200 randomly selected labeled ganglion cells from each retina.

After LGv injections ganglion cells were labeled in only the contralateral retina. These cells ranged from 6-21 um in diameter with a mean of 13.05 um. Most labeled neurons were in the 14-15 um range. After iontophoretic injections of the optic tract (which should have labeled all ganglion cell types) HRP-containing ganglion cell somata in the contralateral retina ranged in diameter from 3-14 um. Their mean diameter was 5.8 um with the highest frequency of labeled neurons in the 5-8 um range. Thus the iontophoretic injections of the optic tract did not label all sizes of ganglion cells. The pressure injection of the optic nerve labeled ganglion cells ranging from 6-18 um in diameter; again, not all of the ganglion cells (particularly the larger cells) were labeled after injections of the optic nerve.

While the present results are preliminary, it appears that not all sizes of ganglion cells project to LGv and those labeled by LGv injections include cells of larger soma sizes. The cells labeled by LGv injections differ from those labeled by dorsal lateral geniculate injections. Ganglion cells labeled by LGd injections are predominantly 8-12 um in diameter (Kicliter and Lugo-Garcia, Neurosci. Abstr. 10: 460, 1984). The present results provide further evidence that different retinal projection targets receive projections from specific classes of retinal ganglion cells. (Supported, in part, by NIH grant NS-07464.)

70.3 SIZES OF RETINAL GANGLION CELLS WHICH PROJECT TO THE GROUND SQUIRREL SUPERIOR COLLICULUS. E. Kicliter and N. Lugo-Garcia. Lab. of Neurobiology and Dept. of Anatomy, Univ. of Puerto Rico Sch. of Med., San Juan, PR 00901.

Three functional types of optic nerve fibers have been described in ground squirrels: 1) contrast-sensitive; 2) opponent color; and 3) directionally selective (Michael, J. Neurophysiol. 31: 249-282, 1968; Gur and Purple, Vision Res. 18: 1-14, 1978; Jacobs and Tootell, J. Neurophysiol. 45: 891-902, 1981). The first two types terminate in the dorsal lateral geniculate nucleus while the directionally selective units project to the superior colliculus (SC, Michael, J. Neurophysiol. 35: 815-832, 1972; 36: 536-550, 1973). We wished to determine the soma sizes of ganglion cells projecting to the ground squirrel SC in order to compare their sizes with those of the entire population of ganglion cells. Thus we could resolve whether directionally selective ganglion cells are associated with a particular soma size.

Horseradish peroxidase (Sigma type VI HRP, 10% in 0.01 M NaCl) was iontophoretically injected into either the optic tract (N=3) or the superficial tectal layers (N=3) of thirteen-lined ground squirrels. After three day survival periods the retinas of these animals were reacted according to the Hanker-Yates method (Histochem. J. 9: 789-792, 1977). Profiles of labeled ganglion cells were drawn with a camera lucida and the equivalent circle diameter of each of these cells was calculated with a Zeiss ZIDAS image analysis system. In each retina 200 randomly selected labeled cells were measured and a histogram was constructed to illustrate the relation between cell size and frequency.

After injections of the optic tract (which should have labeled all ganglion cell types) labeled ganglion cell somata in the contralateral retina ranged in diameter from 3-14 μm. Their mean diameter was 5.87 um and most labeled neurons were in the 5-8 um range. After SC injections (which only labeled cells in the contralateral retina) cells containing HRP also ranged in diameter from 3-14 um. However, after SC injections the mean diameter of labeled neurons was 7.35 um and most cells were in the 6-9 um range.

These experiments indicate that a wide range of ganglion cells (by soma size) project to the SC. Thus the directionally selective functional ganglion cell type may be associated with a variety of morphological types.

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70.4 SOMA SIZE DIFFERENCES BETWEEN TWO PHYSIOLOGICALLY DISTINCT CLASSES OF W-CELLS IN THE CAT'S RETINA. L. R. Stanford. Dept. Struct. Func. Sci. and the Waisman Center for Mental Retardation and Human Development, Univ. of Wisconsin-Madison, Madison, WI 53705.

Retinal ganglion cells in the cat were recorded transclerally and physiologically classified using a battery of response measures. These measures included linearity of spatial and temporal summation, latency to antidromic stimulation of the optic chiasm and optic tract, sustained or transient center response, presence or absence of center/surround organization, and briskness of response to stimulation of the receptive field center. Following physiological classification, the ganglion cells were impaled, retested, and iontophoretically injected with HRP. Thirteen retinal W-cells have thus far been recovered that have dendritic arbors consistent with the gamma cell classification. Physiologically, this sample includes linear and non-linear response types, ON, OFF, and ON/OFF center cells, and phasic and tonic W-cells. Included here are only those neurons that were distinguished as W-cells on the basis of their sluggish responses to stimulation of their receptive field center and/or their long latencies to stimulation of the optic chiasm. Morphologically, all of these W-cells were classified as gamma cells on the basis of their large, sparse dendritic arbors, a feature that readily distinguishes these ganglion cells from retinal alpha cells and beta cells. This sample of gamma cells, however, can be divided into two distinct subpopulations on the basis of soma size. One subpopulation (10 cells) has an average soma size area of 163.9μm^2 (range 112.6μm^2 - 226.7μm^2). The second subpopulation (3 cells) has an average soma size of 387.7μm^2 (range 312μm^2 - 440.5μm^2). No overlap in the soma size distributions has been seen between these two morphological classes at any retinal eccentricity. The two classes can also be distinguished physiologically. Every neuron with a small soma responded phasically to stimulation of its receptive field center while every gamma cell with a larger soma had a tonic response. The tonic W-cells had soma sizes that were in the same size range as cells that have beta morphologies and have been identified as X-cells in these experiments. This group of W-cells probably corresponds to the cat retinal ganglion cells with dendritic morphology typical of a gamma cell, but with beta cell-sized somata, described by Stone and Clark (J. Comp. Neurol. 192:211, 1980). Although it is not possible, from these data, to estimate the relative number of W-cells that have medium-sized somata, it is obvious that they may constitute a significant fraction of the W-cells in the cat. These data emphasize that soma size is not a reliable morphological criterion for distinguishing W-cells from X-cells in the cat retina.

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70.5 TEMPORAL FLOW OF VISUAL INFORMATION FROM GANGLION THROUCH GENICULATE CELLS. S. Lehmkuhle, A.K. Sestokas, and K.E. Kratz. Dept. of Psychology, Brown Univ., Providence, RI 02912 and Dept. of Anatomy, Louisiana State Univ. Medical School, New Orleans, LA 70112.

We recorded extracellular potentials from retinal ganglion cells (RG) in the optic tract and from dorsal lateral geniculate nucleus cells (LGN) in the A laminae of anesthetized, paralyzed cats. Cells were classified as X or Y by conduction latency to optic chiasm stimulation and linearity of spatial summation. Instantaneous discharge frequency (IF) profiles were analyzed on a trial-by-trial basis to measure response latency and amplitude to 100 msec presentations of sinewave gratings that varied in spatial frequency and contrast. Three measures of response latency were calculated: onset latency, peak latency, and response rise time. Onset latency was defined on each trial as the time at which the IF exceeded the baseline firing rate by 2 standard deviations; peak latency was the time at which the IF reached its peak value on each trial; and rise time was the difference between onset and peak latencies.

The onset latencies for RG Y-cells were about 10 msec shorter than RG X-cells at low spatial frequencies ($\leq$.25 c/deg) but about 10 msec longer at higher spatial frequencies ($\geq$.75 c/deg). Peak latencies of RG Y-cells, however, were similar to RG X-cells for all spatial frequencies and contrasts studied. The rise times of RG Y-cells were longer primarily due to the high IF's elicited from RG Y-cells. The longer rise times of RG Y-cells can explain the similarity between peak latencies of RG X- and Y-cells even though their onset latencies are different.

At lower spatial frequencies, the onset latencies of LGN Y-cells were about 10 msec shorter and the peak latencies of LGN Y-cells were about 20 msec shorter than LGN X-cells. The rise times of LGN Y-cells were shorter than LGN X-cells. The faster rise times of LGN Y-cells occurred despite the higher IF's elicited from LGN Y-cells.

The onset latencies of LGN cells were nearly 20 msec longer than their RG counterparts although the conduction latencies to optic chiasm stimulation were only 1 msec longer. Surprisingly, the peak latencies of LGN Y-cells were nearly identical to RG Y-cells because of the fast rise times of LGN Y-cells. The peak latencies of LGN X-cells remained 10 msec longer than RG X-cells.

These measures of temporal flow of visual information from RG through LGN cells suggest that the transient responses of LGN Y-cells speeds up processing of lower spatial frequencies.

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70.6 RESPONSE AMPLITUDE AND VARIABILITY OF GANGLION AND LGN X- AND Y-CELLS IN THE CAT. A.K. Sestokas, S. Lehmkuhle and K.E. Kratz, Dept. of Psychology, Brown Univ., Providence, RI 02912, and Dept. of Anatomy, Louisiana St. Univ. Med. School, New Orleans, LA 70112

We recorded the responses of retinal ganglion (RG) and lateral geniculate nucleus (LGN) neurons to identical visual stimuli in order to compare response amplitudes and variabilities in these closely related structures. X- and Y-cells were tested in anaesthetized, paralyzed cats with repeated, 100-msec presentations of sinewave grating stimuli at spatial frequencies between .17 and 2.0 c/deg, and contrasts between 5% and 40%. Each stimulus was pulsed 24 times at a rate of 1 pulse/sec. Mean discharge rate and peak discharge rate were measured in the 200 msec period that followed stimulus onset in each trial.

In general, Y-cells responded most vigorously at low spatial frequencies and least at high spatial frequencies. X-cells responded most vigorously at intermediate spatial frequencies and less at high and low spatial frequencies. Both X- and Y-cells had larger responses to high contrast gratings than to low. Under optimal stimulus conditions the responses of Y-cells were larger than those of X-cells. RG X- and Y-cells had higher mean discharge rates than LGN X- and Y-cells, respectively, tested under similar stimulus conditions. This may be due in part to the higher spontaneous discharge rates of RG cells. However, the results for peak discharge rates did not follow the same pattern. Peak discharges of LGN X-cells were similar to those of RG X-cells. Peak discharges of LGN Y-cells were slightly smaller than those of RG Y-cells for optimal stimuli, and slightly larger for non-optimal stimuli.

Absolute response variability, measured by response standard deviation across repeated trials, tended to increase with increases in mean response amplitude for both RG and LGN neurons. However, relative response variability, measured by coefficients of variation (standard deviation/mean response across trials), decreased with increases in response amplitude, which suggests that relative noise levels in the neural signals may decrease with increasing signal strength. Coefficients of variation were generally larger for peak discharge rate than for mean discharge rate, indicating that the latter is a more reliable measure of response amplitude. Coefficients of variation were significantly larger for LGN cells than RG cells in all stimulus conditions. The larger relative variability of LGN cells may be a direct consequence of their lower discharge rates.

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70.7 SELECTIVE DEGENERATION OF P-BETA RETINAL GANGLION CELLS IN THE MACAQUE. T. A. Eskin* and W. H. Merigan (SPON: T. Pasternak). Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Chronic acrylamide intoxication in the macaque induces retinal ganglion cell degeneration which the following evidence indicates is confined to the medium-sized (P-beta) cells. Such cells subserve high visual acuity and color opponency in the macaque. Intravitreal horseradish peroxidase injection demonstrates markedly reduced anterograde axonal transport to parvocellular layers of the lateral geniculate nucleus, (which receives P-beta cell input), but normal transport to magnocellular layers, (which receives P-alpha cell input). Cytochrome oxidase histochemistry in the lateral geniculate nucleus shows little activity in parvocellular but normal activity in magnocellular layers. Selective reduction of the cytochrome oxidase staining in parvocellular layers suggests that the parvocellular neurons have undergone deafferentation and transsynaptic atrophy.

Cytochrome oxidase histochemistry in striate cortex in these animals reveals virtually no cytochrome oxidase activity in layer 4A and greatly reduced activity in the lower two-thirds of layer 4C-beta. These layers receive direct input from parvocellular layers of the lateral geniculate nucleus. In contrast, cytochrome oxidase activity appears normal in layer 4C-alpha (which receives input from magnocellular layers of the lateral geniculate nucleus) and the upper third of layer 4C-beta. Many neuronal perikarya are still present in the affected layers. Of particular interest is the preservation of cytochrome oxidase "blobs" or "puffs" in layers 2 and 3, which have been reported to be involved in the color opponent visual pathway.

Supported by NIH grants ESO 1885 and ESO 1247.

70.8 HISTOCHEMICAL LOCALIZATION OF CYTOCHROME OXIDASE IN THE NORMAL AND DENERVATED GOLDFISH OPTIC TECTUM: A COMBINED GOLGI-CYTOCHROME OXIDASE STUDY. G.H. Kageyama* and R.L. Meyer , Dev. Biol. Center, University of California, Irvine, CA 92717.

The histochemical localization of cytochrome oxidase (C.O.) was examined in the optic tectum and retina of normal and monocularly enucleated (or optic nerve crushed) goldfish. In the optic tectum several distinct C.O.-reactive bands were observed. The most intense staining was localized within two narrow (10-16 μm) darkly reactive bands at the top and bottom of the stratum fibrosum et griseum superficialis (SFGS). Monocular enucleation or optic nerve crush resulted in a notable decrease in the overall staining intensity of the contralateral SFGS. Normally the deeper of the two SFGS sublaminae contained a plexus of intensely reactive large diameter (2-3 μm) puncta (presumed axon terminals). These terminals disappeared following either enucleation or nerve crush, indicating that they were of retinal origin. A class of large, darkly reactive retinal ganglion cell was observed in the retina. Based on a correspondence in relative size, C.O.-reactivity and response to enucleation or nerve crush it is likely that the large reactive puncta in the deep SFGS represent the terminal arborizations of the large darkly reactive ganglion cells. Stratum superficialis (SS) was darkly stained, while stratum opticum (SO) was only lightly stained. The C.O. reaction product within stratum marginalis (SM) appeared to be localized predominantly within the dendrites of type I cells, based on their oblique, rather than horizontal orientation. The C.O. staining within stratum griseum centrale (SGC) and stratum album centralis (SAC) consisted of four to six moderately reactive bands. These bands often appeared to be more distinctly segregated within the dorsomedial tectum than in the lateroventral tectum. The perikarya in stratum periventriculare (SPV) were predominantly moderately to lightly reactive. Neurons with darkly reactive perikarya were sparsely distributed throughout the different layers of the tectum. In combined Golgi-C.O. stained sections, Golgi impregnated neurons had dendrites that arborized preferentially within the C.O.-rich bands of the SS, SGC, and SAC as well as the SFGS. These observations suggest the presence of a laminar and sublaminar histochemical organization in the goldfish optic tectum that correlates with the stratified distribution of tectal neuron dendrites and presumed sites of synaptic input. The presence of two C.O.-reactive laminae and a specific class of large diameter C.O.-reactive retinal axon terminals in the deeper portion of the SFGS suggests that there is functional segregation of retinal input to distinct sublaminae within this layer.

This work was supported by NIH Grant No. 15381 and a Monsanto Postdoctoral Training Fund.

70.9 FURTHER EVIDENCE INDICATING THE EXISTENCE OF VARIOUS POPULATIONS OF PEPTIDE-CONTAINING RETINAL GANGLION CELLS IN RANA PIPIENS. Rodrigo O. Kuljis and Harvey J. Karten. Departments of Neurology, Psychiatry and Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York, 11794, U.S.A.

Previous studies have indicated that various populations of putative peptidergic retinal ganglion cells (RGCs) terminate at different depths of the frog's retino-recipient tectal neuropil (RRTN). To date, the evidence was based on the visualization of peptide-like immunoreactivity (PLI) in the RRTN (Kuljis & Karten, JCN '82), the modifications of the latter PLI after retinal deafferentation (Kuljis & Karten, JCN '83), the visualization of PLI in optic nerve axons under conditions of axoplasmic flow blockade (Kuljis et al., JCN '84), and the observation of PLI in regenerating retinofugal axons (Kuljis & Karten, JCN '85). All of these observations relied on the visualization of PLI in portions of the protoplasm of the RGCs other than the soma, and more importantly, often under conditions allowing the possibility of expression of new peptide phenotypes in response to trauma from the experimental manipulations. The present study eliminates these difficulties, by directly demonstrating PLI in RGC somata, and avoiding the need for experimental traumatic manipulations.

Fifteen Rana pipiens tadpole eyes, optic nerves and brains were processed for substance P (SP) and leucine enkephalin (LENK) PLI by the fluorescent, peroxidase-antiperoxidase and avidin-biotin methods. Stages analyzed span from stage 25 of Shumway ('40) to stage XXV of Taylor and Kollros ('46). Some of the specimens were generously provided by Dr. M. Constantine-Paton.

Between stages 25 of Shumway to V of Taylor and Kollros, first the RGCs somata and then the optic nerve exhibit SP and LENK PLI. Subsequently, the RGCs and optic nerve gradually loose PLI, progressively resembling adult animals in which PLI is normally not demonstrable at this level by our methods (Kuljis, et al., '84). By stage VII-XII of Taylor and Kollros the basic pattern of PLI has been established in the RRTN, and subsequently continues to approach that of the adult. Experiments are still in progress to establish further details on the sequence of events.

These results provide the first direct evidence of PLI in the somata of RGCs, inferred in all previous experiments. Thus, our observations support the previous contention that different populations of RGCs contain different transmitters/modulators, and end at different depths of the RRTN, presumably subserving different functional roles in the processing of visual information. Supported by EY 04796 to HJK.

70.10 FUNCTIONAL AND MORPHOLOGICAL CORRELATION OF FROG RETINA GANGLION CELLS : U. Grüsser-Cornehls* and G. Kahle* : (SPON : European Neuroscience Association), Department of Physiology, Freie Universität Berlin, Arnimallee 22, D - 1000 Berlin 33 (Germany)

The categorization of frog (Rana esculenta) retina ganglion cells recorded intracellularly into classes 0 to 5 on the basis of their neurophysiological properties (Grüsser,O.-J. and Grüsser-Cornehls,U., Frog Neurobiology, R. Llinas and W. Precht, eds., 297, Springer-Verlag Berlin 1976) was followed by iontophoretic staining with Lucifer yellow. In this way a correlation could be made for the first time between some morphological and electrophysiological ganglion cell types in the frog's retina. A close affiliation between rapid Golgi stained cells and those with the Lucifer yellow (flat-mount) method was discovered.

By incompletely stained neurons, only the soma size was defined for each neurophysiological cell class. As a correlation between soma size and diameter of the dendritic tree has been determined through extensive morphometric studies (Kalinina, A.V., Vision Res. 14:1305, 1974), the latter was calculated for our neuron sample and subsequently the presumable diameter of the respective receptive field. A good correlation was established between field sizes determined morphometrically and those electrophysiologically. With some reservation one can say that in the frog retina the extension of the dendritic branches of a ganglion cell probably determines the diameter of the excitatory receptive field of that neuron.

Electrophysiologically determined retinal ganglion cell class	mean Soma-diameter (μ)	Diameter of dendritic tree (μ)	Calculated ERF (40 μ in dia on the retina ≈1° of visual field) ***	Electrophysiologically determined ERF ****
0	8	200 – 250	5° – 6.25°	4° – 15°
3	12.8	300 – 350* (200 – 250)**	7.5° – 8.75° (5° – 6.25°)	6° – 10°
4	15	350 – 450* (300)**	8.75° – 11.25° (7.5°)	10° – 15°
4a	6.5	150 – 250* (130 – 150)**	3.75° – 6.25° (2.5° – 3.5°)	4° – 7°

* Calculated after own measurements
** Calculated after Kalinina, 1973
*** Maturana et al. (1960)
**** After Bäckström and Reuter (1974) and Grüsser and Grüsser-Cornehls (1976)

70.11 Quantitative Relationships between Morphology and Receptive Field Characteristics of Frog "Off" Centre Ganglion Cells Stained with Horseradish Peroxidase (HRP). R. V. Stirling* and E. G. Merrill* (SPON: S. Judge). NIMR, Mill Hill, London NW7 1AA and University Laboratory of Physiology, Oxford OX1 3PT, U. K.

We have recorded intracellularly from the axons of frog retinal ganglion cells in the optic nerve, characterized their receptive fields (RFs) and then injected them with HRP. Both the cell in the retina and the central projections were subsequently stained with a modification of the cobalt/nickel intensification of the diaminobenzidine method (Adams, J. C., J. Histochem. Cytochem.., 29: 775, 1981). Stained axons in the optic nerve were examined with electron microscopy. RFs were tested with dark and light spots, annuli and square wave reversal gratings. Although most of the ganglion cell classes previously recognized (Maturana, Lettvin, McCulloch & Pitts, J. Gen. Physiol., 43:129-175, 1960) have been filled, we discuss here only our results for the "off" centre ("dimming detector") cell class.

The typical dimming detector RF center size was about 15°, with a range of less than 8° to more than 35°. A third of these cells had isolated excitatory "on" regions in their surrounds (Keating, M. J. & Gaze, R. M., Q. J. Exp. Physiol., 55: 129-142, 1970), about half had inhibitory surrounds, and about a third were non-linear (ie. they responded with frequency doubling at all positions of reversal gratings in their RF centers) (Gordon, J. & Shapley, R. M., Visual Psychophysics and Physiology, ed. Krauskopf, J. & Wooten, B. R., New York: Academic Press, 1978).

In spite of this heterogeneity, dimming detectors all had: (a) a large ganglion cell (mean diameter, 21.9 um.), with a large dendritic arbor (400-1000 um. diameter) confined to a single stratum in the outer third of the inner plexiform layer, (b) a medium-sized myelinated axon (2.4 um., mean diameter), (c) a small contralateral pretectal arbor, and (d) a contralateral tectal arbor near layer 8. Cells with "on" regions in their surrounds had compact tectal arbors (120-150 um.) above layer 8; the others had large (300-550 um.) terminal zones in and below layer 8.

In this presentation we will examine the relationships between RF size, dendritic spread and tectal arbor size for these cells. We will also describe their RF center properties and surround features quantitively.

70.12 OPTIC AXONS IN THE DIENCHEPHALON OF CHANNEL CATFISH. RETINAL ORIGINS AND DISTRIBUTION PATTERNS. S.M. Fraley and S.C. Sharma. Dept. of Ophthalmology, NY Medical College, Valhalla, NY 10595.

The retina of many siluroid fishes possesses multiple optic papillae, each containing axons from a restricted portion of the retina. In channel catfish, papillar axons are topographically ordered in the optic nerve (Dunn-Meynell, A. and Sharma, S.C., Soc. Neurosci., 10:466, 1984) and the diencephalon of these fish receives optic axon innervation (Rao, P.D. and Sharma, S.C., J. Comp. Neurol., 210:37, 1982). Since several nuclei in the diencephalon of goldfish were observed to contain ordered maps of the retina (Fraley, S.M. and Sharma, S.C., Cell Tiss. Res., 238:529, 1984), and we were interested in determining whether corresponding diencephalic maps existed in channel catfish. We therefore investigated retinal topography in the diencephalon of pigmented Ictalurus (Ameiurus) punctatus by applying the horseradish peroxidase (HRP) to isolated optic papillae. Wholemounts of the retina and sections of the brain were reacted with chromagen to visualize HRP-labeled ganglion cells and axons.

The optic tracts of catfish consist of numerous medial fascicles formed by dorsal retinal axons traveling deep in the diencephalon and of a lateral optic tract formed by ventral and dorsal retinal axons traveling along the outer margin of the diencephalon. The nucleus of the dorsolateral thalamus (NDL) is served by the dorsomedial fascicle of the optic tract and this fascicle is formed by axons departing from the medial fascicles and lateral optic tract. The axons are topographically arranged in the NDL such that dorsal retinal axons project to its ventral aspect and ventral retinal axons project to its dorsal aspect. Axons from both dorsal and ventral retina also project to other diencephalic nuclei, including lateral geniculate and pretectum. The suprachiasmatic nucleus (SCN) receives projections primarily from dorsal retina. These axons project densely along the caudal aspects of SCN whereas ventral retinal axons project only sparsely at its rostral pole. The retinal maps in the SCN of catfish and goldfish do not correspond because, in the latter species, the nucleus is innervated primarily by axons from ventral retina. However, the retinal maps correspond closely in other nuclei, including NDL. There are also between-species similarities with respect to the pathways by which retinal axons reach diencephalic targets. We have determined that the medial fascicle in goldfish diencephalon, the fasciculus medialis tractus opticus, is composed of dorsal retinal axons from the center (oldest) retina. The multiple medial fascicles of catfish are also composed of dorsal retinal axons, and we are presently studying whether the alignment of these fascicles in the diencephalon represents an age-related ordering.

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70.13 HUMAN RETINO-HYPOTHALAMIC PATHWAYS. Betty M. Johnson and Alfredo A. Sadun, Dept. of Ophthalmology, USC School of Medicine, Los Angeles, CA 90033.

Retinofugal projections to certain hypothalamic nuclei have been suggested in the cat, rat and monkey (R.Y. Moore, Brain Res., 1973). Until recently, a reliable method for tracing retinofugal pathways in man has not existed. However, the paraphenylene-diamine staining technique (PPD) permits tracing of degenerated axons in the human visual system, even after extensive survival periods (Sadun et al, J. Neuropath. Exp. Neurol., 1983).

The PPD method was applied to brain specimens from five human cases in which there have been documented unilateral damage to the optic nerve. Degenerated axons, found only in the optic nerve ipsilateral to the lesion, were traced to the optic chiasm. At the level of the optic chiasm, one column of degenerated axons were noted to ascend dorsally and terminate in the suprachiasmic nucleus (SCN) of the hypothalamus. A second column of degenerated axons seemed to proceed more posteriorly and laterally and these were seen to terminate in both the anterior and posterior lobes of the supraoptic nucleus (SON) of the hypothalamus. A third column of degenerated profiles were seen to ascend dorsally alongside the third ventricle. These degenerated axons appeared to terminate in the rostral and medial portions of the paraventricular nucleus (PVN) of the hypothalamus.

The SCN, SON, and PVN of the hypothalamus contribute fiber bundles which course through the infundibulum to the posterior lobe of the hypophysis. In addition, several pathways from these nuclei are thought to project to brain stem and spinal cord nuclei. Oxytocin and vasopressin are among the neurotransmitters known to be synthesized by these nuclei. The SCN and PVN have been implicated in the control of circadian rhythms in several mammals. The demonstration of a visual input to these hypothalamic nuclei in humans suggest that this may be a means by which visual information entrains the diurnal cycle in man. In fact, disruption of water, carbohydrate, and insulin balance has been described in the blinded (Hollwich, Ann. N.Y. Acad. Sci., 1964). Thus, these three hypothalamic nuclei shown to receive a direct retinofugal input, may play an integral role in the diurnal regulation of certain autonomic functions in man.

RETINA I

71.1 THE TRANSITION TEMPERATURE IN ARRHENIUS PLOTS OF ATP-DEPENDENT CALCIUM ION UPTAKE IN RAT RETINA IS INCREASED BY TAURINE. J.B. Lombardini, Depts. of Pharmacology and Ophthalmology & Visual Sciences, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

The rat retina contains high concentrations of taurine (50 mM). However, there is little information as to the role for taurine in the retina at a physiologic level and the mechanism of action at a molecular level. Taurine appears to be participating in at least two different functions: 1) as a modifier of calcium fluxes, and 2) as a membrane stabilizer. Here we show that taurine raised in a dose dependent fashion the transition temperature of the discontinuity observed for ATP-dependent calcium ion uptake in rat retinal membrane preparations. (Calcium ion concentration was 10 μM.) In the absence of taurine the transition temperature was 17.9 ± 4.9°C while the addition of 20 mM taurine to the incubation system increased the transition temperature to 25.4 ± 0.8°C. The apparent activation energies obtained from the Arrhenius plots were 5.96 ± 0.76 and 3.41 ± 0.68 kcal/mole for above the transition temperatures in the ATP dependent and taurine-stimulated ATP dependent calcium ion uptake systems ($P < 0.05$). No significant differences were observed for the activation energies below the transition temperatures when taurine was added to the system (22.5 ± 3.6 vs. 16.2 ± 1.2 kcal/mol). Arrhenius plots of the maximum velocities for calcium ion uptake over a range of calcium ion concentrations (5 - 300 μM) also demonstrated a taurine effect. The transition temperatures for the ATP-dependent calcium ion uptake was 13.5°C while a value of 25.5°C was observed in the presence of taurine (20 mM). These data suggest that taurine is inducing a conformational change in the transport proteins associated with calcium ion uptake. (Supported by NIH grant EY04780).

71.2 PIGMENT EPITHELIAL INVOLVEMENT IN LOW INTENSITY CYCLIC LIGHT TOXICITY. S.L. Semple-Rowland* and W.W. Dawson, Departments of Ophthalmology and Neuroscience, University of Florida, Gainesville, FL 32610

The results of many studies on light damage suggest that the primary site of retinal damage is the photoreceptor outer segment and that light damage is a rhodopsin-mediated phenomenon. In the present study we have investigated the morphological and physiological effects of long term exposure to 20, 10, and 5 FtL cyclic light (12:12 L:D) on the retinas of albino rats raised from birth under 0.5 FtL cyclic light. Animals were exposed to these intensities for various durations of time (1-83 days) beginning at 15-17 weeks of age. We found that 20 FtL light causes severe retinal damage in these animals, and that 10 and 5 FtL light levels appear to approach threshold for producing irreversible damage. The primary morphological change in the damaged retinas was a rapid and significant loss of photoreceptor nuclei accompanied by a loss of the outer segment layer. Changes in the electroretinogram (ERG) included a decrease in the amplitude of the b-wave and an increase in its peak-latency relative to the 0.5 FtL controls. Examination of the d.c. ERGs from our animals reared under 0.5 FtL cyclic light showed that the c-wave, a prominent signal of pigment epithelial (PE) origin, was absent or greatly attenuated. This finding was especially interesting since the retina and PE histology appeared normal in these animals. To investigate the effects of cyclic light on PE function, we recorded the c-wave and changes in the d.c. potential in response to a low dose bolus of intravenous sodium azide in our control animals and in animals raised in constant darkness. Sodium azide was used as a second measure of the integrity of the PE. The azide induced potential is specific, direct and independent of a stimulus light. This potential arises from interference with ionic exchange processes across the PE cells' membrane (Noell, W.K., Air Univ. Sch. of Aviation Med., Proj. #21-1201-0004, Report 1, 1953). In this experiment, the c-waves were recorded under two light adaptation conditions followed by azide (40mM, 0.1 cc/kg) injection through a chronically implanted jugular catheter. The amplitudes of the c-waves and the azide responses from the animals raised under 0.5 FtL cyclic light were significantly smaller ($p<.05$) than those recorded from animals raised in constant darkness. From these results, we conclude that very low levels of cyclic light may disrupt the electrical properties of the pigment epithelium of the albino rat in the absence of histopathology (LM) of the retina or PE. These results suggest that the toxic effect of light on the PE may be primary and that visual cells may become secondarily involved due to (a) abnormal PE function , or (b) a synergy of PE and photoreceptor, functional unit, toxic responses. Supported by a grant from Sigma Xi and NIH grant R24 RR01635.

71.3 ROD SELECTIVE DEFICIT PRODUCED BY DEVELOPMENTAL LEAD EXPOSURE: REGIONAL RETINAL DIFFERENCES. D.A. Fox and L.W.-F. Chu*. College of Optometry, University of Houston, Houston, TX 77004.

Our previous ERG and cyclic nucleotide metabolism studies have established that low-level developmental lead exposure (0-21 days postnatal;0.2% lead acetate to dam) produces a longterm rod selective deficit in Long-Evans hooded rats (Neurosci. Abs. 9: 89, 1983; IOVS Suppl. 25: 287, 1984). To determine the morphological correlates of this rod-mediated deficit we quantified the no. of rod and cone photoreceptor cells (nuclei) in the posterior or central (POST) and peripheral (PERI) quadrants of the temporal retina of 90 day old control and developmentallylead-exposed rats (n=6-12 retinas/group). Nuclei were identified by light microscopy on the basis of nuclear heterochromatin pattern and staining (LaVail, IOVS 15: 64, 1976). We also measured the length of rod outer segments (ROS) and the thickness of the outer and inner nuclear layers (ONL and INL), inner plexiform layer (IPL), pigment epithelium (RPE) and total neural retina (TNR) in superior (S) and inferior (I) retina. Utilizing EM, ultrastructural alterations produced by lead were examined. In controls, the no. of rod and cone nuclei/100μm of POST retina is 140 and 2.6, respectively, while in PERI retina it is 81 and 1.5, respectively. In controls no differences in the no. of nuclei are observed between S and I retinal quadrants. In contrast, in the lead rats the no. of rod nuclei are decreased 17% and 28% in the POST S and I retina, respectively, and 13% and 22% in the PERI S and I retina, respectively. No change in the no. of cone nuclei is observed in leaded rats. In controls the ROS in S retina are 20-25% longer than in I retina (30 vs 24μm), as previously reported (Battelle and LaVail, 1978). In lead rats there is a 9% and 12% decrease in ROS length in S and I retina, respectively. Similarly there is a 14-16% and 22-29% decrease in thickness of ONL, INL, IPL and TNR in S and I retina, respectively. No between group differences in RPE thickness are observed. Thus the effects of lead are greater in (1) POST than PERI and (2) I than S retina. The two most prominent ultrastructural alterations are random patches of swollen and necrotic ROS (especially in proximal one-half) and the presence of numerous glycogen granules within the mitochondria of rod inner segments. There is also morphological evidence of an increased permeability of the blood-retinal barrier. These quantitative histological results confirm our ERG and cyclic nucleotide studies demonstrating that developmental lead exposure produces a longterm rod selective deficit. They also extend these findings by showing regional differences in photoreceptor and inner retinal degeneration and produce evidence of some general retinal damage---glycogen and blood-borne macrophages. It remains to be established that these findings have relevance to human pediatric lead exposure or retinal degenerations such as retinitis pigmentosa.

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71.4 **TELODENDRITE NETWORK OF HRP INJECTED CONES: ANATOMICAL AND FUNCTIONAL PROPERTIES IN THE WALLEYE RETINA.** Timothy W. Kraft[1], and Dwight A. Burkhardt[2]. Departments of Physiology[1] and Psychology[2], Univ. of Minnesota, Minneapolis, MN 55455.

Intracellular recordings from walleye cones were obtained using an isolated, superfused retina-pigment epithelium cell preparation, and triangular glass pipets filled with 10% HRP, 5% Lucifer Yellow in 0.5M LiCl (170-250MΩ). Iontophoretic injection of HRP into physiologically identified cones resulted in cells filled with a dense reaction product. Twenty-five of 46 injected cells were recovered; 16 cones showed several basal processes, or telodendrites projecting radially from the cone pedicle. Serial sections through the pedicle region (0.7-1.2μm) were photographed with phase contrast to reconstruct the telodendrite pattern and neighboring unstained pedicles.

Three to five fine telodendrites (0.3-1.0μm dia.) extend laterally into the outer plexiform layer for distances up to 35μm. En passant and terminal swellings were frequently noted. The diameter and length of the telodendrites increased as the diameter of the inner segment increased. Therefore each cone was able to make direct contacts only with neighbors within a distance of 2-3 cone diameters. Over 65% of the telodendrites were seen to end in close apposition with neighboring pedicles. Previous works suggest that electrical coupling may occur between walleye cones via gap junctions between telodendrites and neighboring pedicles.

Compartment models of individual and electrically coupled cones were used to measure the attenuation of signals passively spreading within and between photoreceptors via gap juntions. Results suggest that the signal generated in the photoreceptor inner segment spreads with little loss to the distal tips of the telodendrites. However, because of the cell geometry, membrane potential changes introduced in the telodendrites are attenuated considerably in the pedicle, and dramatically by the time they reach the inner segment.

Modeling electrical synapses as 2500MΩ junctions accurately predicts summation in the near surround which has been measured physiologically. However, this type of a network of interconnections should only produce weak spectral interactions.

71.5 RECEPTOR ORGANIZATION IN THE GRAY SQUIRREL RETINA. H.M. Petry, Dept. of Psychology, SUNY at Stony Brook, NY 11794.

The eastern gray squirrel (Sciurus carolinensis) is a diurnal mammal with a cone-dominated retina. Its photoreceptors are organized into two tiers: an inner, more vitreal tier and an outer, more scleral tier. Because the receptors appear uniformly cone-like when viewed under the light microscope, the gray squirrel retina was long thought to be "all-cone". But, electrophysiological (Green & Dowling, 1975) and electron microscopic (Cohen, 1964; West & Dowling, 1975) analyses have indicated the presence of a substantial population of rods. Furthermore, the EM analysis showed that cone and rod receptors appear to be spatially segregated. Cones appear to be located in the outer tier of receptors and rods in the inner tier. However, data previously presented at these meetings (Petry, 1982) suggested that this segregation may not be complete. Intravitreally-injected Procion yellow dye, which selectively stains short-wavelength-sensitive (SWS) cones in the monkey retina (DeMonasterio et al., 1981), was shown to be taken up by receptors in the gray squirrel's "rod" tier. The present study was undertaken as an alternative means of identifying SWS cones in the gray squirrel retina using repeated exposures of moderately intense short-wavelength light. In monkeys, such a procedure has been shown to produce selective damage to SWS cones, but not to other cone types or rods (Sperling et al., 1980).

Anesthetized gray squirrels were given daily 40 min exposures of one eye to moderately-intense (1.5 J/cm^2, daily) blue light over a 7 or 10 day period. The blue light (451nm, ±3nm) was presented in Maxwellian view and subtended a visual angle of 65 deg. centered on the retina. Post-exposure survival time ranged from 14-21 days. Light microscopic examination of the exposed retinas showed that light-induced cellular damage, (e.g., gaps in the receptor mosaic), was restricted to that area of the retina that had been exposed to the blue light. Furthermore, this damage was observed only in the inner tier of receptors and never in the outer tier.

These results confirm the Procion yellow findings and indicate that the gray squirrel's SWS cones are located, with its rods, in the inner tier. Such a receptor arrangement would be adaptive to the species for two reasons. First, by the layering of the dichromatic gray squirrel's other cone type, the long-wavelength-sensitive (LWS) cones, in the outer tier and the SWS cones in the inner tier, the effects of chromatic aberation on the retinal image would be minimized. Second, because a portion of the inner tier receptors are SWS cones and not rods, then West & Dowling's estimate of a total of 40% rod receptors would be reduced proportionately. Thus, fewer rods and more cones must compose the gray squirrel's retina, allowing for more of its receptors to function under the photopic light levels in which this diurnal squirrel is usually active.

71.6 **INHIBITION OF THE ELECTROGENIC SODIUM PUMP IN ROD PHOTORECEPTORS DURING MAINTAINED ILLUMINATION.** H. Shimazaki and B. Oakley II. Depts. of Electrical and Computer Engineering & Biophysics, & the Bioengineering Program, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

In our earlier work involving measurement of the light-evoked decrease in extracellular potassium ion concentration, $[K^+]_o$, in the subretinal space (J. Gen. Physiol. 84:475-504, 1984), we hypothesized that the Na^+/K^+ pump in the membrane of the rod inner segment becomes inhibited progressively during maintained illumination. In order to test this hypothesis directly, we recorded light-evoked changes in membrane voltage from rod photoreceptors in the isolated retina preparation of the toad, **Bufo marinus**. During our standard test conditions, the retina was superfused with pharmacological agents that blocked voltage-dependent conductances (and, in some experiments, calcium-dependent conductances, as well). Under these test conditions, the amplitude of the hyperpolarizing photoresponse became much greater than under control conditions. Results of several experiments support the conclusion that this increase in photoresponse amplitude was due primarily to current from the rods' electrogenic Na^+/K^+ pump, flowing across a high membrane resistance (which, in these experiments, resulted from a combination of the pharmacological agents and light). A similar conclusion was reached earlier by V. Torre (**J. Physiol. 333**:315-341, 1982).

Having established that, under our standard test conditions, a major component of the rods' photoresponse is produced by the rods' electrogenic Na^+/K^+ pump, we next recorded rod responses to continuous illumination under these test conditions. At the onset of a period of continuous illumination, the rod membrane first hyperpolarized by approximately 50 mV, and then began to repolarize, and after 180 seconds of illumination, the membrane voltage had recovered by as much as 77% of its initial hyperpolarization. Both the enhanced hyperpolarization at light onset and the slow depolarization during maintained illumination were blocked by superfusion with 10 micromolar strophanthidin. These results support the hypothesis that the rods' electrogenic Na^+/K^+ pump becomes inhibited progressively during maintained illumination. Moreover, the time course of the rod membrane repolarization during maintained illumination had a time course that was slightly more rapid than that of the recovery of the light-evoked decrease in $[K^+]_o$. This latter observation provides additional evidence in support of the hypothesis that inhibition of the rods' Na^+/K^+ pump produces the significant recovery of $[K^+]_o$ in the subretinal space observed during maintained illumination.

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71.7 DIFFUSIONAL RESTRICTION AND DYE COUPLING IN INSECT BRAIN SLICES. M. Järvilehto*, I.A.Meinertzhagen and S.R. Shaw (SPON: D. Mitchell) Department of Psychology, Dalhousie University, Halifax, N.S., Canada, and Department of Zoology, University of Oulu, Finland.

Neurons in the peripheral visual system of certain insects are uniquely anatomically identifiable, and in the neuronal ensembles (cartridges) of the lamina in Diptera, all the connections have been documented. Exploitation of these resources for physiological purposes has been hindered, however, because recovery of dye-injected cells is time consuming. We have therefore developed a perfused dipteran brain slice preparation that includes the eye, in which dye injections can be watched directly by epifluorescence. Visual stimulation so far has been crude, but sufficient to confirm that cells in the slice can remain viable for >3h. We report here two findings made with this slice preparation.

First, we frequently observed unexpected and almost immediate dye-coupling between cells in the optic lobe, always radiating from the position of the microelectrode tip. These occurrences were neither consistent from site to site, nor expected from the known ultrastructure. For example, the photoreceptor terminals are known not to be coupled via gap junctions either to postsynaptic monopolar neurons or to epithelial glial cells (EGCs), yet sometimes one or other type stained along with a terminal, when sites in the lamina were injected with Lucifer Yellow or other dyes. We suspect that such anomalous cases of dye-coupling are artefactual, perhaps due to genesis of relatively low impedance pathways through cells with surface membranes damaged by the micropipette tip.

Second, local dye ejection revealed a major restriction upon extracellular diffusion in the lamina synaptic zone, compared to other parts of the brain examined. In the medulla, chiasm and retina, a current pulse produced a halo of dye fluorescence around the pipette tip, that dissipated a few seconds after current-off, indicating a relative lack of constraint upon local extracellular diffusion. In the lamina, currents of 1 - 20nA applied for many minutes produced no lateral diffusion, only intense local columnar staining, which usually spread no further laterally than 1 - 2 cartridge widths, then persisted indefinitely. Subsequent histology disclosed one or more stained EGCs at the site. The EGCs in some cases appeared to form a dye-coupled network. This direct demonstration of compartmentalization in the lamina complements predictions made on other grounds (review: Shaw, S.R., J. Exp. Biol. 112: 225, 1985). Since the extracellular route is blocked, the laterally spreading photocurrents that can be observed in the lamina must be travelling in part intracellularly. The glial network is indicated as the probable conduit for these currents, which have been implicated in light adaptational and lateral inhibitory control. Supported by NSERC, Canada (I.A.M., S.R.S.), and NSERC International Exchange Award, and Finnish Academy (M.J.).

71.8 GABA (γ aminobutyric acid) ANTAGONISTS STIMULATE RELEASE OF DOPAMINE FROM INTERPLEXIFORM CELL PROCESSES IN THE CARP OUTER PLEXIFORM LAYER. Patricia O'Connor*, Charles L. Zucker and John E. Dowling, Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

In the outer plexiform layer (OPL) of teleost fish retina, the spatial properties of horizontal and bipolar cells are modified by dopaminergic interplexiform cell (DA-IPC) input. It has been shown in several species including carp (Cyprinius carpio), that dopamine, as well as the GABA antagonists picrotoxin and bicuculline (pic/bic), decrease electrical and dye-coupling between horizontal cells. The effects of GABA antagonists are blocked by the dopamine antagonist haloperidol suggesting that these agents disinhibit the DA-IPC causing dopamine release. In this study, the ability of pic/bic to release endogenous dopamine and activate the dopamine sensitive adenylate cyclase in isolated intact carp retina was investigated. Additionally, the cellular localization of this release has been determined autoradiographically.

HPLC analysis showed that pic/bic (100 μM) stimulated a 4-fold increase in endogenous dopamine release over a basal efflux of 1.25 pmole/mg protein. The release evoked by these agents was calcium dependent. Pic/bic, at concentrations which evoked dopamine release, stimulated an increase of 300 pmole/mg protein of cAMP above control levels. This activation of adenylate cyclase was blocked by preincubation with 100 μM haloperidol.

Retinas were preloaded with 0.2 μM ^{3}[H]dopamine, incubated with or without pic/bic and processed for light microscopical autoradiography. 1.5 μm plastic sections of control and antagonist treated retinas were processed on the same slides to prevent the variability common to autoradiographical techniques. Picrotoxin (100 μM) caused a reduction in grain density over the outer plexiform and horizontal cell layers by about 40% compared to control retinas. Bicuculline caused a similar reduction but to a lesser degree (20%).

It thus appears that the regulation of horizontal cell receptive field size by the DA-IPC is subject to GABAergic inhibition provided by amacrine cells in the inner plexiform layer. This inhibition influences the release of dopamine transmitter from DA-IPC synaptic terminals onto horizontal cell bodies in the OPL.

This work was supported by NIH grants EY05631 to P.O.C., EY05738 to C.L.Z. and EY00811 to J.E.D.

71.9 MORPHOLOGICAL EFFECTS OF DOPAMINE ON GOLDFISH HORIZONTAL CELL GAP JUNCTIONS. R.G. Miller and W.H. Baldridge, Dept. of Anatomy, The University of Calgary, Calgary Alberta, Canada.

Horizontal cells are second order neurons in the inner plexiform layer of vertebrate retina. Horizontal cells have been associated with both color vision and contrast regulation, as suggested by extensive gap junction coupling of both their cell bodies and also their axon terminals. Coupling of horizontal cells at gap junctions can be regulated by dopamine (Teranishi et al. J. Neurosci,. 4:1271, 1984) either by direct applications in situ or by light induced release of dopamine from interplexiform cells (Kramer, Inv. Ophthal. 10:438, 1971). Electrophysiologic evidence suggests that dopamine uncouples horizontal cells. The role of gap junctions in horizontal cell coupling has been studied extensively through dye coupling but relatively few studies have characterized morphological changes associated with horizontal gap junction coupling.

In this study, we have investigated the morphology of gap junctions on both horizontal cell bodies and their axon terminals in the goldfish using freeze-fracture electron microscopy. Light or dark (>1 hr.) adapted retinae and retinae from eyes intraocularly injected with various drugs were fixed in glutaraldehyde and prepared for freeze-fracture by standard procedures. We find significant changes in gap junction particle density associated with cell body coupling. No changes in axon terminal junctions were observed. Under conditions of coupling (darkness and no dopamine) gap junctions exhibit a particle density of 3,680 μm ± 280 μm^{-2} (mean ± SEM) compared to gap junctions which are uncoupled (treated with dopamine (in the dark) or exposed to light) which exhibit a density of 2,600 μm^{-2} ± 225 μm^{-2}. The action of dopamine can be blocked in all cases with haloperidol which results in "coupled-like" gap junction particle densities on the order of 3,600 μm^{-2} ± 200 μm^{-2}. Interestingly, gap junctions located at axon terminals appeared as coupled and did not significantly alter particle density under experimental manipulations.

van Buskirk and Dowling (PNAS, 78:7825, 1984) have implicated cyclic AMP as a second messenger of dopamine action in horizontal cells. Preliminary results suggest that vasoactive intestinal peptide (VIP), an adenylate cyclase stimulator, acts to uncouple horizontal cells as indicated by a less dense organization of gap junction particles. Further studies are underway to investigate the role of cyclic AMP on gap junction morphology. Electrophysiologic determination of coupling state prior to fixation is underway to verify presumed coupling states.

71.10 THE USE OF VECTOR PROCESSING SUPERCOMPUTERS FOR THE DISCRETE TIME SIMULATION OF LARGE NEURAL NETWORKS...A CASE STUDY USING A MODEL OF THE FROG RETINA. J.L. Teeters* and Y. Lee* (SPON: M.A. Arbib). Dept. of Computer and Information Science, University of Massachusetts, Amherst, MA 01003.

Discrete time computer simulation at the neuron level is important for the testing of neural network models, but is so computationally expensive that it is often impractical, especially for large networks. On a conventional computer the major bottleneck is that all the neurons must be simulated sequentially in the simulation of each time step before advancing to the next time step. Supercomputers (such as the Cray or Cyber-200 machines) have the capability to greatly reduce this bottleneck by using vector instructions to (in effect) simultaneously simulate many neurons within each time step. However, to take full advantage of this capability the simulation program must be structured so that nearly all of the computations are done by vector instructions. While this is not possible for most typical computer programs, the simultaneous operation of neurons in a network makes programs to simulate them good candidates for vector processing computers.

In the retina model the potentials of individual neurons are modeled by variations of a leaky-capacitor model. The density of each type of neuron and the existence and size of connections are based on anatomical data. A population of neurons is modeled to provide a spatio-temporal pattern of activities for every cell involved in the cone-pathways for a patch of frog's retina. Parameters for each type of model neuron are set so that their temporal activities approximate the typical intracellular recording for the corresponding neurons given the known visual/electrical stimulus patterns.

Simulating a small area (16 visual degrees) of the retina on a VAX 11/780 takes about one hour of computer time for each second of real time. Expanding the area simulated (which is necessary to test some aspects of the model) is impractical if the VAX is used because several weeks of computer time would be required to complete a single run. By carefully restructuring the program to make use of the vector capabilities of the Cyber 205, simulation time for the 16 degree patch was reduced to under thirty seconds for each second of real time. Using the 205, test requiring the simulation of a large visual area are feasible as the time required is under an hour. (This research was supported in part by NIH under grant No. NS14971-06A2.)

71.11 *THE EFFECTS OF DESTROYING THE INDOLEAMINE-CONTAINING RETINAL NEURONS ON THE ELECTRORETINOGRAM OF THE CARP.* *J. L. Cohen and S. Hensley*. Dept. of Anatomy, Wright State University, School Medicine and School of Science and Engineering, Dayton, OH 45435.*

The action of intravitreal injection of 5,7 dihyroxytryptamine is to destroy the indoleamine-accumulating neurons of the retina. In the teleost fish these neurons have been identified as amacrine cells. The electroretinogram is the algebraic sum of the electrical potentials of retinal neurons. We therefore wanted to determine what effects the destruction of the indoleaminergic-amacrine cells would have on the electroretinogram.

Twenty microliters of a solution containing 2 mg of 5,7 dihydroxytrptamine dissolved in 1 ml 0.9% NaCl with 1 mg ascorbic acid added as an antioxidant was injected into the vitreous of the right eyes of carp, 4-6 inches in length on two consecutive days. The left eyes acted as the control. One week after injection, the eyes were removed and the cornea, lens and vitreous cut away. The remaining eyecup was placed in a chamber with moist 100% oxygen blowing on it. A silver-silver chloride electrode was inserted into the vitreous and connected to a Grass P16 AC preamplifier and displayed on an oscilloscope. To determine if the serotonergic cells were indeed destroyed, 20 ul of a solution containing 3.5 mg of 5,6 dihydroxytryptamine dissolved in 0.5 ml of 0.9% NaCl was added to the eyecup at the end of each experiment. The preparation was allowed to incubate for 30 minutes in the dark. The retina was removed and then fixed in a solution containing 4% paraformaldehyde, 0.5 gluteraldehyde, 35% sucrose and 0.1M phosphate buffer. The retina remained in the solution overnight and was then air dryed for 24 hours and examined under a fluorescence microscope.

The b-wave of the electroretinogram was found to be significantly smaller in the treated eyes. Intensity-response curves obtained with 460nm, 520nm and 620nm light stimuli revealed curves with approximately the same slope, but with differences in the maximum amplitude. The amplitudes recorded from control retinas were twice that of treated retinas. The duration of the b-wave was longer than that from control eyes. The a-wave was not affected. Double flash experiments showed that the suppression of the second flash was greater for control retinas than for treated retinas. Fluorescence microscopy revealed the absence of indoleamine-containing cell bodies in the treated retina, thus demonstrating the destruction of the indoleaminergic neurons.

71.12 **THE ROLE OF INDOLEAMINES IN VISUAL PROCESSING IN THE RABBIT RETINA.** **William J. Brunken and Nigel W. Daw, Department of Cell Biology and Physiology, Washington University Medical School, St. Louis, Missouri 63110.**

We have reported previously that the serotonergic antagonists ketanserin and methysergide, both 5-HT2 antagonists, altered the center-surround balance of off-center rabbit ganglion cells in favor of the central (off) mechanism (Brunken et al. '84). Furthermore, 5-methoxy N, N, dimethyltryptamine (5-MDT), a potent agonist at autoreceptors, a subclass of 5-HT1 receptors, produced similar results. Thus, these data provided physiological evidence for the existence of both 5-HT1 and 5-HT2 receptors in rabbit retina. This conclusion has recently been confirmed by binding studies (Redburn et al. '85).

We are now pursuing the question of what is the functional significance of these two serotonin receptor classes. The attempts to elucidate the role of 5-HT1 receptors are limited by the lack of a specific antagonist at this site. We have employed methiothepin, an antagonist potent at 5-HT1 subtypes, including the autoreceptor, and less potent at 5-HT2 receptors. Methiothepin, like both methysergide and ketanserin, reduced spontanteous activity and abolished the on-component of the response to both diffuse or annular stimulation for off-center cells. However, unlike either of these other drugs, methiothepin reduced the sensitivity and responsivity of the central component of the response as well. In some cases, it eliminated the response to small spots centered on the receptive field altogether. We suggest that this latter action is the result of specific antagonism at 5-HT1 receptors.

In order to assess whether or not the blockade of the on-component of off-center cells produced by 5-HT2 antagonism represents a shift specifically in the center-surround balance or rather an interruption of the on-pathways in rabbit retina, we have turned to monitoring the effect of 5-HT2 antagonists on the properties of on-off directional selective ganglion cells. LY-53857, a 5-HT2 antagonist, which has effects on off-center cells similar to ketanserin and methysergide, but unlike these other drugs lacks any histamine or alpha-adrenergic effects, reduced the spontaneous activity and both the leading and trailing edge responses of on/off directional selective cells. Thus, it appears that at least in these cells the endogenous indoleamine modulates (increases) both the on and off inputs. Furthermore, these results are distinctly different from those obtained with the D1 dopamine antagonist Sch 23390, which selectively reduces the leading edge response of on/off directionally selective cells. Thus, we conclude that there is a functional role for an as yet unidentified indoleamine in the mammalian retina.

71.13 EFFECTS OF MORPHINE ON LIGHT RESPONSES OF GANGLION CELLS IN THE LARVAL TIGER SALAMANDER RETINA. S.E. Feingold*,C.B. Watt*, D.M.K. Lam, and S.M. Wu. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

Previously, using immunocytochemical techniques, we have demonstrated the presence of enkephalin-like immunoreactivity in two populations of amacrine cells in the larval tiger salamander retina (Watt et al., Neuroscience Abstract 248.16, 1984). Light microscopy showed heavily stained processes in sublamina 5 and punctate deposits in sublamina 1 of the inner plexiform layer. At the ultrastructural level, enk-immunoreactive processes make synaptic contacts with other amacrine cells, bipolar cells, and possibly ganglion cell processes.

To study the function of these enkephalinergic cells, single-unit ganglion cell light responses were monitored in the superfused salamander eyecup before, during, and after applications of morphine sulfate. In about 1/3 of the on-off cells tested, 1 mM morphine caused a reversible reduction in firing frequency at the onset of broad-field illumination. Little change was observed at the cessation of light stimuli. Approximately 80% of the on-center and off-center cells encountered responded to morphine application. In the on-center cells, a transient on-response to surround illumination appeared in the presence of morphine. In the off-center cells, administration of 1 mM morphine resulted in an increase in firing frequency at the onset of surround illumination and caused no change in the responses to center illumination.

Physiological observations described above suggest that the primary action of morphine is to alter selectively the electrical responses to onsets of broad-field illumination in retinal ganglion cells. This is consistent with the immunocytochemical results since amacrine cells mediate the broad-field inputs to ganglion cells, and cells which send processes to sublamina 5 are thought to be involved in the on-pathway of the inner retina.

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71.14 RETINAL GANGLION CELL MORPHOLOGY IN CATFISH. A.A. Dunn-Meynell* and S.C. Sharma (SPON A.B. Drakontides). Dept. Ophthalmol., New York Med. Coll., Valhalla, New York 10595.

Retinal ganglion cell morphology was examined in the catfish Ictalurus punctatus using horseradish peroxidase (HRP) techniques which permit fine details of the structure of these cells to be discerned. HRP was applied to small lesions in the optic papillae at the point at which they left the retina in 3-5 inch catfish. After 3-5 days the neural retina was isolated, flat mounted and reacted to reveal HRP using diamino benzidine as chromagen.

Ganglion cells could be broadly divided into giant (soma diameter about 15-30 μm, dendritic field area roughly 15 X 10^4 μm^2) and small (soma diameter approximately 7-15 μm, area of dendritic arbor in the order of 3 X 10^4 μm^2). We subdivided the inner plexiform layer (ipl) into 4 laminae (from most chorad - a, to most vitrad - d) to describe the depth of dendritic arbor.

The following general characteristics distinguished the 12 classes of ganglion cells which we delineated: GIANT CELLS; G1 - The axon arises from the soma, thick dendrites ascend to ipl lamina a. In the dorsal retina the dendritic fields of these cells have a marked naso-temporal elongation and the cells have a roughly regular distribution. G2 - The axon arises from a dendrite, the soma has an irregular shape, the dendrites arborize in ipl lamina b. G3 - Typified by their medium diameter dendrites which arborize in ipl lamina d. These cells tend to be smaller than other giant cells. G4 - Found almost solely in a band running nasotemporally along the center of the retina. In this position the dendritic field is elongated dorsoventrally. The main dendrites arborize in lamina c, fine dendrites ascend to arborize in laminae a or b. G5 - The soma is displaced into the inner nuclear layer (inl), the dendrites arborize in ipl lamina a. SMALL CELLS; S1 The axon arises from a round or oval soma, the dendrites (which are fine and are often seen to have an intricate arbor) ascend to ipl lamina b. S2 - These cells have a roughly round soma from which the axon departs. The dendrites are fine and their arbor is unstratified. S3 - The cells are oval with about 3 dendrites which arborize in laminae a and c. S4 - The axon arises from an oval soma, the dendrites form a small arbor in lamina b. S5 - The cells have a round soma with dendrites in lamina d. S6 - The soma is displaced into the inl. The dendrites arborize in lamina a. These cells form a clearly distinct population from the giant displaced ganglion cells (G5). S7 - The axon arises from a dendrite, the dendrites arborize in lamina c.

These results show the ganglion cell population in catfish to be diverse and complex. Further subdivision of cell types may be possible.

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71.15 ELECTRICAL PROPERTIES OF TIGER SALAMANDER RETINAL GANGLION CELLS. Peter. D. Lukasiewicz* and Frank S. Werblin*
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The membrane properties of salamander retinal ganglion cells were studied using "gigaseal" whole cell patch recording techniques. Recordings were made from retinal slices and cells were identified by their locations in the slice. The membrane currents were composed of five components; a fast transient sodium current, a slow transient sodium current, a voltage-dependent potassium current, a calcium-dependent potassium current, and a sustained calcium current.

The fast and slow sodium currents were both activated at potentials more positive than -40 mV and were maximal at -10 mV. Both types of sodium currents were blocked by tetrodotoxin or by substituting choline for external sodium. The voltage-dependent potassium current was activated at potentials more positive than -20 mV and was blocked when the cell was internally perfused with cesium. The calcium-dependent potassium current was activated at potentials more positive than -20 mV and was suppressed by 20 mM TEA, 4 mM cobalt or 1 mM cadmium. The sustained calcium current was activated at potentials more positive than -25 mV and was maximal at +10 mV. The sustained calcium current was blocked by 4 mM cobalt or 1 mM cadmium.

The input resistance of these cells was greater than 2 gigaohms and the resting potential was near -70 mV. We have measured excitatory synaptic currents greater than 50 pA which could bring these cells within the range of activation for all currents described above. Gated currents may thus significantly alter the form of response following synaptic input from amacrine and bipolar cells.

71.16 THE DELTA GANGLION CELL OF THE CAT RETINA. H. Kolb and R. Nelson, Physiology, Univ. Utah Sch. Med., Utah, and Neurophysiology, NINCDS, National Institutes of Health, Bethesda, MD.

A ganglion cell type belonging to the ill-defined W cell class in the cat retina has been studied by intracellular recording, iontophoresis of HRP and electron microscopy. The ganglion cell has a dendritic branching pattern typical of the delta cell described originally by Boycott and Wassle (1974) and further defined by Kolb, Nelson and Mariani (1981). The 20 um cell body gives rise to long curved dendrites that are narrowly stratified in s2, on the sublamina a/b border of the inner plexiform layer. The overall dendritic tree size is 660 um and the dendrites are essentially smooth bearing only a few small spines. Electron microscopy of the delta ganglion cell indicates that it receives cone bipolar synapses primarily from type cb1, and various amacrine synapses. The delta cell dendrite usually shares the cone bipolar ribbon synapse with an A19 amacrine cell dendrite. The A19 characteristically makes a reciprocal synapse back upon the cone bipolar terminal and occasionally also upon the delta ganglion cell dendrite.

The intracellular responses of this ganglion cell are characterized by a complex interplay of inhibitory and excitatory post synaptic potentials. With diffuse stimulation there is a rapid on-hyperpolarization which is modulated by a delayed sustained repolarization punctuated by large, rapid fluctuations in membrane potential, both hyperpolarizing and depolarizing. The larger depolarizations trigger action potentials. The initial hyperpolarizing event reveals a large receptive field with a half width of about 650 um. The edges of the receptive field are characterized by depolarizing events. The cell is not directional along the axis of the receptive field measurement, and appears concentric, with a central region of inhibition being overlapped and flanked by more peripheral excitation. Some differences in the cells behaviour under light and dark adaptation have been noted. Thus, although concentrically organized, the physiology of the delta ganglion cell appears to be more complex than that of the alpha or beta ganglion cells of the cat retina.

71.17 REGULATION OF GABA AND SOMATOSTATIN RELEASE IN RETINA AND OLFACTORY BULB BY VASOACTIVE INTESTINAL PEPTIDE (VIP) AND DOPAMINE. D. Smith*, S.L. Horowitz*, J.F. Cubells and M.H. Makman*. (SPON: E.B. Gardner). Departments of Biochemistry, Molecular Pharmacology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

We have previously reported that VIP stimulates several fold the release of ^{3}H-GABA from retinal synaptosomes that had been prelabeled either with ^{3}H-GABA or with ^{3}H-glutamate in order to assess the release of newly formed ^{3}H-GABA (Soc. Neurosci. Abstr. 10:837, 1984). PHI, secretin and glucagon also stimulated GABA release, with relative potencies resembling those for stimulation of adenylate cyclase (AC) activity. In further studies the VIP-stimulated release of newly formed GABA was not blocked by tetrodotoxin or by substitution of Co^{++} for Ca^{++} in the medium. Glutamate at 500 uM markedly stimulated GABA release. Glutamate at 50 uM only slightly increased GABA release but greatly enhanced the amount of GABA released due to VIP; percent increase by VIP was unchanged. In the presence but not in the absence of glutamate, dopamine (DA) also stimulated GABA release; however, DA was less effective than was VIP. In preliminary studies the selective D_1 receptor antagonist, SCH23390, blocked the effect of DA. Comparable studies have been carried out with synaptosomes prepared from rat olfactory bulb (OB). VIP caused a marked stimulation of GABA release from OB synaptosomes. The relative order of potency of related peptides was similar to retina, except that at high concentration (10 uM) PHI was more potent than VIP in OB, while VIP was more potent in retina. AC may not be involved in the effects of VIP in OB or retina since forskolin was inactive and cyclic AMP analogues had little effect.

Release of somatostatin from bovine retinal and rat OB synaptosomes has also been studied. VIP and also DA stimulated immunoreactive somatostatin release from both retinal and OB synaptosomes. The receptors involved in these effects are under investigation. It remains to be established if GABA and somatostatin are colocalized in neurons containing VIP receptors. Somatostatin itself did not influence basal or VIP-stimulated GABA release. While VIP and DA acting at D_1 receptors may have common effects, differences are also expected on the basis of studies of additivity of VIP-AC and DA-AC. It is of interest that in two sensory systems, retina and OB, VIP exerts similar effects on other transmitters. (Supported by USPHS grants EY-04633 and 5T32-GM-07260.)

71.18 IS RETINAL GANGLION CELL RESPONSE TO MOTION PREDICTABLE FROM THE CELL RESPONSES TO STATIONARY FLASHING STIMULI? M.F. Sarna* (SPON: P. Sterling) Dept. Neurophysiology, Nencki Institute, Warsaw, Poland and Dept. Physiology, University of Pennsylvania, Philadelphia, PA 19104.

Assuming linear spatiotemporal summation within receptive field of a visual cell one should be able to predict the cell response to movement from the cell responses to stationary stimulus flashing on and off with properly adjusted timing in consecutive positions along trajectory of a movement. These experiments were carried out to examine this principle.

Responses of retinal ganglion cells of the pretrigeminal cat to small bar flashing on and off in randomly ordered positions along a line within the cell receptive field were recorded. Peri-stimulus time histogram (PST) was collected for each bar position. The 'predicted' PST to moving stimulus was then constructed from stationary data by summing up PSTs related to succeeding stationary bar positions each PST shifted by the appropriate time. Such a 'predicted' PST was compared with 'actual' PST recorded during stimulus motion along the same line with properly adjusted velocity.

A comparison showed near-perfect matching of both 'predicted' and 'actual' histograms in the case of X-cells, both ON- and OFF-center. In the case of Y-cells matching was much worse, ranging from good for relatively sustained ON-center cells, through poor for transient ON-center cells, to lack of any matching in majority of OFF-center cells. In general, excitatory peaks matched much better than inhibitory valleys, which frequently were hardly seen in 'predicted' PST of Y-cells.

These findings are consistent with the assumption that spatiotemporal summation within X-cell receptive field is nearly linear, whereas within Y-cell is strongly nonlinear. But these results can also be explained, at least roughly, without assuming nonlinearities. Since resting firing level of Y-cells is small, then both strong and weak inhibition produce the same image in stationary PSTs, i.e. a zero line, whereas stronger excitation produces higher peaks than weaker excitation. This leads to underrepresentation of inhibition in stationary responses and consequently in 'predicted' PST.

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71.19 COMPUTER IMAGE ANALYSIS OF LASER INDUCED PUNCTATE LESIONS. H. Zwick*, L. Sherman*, and D. O. Robbins Letterman Army Institute of Research, San Francisco, CA 94925 and Ohio Wesleyan University, Delaware, OH 43015.

In previous experiments we have evaluated the immediate and long term effects of single and multiple laser exposures on spatial vision of rhesus monkeys. For exposure energies ranging from near the morphological damage level to energies several log units below this level, brief (100 msec) foveal exposures of various retinal diameters, produced immediate and transient changes in both visual acuity and contrast sensitivity. The magnitude and duration of these changes in visual sensitivity were related to the energy, duration, and placement of the exposure on the retina but extended beyond what might be expected solely on the basis of local adaptation of foveal receptors. Repeated exposures over days or weeks at energy densities below ED 50 for the single exposure condition ultimately produced long term changes in acuity characteristic of those produced by single exposures of higher energy above the ED 50 level. Exposure of the fovea to a minimal diameter ($\leq 50\,\mu$) single Q-switched pulse of extremely short (15 nsec) duration but high energy density (50 - 100 μJ), on the other hand, produced rather small and extremely variable changes in immediate postexposure acuity despite the fact that these energies densities were significantly above the ED 50 level. Long term shifts in acuity to these high energy, Q-switched pulses were observed only after repeated exposures over several days or weeks. Postexposure sensitivity was, however, marked by increased response variability not evident in pre-exposure testing.

In this paper we have correlated these behavioral findings with funduscopic changes through the use of digital image analysis techniques. Punctate foveal lesions from exposed animals were differentiated from normal retina on the basis of grey scale distributions measured in local foveal regions and referenced to unexposed regions from the same retina. Such relative grey scale distributions are capable of characterizing the degree of focal and non-focal disturbance induced by punctate damage. A second technique involving the use of two dimensional fast fourier transformations over local regions such as the fovea enhanced the contrast of relatively large but low contrast aspects of foveal-macular detail. This latter procedure has demonstrated the full extent of lesions in the fovea that otherwise would be nearly impossible to detect by conventional funduscopy. Correlation of these enhanced morphology disruptions with behavioral changes will be presented.

71.20 COMPARISON OF PATTERN-EVOKED RETINAL RESPONSES AND VISUAL-EVOKED POTENTIALS IN RHESUS MONKEY. R.D. Glickman, F.H. Previc and R.M. Cartledge*+. Life Sciences Div., Technology Inc., San Antonio, TX 78216, and +Radiation Sciences Div., Brooks AFB, TX.

We have recorded the pattern-evoked retinal response (PERR) and the visual-evoked potential (VEP) under identical stimulus conditions from the rhesus monkey. The monkey was anesthetized with barbiturates, paralyzed with gallamine, and mechanically ventilated. Focal VEP's were recorded with bipolar, stainless-steel electrodes implanted in the foveal projection region of both visual cortices. These electrodes measured the potential generated between the cortical surface and a depth of 3 mm. Monocular PERR's were recorded with an ERG-jet electrode (Life-Tech, Inc.). A conductive thread electrode ("D-T-L") on the occluded eye served as a reference. Stimuli subtended 6 or 12 degrees of visual angle and consisted of green and black square-wave, luminance-modulated gratings presented on a CRT monitor. These gratings ranged in spatial frequency from 0.5-8 cpd and were counterphased at 1-20 Hz. Maximum luminance of the gratings was 14 cd/m^2 and the contrast was varied between 30-85%.

Several consistent differences were noted between the rhesus VEP and PERR. With a 1 Hz counterphased grating, the implicit time of the prominent negative wave of the PERR (84 $\pm$ 3.5 msec) was always greater than that of the VEP (72 $\pm$ 7 msec). The VEP peaked in amplitude in response to stimuli of 4 cpd, while the optimal spatial frequency for the PERR was about 1 cpd. Both the VEP and PERR showed the largest amplitude responses to stimuli counterphased at or below 5 Hz. The PERR, however, was detectable at counterphase rates up to 20 Hz (the highest rate tested), whereas the VEP became unreliable above 10 Hz. Signal amplitude was highly dependent on stimulus size and contrast, e.g., the PERR amplitude increased from 1.6 to 6.5 uV as stimulus contrast was varied from 30 to 85% while the VEP amplitude (86 uV) saturated at about 50% contrast. We examined the contribution of the central visual field to the PERR by selectively stimulating parts of the retina. The PERR appeared to be derived uniformly from the central 12^o of visual field that was tested. The PERR has characteristics distinct from those of the VEP, especially with respect to temporal modulation.

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AUDITORY SENSORY ORGANS AND TRANSDUCTION

72.1 THE TEMPORAL ACTIVATION OF AUDITORY NEURONS AS A FUNCTION OF ACOUSTIC FREQUENCY IN THE HATCHLING CHICK. T.A. Jones, H. Brown,*R. Czeranko* and M.M. Beck, Depts. of Oral Biology, University of Nebraska Medical Center, and Animal Science, University of Nebraska, Lincoln Ne. 68583.

A vibration of the basilar papilla (basilar membrane) travels from the base to the apex of the cochlea at a velocity which decreases as a function of distance (Bekesy 1960). The time of its arrival at any particular position will depend on the traveling wave velocities encountered and the distance traversed during travel. Amplitudes of the vibration also change as a function of cochlear position and for a given frequency will be maximum at a particular location. At low intensities, narrow band stimuli may be used to preferentially activate neurons innervating this corresponding region of maximum amplitude. The latency of activation for such neurons will reflect the cochlear travel times for the acoustic stimulus. Tonotopic maps presenting the position of maximum amplitude as a function of frequency have been described for birds and many species of mammals. The corresponding temporal data relating the latency of neural activation as a function of frequency are not available for birds. In the present study, low intensity narrow band stimuli (pure tones:15 msec duration) were employed to estimate the delay in neural activation for various frequencies (approx. 5k, 3k, 1650, 990, 490, 290, 220, and 180 Hz). Tones were presented at a repetition rate of 10/sec. Hy-line chicks (one to three days after hatch) were used in the present study. Auditory responses were recorded using subcutaneous electrodes (vertex=G1; exoccipital=G2;abdominal ground) where electrical signals were led to an amplifier (10,000X; Lf=100; Hf=10,000) and in turn to a computer averager (8.3 microsec/point; 1024 points). Neural and cochlear microphonic (CM) potentials were resolved out of the background electrical noise using computer averaging (n=512). Threshold stimulus levels were determined for each frequency to generate audibility functions. Wide band maskers were also applied to verify neural components and the resulting non-neural waveforms (CM & electrical artifact) were subtracted from the unmasked responses to stimuli of the same frequency and intensity. Latencies of neural response components were then plotted against frequency. Neural response thresholds were below 88dbSPL for all frequencies and were lowest for frequencies between 400 and 5k Hz (range: 30-60dbSPL). The slopes of the latency/frequency curves were nonlinear over the range of frequencies studied. Latency/ frequency slopes were least at frequencies below 290 and above 3kHz presumably reflecting properties of the apex and base of the cochlea respectively in these young birds. (Bekesy, G. v. Experiments in Hearing, New York:McGraw-Hill, 1960)

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72.2 A SURVEY OF SPONTANEOUS OTOACOUSTIC EMISSIONS IN THE NONHUMAN PRIMATE. B.L.Lonsbury-Martin, G.K.Martin, R.Probst,* and A.C.Coats. Dept. of Otorhinolaryngology & Communicative Sciences, Baylor Col. of Med., Houston, TX 77030.

A number of reports have described a relatively high (40%) incidence of continuous, low-level, narrowband signals known as spontaneous otoacoustic emissions (SOAEs) in recordings made from the sealed human ear canal. The subsequent finding of significant differences in incidence and some basic characteristics between SOAEs of humans and those of common laboratory animals raises important concerns regarding the differences in micromechanics between human and animal ears. The present study of nonhuman primate ears was conducted to determine the incidence of SOAEs in an animal ear that is phylogenetically closer to the human than previously studied animals. Both ears of 61 seemingly normal-hearing primates representing four species of Old World macaque and one species each of ape (baboon) and New World monkey (squirrel) were examined. Multiple emissions were discovered in three ears of three different macaques, two Macaca mulatta and one M. nemestrina. Overall, 2.5% of nonhuman primate ears and 5% of the monkeys demonstrated SOAEs. In contrast to what has been typically documented in other laboratory species, the ranges describing frequency, amplitude, and bandwidth characteristics of the monkey emissions were identical to those that have been reported for humans. To further characterize the psychoacoustic properties of monkey SOAEs and to relate the SOAEs to other classes of emissions (evoked, stimulus-frequency, distortion-product), detailed testing of one emitting rhesus monkey was performed. Results from these tests showed additional features highly similar to those that have been described for human ears. The appreciably lower incidence of spontaneous emissions in nonhuman primates, although notably greater than that shown for other laboratory species, suggests that despite similar features, subtle differences in the micromechanical generating mechanism(s) must exist between monkey and human ears. Work in progress is aimed at determining if monkey SOAEs are associated with cochlear pathology.

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72.3 SCANNING ELECTRON MICROSCOPY OF THE COCHLEA IN RATS EXPOSED TO VARYING DURATIONS OF HIGH-INTENSITY, BROAD-BAND NOISE.
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The organs of Corti (OC) of Sprague-Dawley rats exposed to varying durations of high-intensity, broad-band noise (2 school bells ringing at 124 dB SPL) were examined by scanning electron microscopy (SEM). The rats were divided into eight groups (G) of noise exposure (1/5 the noise given daily for 5 consecutive days): G-1 (7.5 min); G-2 (10 min); G-3 (15 min); G-4 (25 min); G-5 (50 min); G-6 (75 min); G-7 (120 min); G-8 (0 min).

Cochleas from each group were examined 21 or 28 days post noise exposure. Each rat was perfused through the heart with 3% buffered glutaraldehyde and the cochleas removed and stored in the perfusate over night. After rinsing with water, the temporal bones were post-fixed for one hour in osmium tetroxide, decalcified in EDTA, then dehydrated to 70% ethyl alcohol for microdissection. After microdissection, the entire organ of Corti (OC) was prepared for SEM.

The OC contained damage in proportion to the length of noise exposure. Animals exposed to short noise durations (the 7.5, 10, and 15 minute groups) had minor damage while animals exposed to long durations of noise (the 25, 50, 75 and 120 minute groups) had more extensive damage. Hair cell loss and stereocilia loss, elongation, clumping and/or fusion was most extensive in the long duration groups. Inner hair cells were affected first and sustained more damage than outer hair cells. For this particular noise stimulus, the basal turn contained more damage than the hook, middle turn, and apex at similar durations of noise exposure. Headplate (HP) damage in the short duration groups was slight with the HPs being curved in different areas; however, in the long duration groups, up to 100% of the HPs were sloughed off and replaced by supporting cells. A smaller, yet progressive increase in damage was seen in the short duration groups.

Animals studied at 28 days post noise exposure (dpe) in the 25 and 120 minute groups exhibited more damage than the 21 dpe animals of the same groups. It seems likely that cells which appeared to be only damaged in the 21 dpe group were actually dying, because by 28 dpe, more damage was apparent. However, there was little difference in the damage sustained in the 21 vs. 28 dpe animals in the 7.5 and 10 minute groups. It seems likely that cells in the 21 dpe groups were insufficiently damaged to cause cell death great enough to manifest itself in the 28 dpe animals, or that healing had occured.

These data show morphologically demonstratable damage to the OC with increased durations of exposure to high intensity, broad-band noise. Furthermore, the extent of cochlear damage in long exposure times is not complete until at least 28 days after noise exposure. (Supported in part by The Deafness Research Foundation)

72.4 DOSE-EFFECTS OF GENTAMICIN IN NOISE EXPOSED RATS.
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Ototoxic interaction between aminoglycoside antibiotics and noise has been studied in the guinea pig. Studies of this interaction are lacking in the rat. The purpose of this study was to investigate the dose effects of gentamicin (GM) and noise on cochlear function in the rat. The experiments employed the following 8 groups (G). In each group (n=10): GIa - GM 50 mg/kg/d; GIb - GM 100 mg/kg/d; GIc - GM 150 mg/kg/d; GId - Saline only; GIIa - Noise then GM (N/GM) 50 mg/kg/d; GIIb - N/GM 100 mg/kg/d; GIIc - N/GM 150 mg/kg/d; GIId - N/Saline. The indicated doses were divided into 3 equal doses and administered i.m. every 8 hours for 10 days. Rats in GII were exposed to broad band noise (124 dB SPL peak intensity) 90 seconds/day for 5 days prior to GM or Saline treatment. Cochlear round window membrane recordings were assessed 28 days post noise exposure and 18 days post drug treatment. All recordings were made on day 29 of the experiment.

Depressions of auditory nerve action potentials (N_1) and the a.c. cochlear potentials (ACCP's) produced by gentamicin alone bore a direct relationship to dose of the drug, with larger depressions being produced as the dose of gentamicin was increased. Noise/Saline treatment significantly depressed N_1 potentials (compared to Saline alone); with no effect on the ACCP's at 4, 12 and 20 kHz. The combined effects of noise and low dose gentamicin on N_1 and on the ACCP's at 4 kHz were significantly greater than those due to noise or gentamicin alone, demonstrating a classical noise-drug ototoxic interaction. The lack of interaction of noise and low dose gentamicin on the higher frequencies (12 and 20 kHz) was due to the noise used; noise of this duration and intensity has little effect on the areas of the cochlea corresponding to the higher frequencies. There was no detectable interaction between noise and the two higher doses of gentamicin; this was due to these doses producing an overwhelming cochlear toxicity by themselves. The effect that noise had on this dose-effect relationship is relatively straightforward, if one recalls two facts: 1) N_1 is the most susceptible indicator of noise trauma and 2) the 4 kHz region of the cochlea appears to be most susceptible to noise-induced damage. Thus, in these experiments, it is probable that noise trauma to a frequency-selective area of the cochlea facilitated entry of gentamicin into that area of the cochlea.

72.5 SALICYLATE-RELATED CHANGES IN INTRACOCHLEAR CALCIUM AS A CAUSE OF AUDITORY DYSFUNCTION.
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Both the coupling of stereocilia to the tectorial membrane and the rigidity of ciliary anchoring to hair cells are crucial to normal cochlear micromechanics. According to previous theories, partial de-coupling and/or loss of ciliary stiffness may result in tinnitus, recruitment, or disorders in speech perception. We hypothesize that salicylate-related auditory dysfunction, particularly tinnitus of cochlear origin, may result from salicylate-induced changes in calcium concentration within perilymphatic fluids. Our hypothesis is based upon observations that: i) salicylates, which are known to cause tinnitus, evoke their activity on the auditory system at the level of the cochlea; ii) administration of salicylates decreases serum calcium; and iii) decrease of calcium concentration in perilymph results in de-coupling of stereocilia from the tectorial membrane as well as decreased stiffness of ciliary anchoring.

To test our hypothesis, we performed the following set of experiments. Guinea pigs and rats were anesthetized with pentobarbital (50 mg/kg). Samples of blood, cerebrospinal fluid and perilymph were taken at different times after i.p. injection of sodium salicylate (464 mg/kg) and evaluated for total calcium by means of the Atomic Absorption Method, or for free calcium by a calcium selective electrode. After salicylate administration, the level of total and free calcium in serum, cerebrospinal fluid and perilymph decreased to about 80% of control values, although with different time courses. Characteristics of cochlear microphonics and whole nerve action potential were measured before and two hours after salicylate administration. Before salicylate, the characteristics were linear for low levels of stimuli and showed saturation for high levels. After salicylate, microphonic characteristics showed recruitment-type nonlinearities for low levels of stimuli. In identical experimental situations, recordings of spontaneous activity of single cells residing in the inferior colliculus and lobulus V of the cerebellar vermis revealed increased spontaneous activity in the inferior colliculus as contrasted with no change in the cerebellum.

Our data support the hypothesis that salicylate may influence the auditory system by decreasing calcium concentration in cochlear fluids, resulting in de-coupling of cilia from the tectorial membrane and/or decreasing the stiffness of ciliary anchoring to hair cells, thereby affecting the mechano-electrical transduction properties of the cochlea. (Supported by NIH Grant #NS22024)

72.6 ACTION OF SALICYLATE ON THE ACTIVITY OF AFFERENT FIBERS IN THE XENOPUS LAEVIS LATERAL LINE.
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The mechanism for the reversible hearing loss induced by aspirin is unknown. Salicylate suppresses the compound action potential and single fiber activity of the auditory nerve with no effect on cochlear microphonics (Bobbin and Thompson, Ann. Otol. Rhinol. Lar. 87:185-190, 1978; Evans et al., In: Tinnitus, Ciba Foundation Symposium 85, pp. 108-138, 1981). We examined the action of salicylate on the isolated lateral line of Xenopus laevis to explore further the mechanism of action of salicylate at the hair cell-afferent nerve fiber junction.

The compounds were dissolved in a frog Ringer solution and then applied to the serosal surface of an isolated frog lateral-line stitch utilizing techniques previously described (Bledsoe and Bobbin, Neurosci. Lett., 32:315-320, 1982).

Sodium salicylate (0.3-2.5 mM) reversibly suppressed the spontaneous activity of the lateral line nerve and the increase in spontaneous activity evoked by kainate (10-20 μM) and glutamate (1-2 mM). Salicylate had little (10%) effect on the magnitude of the difference between spontaneous activity and the excitation induced by water motion. After washing out the salicylate the excitation to kainate, glutamate and water motion were greater than their responses before salicylate.

In previous studies it was found that glutamate and kainate induce excitation in the presence of low calcium/high magnesium, demonstrating an action postsynaptically on the nerve. Together these results indicate that salicylate acts on the nerve to suppress spontaneous activity and the responses to kainate and glutamate, but not water motion. Future experiments will test salicylate against NMDA, quisqualate and other agonists.

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72.7 POTASSIUM GRADIENTS IN SCALA TYMPANI OF THE GUINEA PIG IN THE ABSENCE OF SOUND STIMULATION. P. Dulguerov*, M. Zidanic*, W.E. Brownell and G.A. Spirou*. Dept. of Otolaryngology-HNS, Neurosciences and Biomedical Engineering, John Hopkins Medical School, Baltimore, MD 21205.

Potassium-sensitive electrodes and current density analysis techniques were used to assess potassium activity gradients in scala tympani of the guinea pig cochlea.

The presence of a high extracellular potassium concentration in endolymph (150mM) coupled with the positive endocochlear potential (+80mV) constitute chemical and electrical gradients that drive potassium out of scala media and into the surrounding perilymphatic and intracellular spaces. Ionic outflow is restricted by tight junctional complexes located at the apexes of all cells bordering scala media. Tracer flux experiments (Konishi & Salt, Exp. Brain Res. 40:457) suggest that the receptor cells of the organ of Corti are one of the major pathways of potassium efflux from scala media. This hypothesis would be supported by the existence of potassium gradients in scala tympani with the maximum potassium activity near the organ of Corti.

Double-barrelled potassium-sensitive microelectrodes of the liquid membrane type, using potassium-tetra-p-cholorophenylborate as an ion-exchanger, were made according to the method developed by Coles and Tsacopoulos (J. Physiol. 270:12-14P, 1977). The micropipettes were introduced through a 50-150µ fenestration in the bony otic capsule into scala tympani of the third turn of the guinea pig cochlea. DC potentials and changes in K+ activity were measured along a radial track parallel to the basilar membrane. The potassium activity was obtained by subtracting the DC potential recorded by the reference barrel from the signal of the potassium barrel. Potassium activity gradients were computed using the first derivative of the potassium activity profiles (Brownell et al., J. Acoust. Soc. AM. 74:792).

Preliminary results have confirmed the radial standing currents described in our laboratory (Zidanic et al. ARO abstract #114,1984) and have shown an increase in potassium activity in the perilymphatic spaces beneath the organ of Corti. A biological origin of these potassium gradients is indicated by their disappearance postmortem.

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72.8 POSTNATAL DEVELOPMENT OF THE CORTI-ORGAN IN GERBILS. V. Bruns*, S. Anton, Ch. Wied, B. Gottschalk and W. Plassmann. Zool. Dept. Univ. Frankfurt, D-6000 Frankfurt/M. (* Sponsor: G. Neuweiler)

In mammalian cochlear ontogeny, both the structure-function relation and the differentiation of the developmental pattern in baso-apical direction were unclear. This study attempts to shed some light on these aspects. Gerbils were chosen for two reasons: (1) hearing begins late after birth, which makes the animal suitable for postnatal analysis; (2) the cochlea is morphologically specialized, which provides ample opportunity to study the development of these specializations during ontogeny. The structure of the Corti-organ was studied in 7 postnatal stages during 4th-28th day after birth (DAB) as well as in adult animals, by analyzing semi-thin sections of 10 equidistant locations in the cochlea of each animal. The daily changes in the hearing thresholds were recorded by means of cochlear microphonics during 16th-25th DAB and compared with those of adult.

Hearing begins in gerbils on the 16th DAB and the auditory threshold curve at that age shows maximum sensitivity in the frequency range of 10 kHz and an upper frequency limit of 20 kHz. Development towards the adult stage reveals an expansion of the high-frequency range from 20 kHz to 40 kHz and an extraordinary sensitivity increase at about 2 kHz. Both length and width of the basilar membrane reach their final values as early as on the 12th DAB. After the 16th DAB, changes occur only in the fine structure of the Corti-organ. The filaments of the basilar membrane, for instance, are predominantly formed between the16th and 24th DAB, which strengthens the membrane`s stiffness and makes a shift towards higher frequencies likely.

As early as on the 4th DAB, the stereocilia of the outer hair cells have the same length throughout the cochlea. Starting with the 8th DAB, they grow in length towards the apex and reduce in length towards the basis. These factors can be assumed to shift local mechanical resonance on the basilar membrane. During their postnatal development, the special cochlear structures of adult gerbils- e.g. the conspicuous thickness of the basilar membrane and the mass of the tectorial membrane- grow disproportionately in the upper middle region of the cochlea. Since the final forms of these structures can be correlated with the 2 kHz frequency range on the basilar membrane, it is suggested that their functioning as resonators may explain the remarkable sensitivity of adult gerbils with respect to low frequencies.

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72.9 DEVELOPMENT OF HAIR CELLS: LOCATION-SPECIFIC PATTERNS OF CILIA NUMBER, CILIA LENGTH, AND BUNDLE ORIENTATION DIFFERENTIATE AFTER DENERVATION AND TRANSPLANTATION OF EMBRYONIC COCHLEAE. J. T. Corwin and D. A. Cotanche. Dept. of Zoology and Bekesy Lab., Univ. of Hawaii, Honolulu, HI 96822 and Dept. of Otolaryngology, Med. Univ. of South Carolina, Charleston, SC 29425.

Work in several laboratories, in particular work by Tilney and colleagues, has demonstrated that hair cells develop ultrastructural phenotypes that are specific for each hair cell location in the basilar papilla of the chick cochlea. Hair cells express these individual phenotypes in the form of sensory cilia bundles that differentiate by embryonic day 9-10, simultaneously with the first extensive formation of afferent synapses in this epithelium.

The dependence of hair cell differentiation on synatogenesis was tested by transplanting 43 surgically denervated otocysts from donor chick embryos onto the chorioallantoic membranes of host embryos. Transplants were then grown for twelve days before they were fixed. The basilar papillae from the experimental ears and from control ears transplanted with innervation intact were examined by scanning electron microscopy. Denervated cochleae that were transplanted on embryonic day 5 contained thousands of hair cells with cilia bundles that were indistinguishable from those in normal embryos at equivalent chronological stages. The transplanted papillae were much shorter and wider than those from normal embryos, but at each level in the base-to-apex axis of the cochlea the cilia bundle characteristics were appropriate for the hair cells at that relative position in a normal cochlea. The hair cell cilia bundles were uniformly oriented as in normal cochleae. They also developed the normal gradients of cilia length and number. Hair cells at the bases of the denervated cochleae possessed short cilia bundles with more than 200 stereocilia on each cell surface; those at the apexes possessed longer cilia bundles with as few as 40 cilia on each cell; and cells in intermediate positions had intermediate cilia lengths and numbers that graded between those extremes just as in normal cochleae.

The massive formation of synapses that occurs on embryonic days 9 & 10 is not necessary to trigger or to instruct hair cells that participate in the location-specific differentiation of cilia bundles that occurs on days 9 & 10. However, it is possible that the presence of the nerve before denervation at stage 25 (Embryonic Day 5) has influences that last until days 9 & 10. It is also possible that the factors that control the development of these location-specific features may relate to location themselves, this hypothesis is suggested because the ultrastructural gradients in transplanted basilar papillae develop normally for each relative location in the base-to-apex axis of the cochlea even though the dimensions of those papillae were shorter and wider than those of normal papillae. (Supported by grants from NINCDS and the Deafness Research Foundation to JTC and a NRSA Fellowship to DAC.)

72.10 MORPHOLOGY OF GERBIL COCHLEAE AND SOUND LOCALIZATION IN MERIONES UNGUICULATUS. W. Plassmann, M. Kausch, R. Kuhn, W. Peetz and B. Gottschalk. Zoological Institute, University of Frankfurt, 6000 Frankfurt, West Germany.

The auditory system of Gerbils is particularly sensitive in the range of medium and low frequencies. Comparative study of serial sections through the cochleae of Meriones unguiculatus, Meriones tristrami, Psammomys obesus, Pachyuromys duprasi and Tatera spec. reveals - among other things - that the basilar membranes of these Gerbils are by far longer, wider and thicker than those of rats, whereas the number of acoustic neurons in the nerve VIII is inferior to that of rats. Comparison of the above Gerbil species with each other also shows morphological differences in cochlea structure which are partly reflected by the different 1 uV CM-isopotential curves.

Localization of sound might be problematic in Gerbils because their small heads are of limited value for the measuring of intensity and time differences. Behavioral studies of sound localization showed, that Meriones unguiculatus is well able to localize sound sources both in low and high frequency ranges, exept for frequencies between 4 and 5 kHz. Experiments involving delivery of stimuli of different shapes indicate that the animals may evaluate phase differences in the frequency range below 4 kHz. CM-potential recordings under the condition of stimulus presentation from different directions yield the following two results: (1) A sound shadow exceeding 6 dB occurs only in the frequency range above 4 kHz; (2) phase differences smaller than 180 , which are necessary for correct evaluation of sound directions, can only be obtained in the frequency range below 3 kHz. The maximum interaural time differences calculated from the phase differences for individual frequencies reach values of 120-160 usec. The difficulty of sound localization in the range of 4-5 kHz may be explained by the following physiological findings: the phase differences above 3 kHz exceed 180 and therefore prevent directional hearing based on evaluation of phase differences; it is only above 4 kHz that the intensity difference due to sound shadow effects is large enough to permit directional hearing. Thus, the frequency range around 4 kHz appears to constitute the transitional zone between the two types of evaluations.

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72.11 HAIR-CELL INNERVATION BY SPIRAL GANGLION CELLS IN ADULT MICE. A.M. Berglund* and D.K. Ryugo. Dept. of Anatomy & Cellular Biology, Harvard Med. School, Boston, MA 02115, and Eaton-Peabody Lab, Mass. Eye & Ear Infirmary, Boston, MA 02114.

The mammalian cochlea contains two types of acoustic receptors: inner (IHCs) and outer (OHCs) hair cells. HRP methods have shown that in adult cats, type I spiral ganglion cells innervate IHCs, while type II ganglion cells innervate OHCs (Kiang, N.Y.S. et al., Science 217:175, 1982). In contrast, Golgi descriptions in neonatal rodents have suggested that a single ganglion cell can innervate both receptor types (e.g., Lorente de No, Laryngoscope 47:373, 1937). The present study sought to determine whether the innervation of both receptor types by a single neuron reflected maturational or species differences.

Individual spiral ganglion cells (n=34) in adult mice were labelled using HRP-DAB methods and reconstructed through serial sections from their cell bodies to their peripheral terminations. All ganglion cell somata are bipolar in shape and roughly similar in size. Type I neurons (n=21) emit peripheral processes that are very thin (~0.5 um) in the vicinity of their somata. Within 25 um of the cell body, each process abruptly thickens and maintains a relatively constant diameter (1-1.4 um) as it travels radially and unbranched through the osseous spiral lamina. After passing through a habenular opening, the fiber becomes thinner. Most (19) contact a single IHC via one terminal swelling. Two fibers branch: one forms two terminals against the same IHC, while the other sends a terminal to each of two adjacent IHCs. In contrast, type II neurons (n=13) emit peripheral processes that exhibit a maximum diameter in the vicinity of the cell body. These processes gradually become thinner as they project radially through the osseous spiral lamina, but reach a relatively constant diameter (~0.4 um) as they pass through the IHC region. They cross the tunnel of Corti at low levels before entering the OHC region; then they turn basally and spiral for up to 500 um before emitting short collaterals with bouton-like endings to 3-10 OHCs.

In conclusion, no ganglion cell innervates both types of acoustic receptors in the adult mouse. Previous observations of IHCs and OHCs innervated by the same neuron in neonatal rodents is presumed to represent a transient developmental characteristic. The segregated innervation of IHCs and OHCs by type I and type II ganglion cells has now been shown for several species, and may therefore reflect a generalized plan of afferent organization for the mammalian cochlea.

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SENSORY SYSTEMS: AUDITORY PATHWAYS II

73.1 **COMPARISONS OF SYNAPTIC INPUTS TO THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS FROM THE ANTEROVENTRAL AND DORSAL COCHLEAR NUCLEUS.** D. L. Oliver and C. Krevolin*, Dept. of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

Neurons in the central nucleus of the inferior colliculus (ICC) integrate acoustic ionformation from the cochlear nucleus, the superior olivary complex, and the lateral lemniscal nuclei. The response of a neuron in the ICC to sound depends, in part, upon which sources provide the most numerous inputs. Since the subdivisions of the ICC may receive different combinations of ascending inputs, it is important to determine the relative contribution of axonal endings from afferents which synapse in that subdivision. In the present study, we used EM autoradiography to visualize axonal endings in the ICC after small injections of 3H-leucine and/or 3H-proline in the anteroventral (AVCN) and dorsal (DCN) cochlear nucleus of the cat. Samples were from regions of the ICC in which heavily labeled bands of afferents were identified in adjacent LM autoradiography sections. The hypothetical grain procedure was used to verify significant labeling and identify radioactive sources.

Axonal endings in the ICC from both the AVCN and DCN contained round synaptic vesicles and are probably excitatory. After injections, labeled endings in both contralateral and ipsilateral pars lateralis of the ICC were densely packed with small-to-medium sized, round synaptic vesicles and made one or two asymmetric synaptic contacts on dendritic shafts. Less often, synapses with labeled endings occurred on smaller dendritic processes or on cell bodies and primary dendrites. Endings in these samples were labeled with 1.9-2.9 grains/um^2 and contained 29-40% of the radioactivity. Unmyelinated axons also were labeled routinely. Injections in the DCN produced comparable labeling of axonal endings in the contralateral pars medialis and centralis of the ICC. Synaptic vesicles in these endings averaged 47 nm or less.

Although axonal endings with round synaptic vesicles represent 60% of all endings in the ICC, they may originate from different sources in each subdivision. For example, pars lateralis receives bilateral projections from the AVCN but lacks projections from the DCN. The AVCN projections may account for a third of all round-vesicled endings with nearly equal numbers from both sides. These endings could contribute to bilateral excitation in time-sensitive units found in pars lateralis. In contrast, pars centralis and medialis receive direct projections from the contralateral AVCN and DCN which account for about 25% of the round-vesicled endings. These projections could contribute to contralateral excitation in the central and medial ICC.

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73.2 DISCRETE BILATERAL GABAERGIC NEURON POOLS AT THE COMMISSURES OF SUPERIOR AND INFERIOR COLLICULI IN THE RAT. D. E. Vetter and E. Mugnaini, Lab. of Neuromorphology, Univ. of Conn., Storrs, CT 06268

Immunocytochemical stains provide useful vistas of brain organization by highlighting special neuronal pools and pathways that lack distinctness in standard histological sections. Using a serum against glutamic acid decarboxylase (GAD), the synthetic enzyme for the inhibitory neurotransmitter GABA, we have observed discrete and sizeable assemblies of GABAergic neurons situated at the commissures of the superior (SC) and inferior (IC) colluculi in rat. Sprague-Dawley rats were perfused with a zinc-aldehyde fixative. Frozen serial sections of the brain stem cut in the three standard planes were immunoreacted with a double PAP procedure using the sheep antiserum to rat brain GAD obtained by Oertel et al., (1981). In the paramedian region where the ICs are joined at the midline two bilateral cell groups made up of small neurons appeared immunostained. One of these cell groups is situated rostrally and laterally in the commissure of the IC and occupies a triangular region bordered dorsally by the pia, and laterally by the external cortex of the IC. The other cell group is more extensive and appears prominent at all levels of the IC. The cells show orientation of their dendritic arbors, which are flattened in the sagittal plane where they are traversed by bundles of commissural fibers. Ventrally and caudally this group is contiguous, if not continuous, with the dorsal portion of the periaqueductal grey matter (PAG) which also contains numerous GABAergic small neurons. The paramedian region of the superior colliculus is also populated by numerous neurons. GAD-immunostaining of the superficial grey layer, including the stratum zonale is strikingly dense and this helps distinguish the laminated parts of the superior colliculus. A bilateral subcortical assembly of closely packed small GABAneurons is present at the medial edges of the intermediate and deep grey layers of SC. Sagittal sections suggest that the median GABAergic cell groups of SC and IC may form a continuous cell column. Numerous GABAergic terminals are present in the neuropil pertaining to these cell groups, but the source of this inhibitory input is not known.

A battery of traditional and immunocytochemical methods will be necessary to characterize further these discrete assemblies of GABAergic neurons and to determine whether they constitute definite nuclear subdivisions deserving separate names.

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73.3 COMMISSURAL CELLS OF THE EXTERNAL NUCLEUS OF THE RAT INFERIOR COLLICULUS: NEURONAL ARCHITECTURE AND CELLS OF ORIGIN. K.D. Games, M.C. Townsley*, and J.A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

The external nucleus (ICX) of the inferior colliculus (IC) has somatic sensory as well as auditory functions, receiving axons from dorsal column and pontine nuclei, and projecting to the superior colliculus and thalamus. We characterized ICX cells participating in the intercollicular commissural system of the inferior colliculus.

Neurons in the rat ICX were studied in Golgi preparations and in Nissl stained material. The ICX formed the lateral rind of the inferior colliculus and was bordered dorsally by the dorsal cortex and medially by the central nucleus. The ICX was distinguished from the dorsal cortex, whose cells often had smaller somata and dendritic fields, and from central nucleus cells, which had disc-shaped dendritic fields and formed fibrodendritic laminae. We subdivided the ICX into dorsal and ventral parts. In Nissl preparations, the ventral part had significantly larger somata (mean: 113 sq. μm, s.d.: 52) than the dorsal part (mean: 72 sq. μm, s.d.: 32; $p<0.01$). The dorsal part had more cells per unit area.

We made pressure injections of 15-30% horseradish peroxidase in the contralateral IC. TMB-labeled somatodendritic profiles in the ICX were correlated with the cells identified in Golgi preparations. Multipolar cells had three or more dendrites radiating from the cell body. Bipolar cells had two primary dendrites emerging from the somatic poles. Projection cells included small, medium-sized, and large bipolar cells and medium-sized and large multipolar cells. The small multipolar cells were not identified in our TMB-reacted material. Most commissural cells were medium-sized (72%; 81-201 sq. μm) or large (23%; >201 sq. μm). Small cells (<81 sq. μm) constituted only 5% of labeled cells. The mean somatic area of commissural cells was 148 sq. μm (s.d.: 68). We found no significant difference in somatic size or in the form of labeled cells between the dorsal and ventral parts of ICX; however, the dorsal part contained more labeled cells.

In summary, bipolar and multipolar cells are the principal commissural neurons of the ICX. Besides their commissural projections, many of these cells could project to the thalamus, auditory brain stem, and participate in the intrinsic organization of the IC. This suggests that, whatever the functional role of the external nucleus in the commissural system, it may differ from the commissural pathways of the dorsal cortex and central nucleus.

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73.4 EFFECTS OF GABA ON INFERIOR COLLICULUS NEURONAL RESPONSES TO ACOUSTIC STIMULI. C.L. Faingold, G. Gehlbach* and D.M. Caspary (SPON: H.R. Konrad). Dept. Pharmacology Southern Illinois University, School of Medicine, Springfield, IL 62708

The responses of inferior colliculus (IC) neurons to acoustic stimuli in many cases exhibit periods of suppressed firing. Recent evidence indicates that IC neurons exhibit processing of acoustic input beyond that seen in more caudal auditory nuclei. Intracellular studies have reported that inhibitory postsynaptic potentials are involved (Nelson and Erulkar, J. Neurophysiol. 26:908-923, 1963). Neurotransmitter mechanisms which may subserve inhibition in the IC are not well established. High levels of GABA as well as the enzymes which synthesize and metabolize GABA are present in IC, and GAD-immunoreactive terminals on IC neurons are observed which may arise, in part, from projections of the dorsal nucleus of the lateral lemniscus (Adams and Mugnaini, Brain Res. Bul. 13:585-590, 1984). Release of GABA from IC has been demonstrated in vitro. This study was initiated to examine the criteria of mimicry and pharmacological identity for establishing GABA as an inhibitory neurotransmitter in the IC. Sprague-Dawley rats were initially anesthetized with ketamine and subsequently paralyzed with gallamine treithiodide and respired. The animal's comfort was maintained using a semichronic adapter with local anesthetic infiltration. Response patterns at characteristic frequency were evaluated using poststimulus time histogram analysis. Iontophoretic application of GABA inhibited the firing of most IC neurons. The effect was rapid in onset, occurred in a large majority (over 90%) of neurons in the central nucleus of IC and showed a rapid offset. Of the other putative inhibitory neurotransmitters reported to be present in IC (Guth and Melamed, Ann. Rev. Pharmacol. Tox. 22; 383-412, 1982) glycine also produced inhibition with a rapid onset and offset but was less potent than GABA. Norepinephrine and 5-hydroxytryptamine were much less effective at producing inhibition and usually displayed a much longer time of onset. The benzodiazepine, flurazepam, which is postulated to act at the GABA receptor complex inhibited IC neuronal firing and enhanced the action of GABA. The inhibitory action of iontophoretically applied GABA was enhanced by simultaneous application of the GABA uptake inhibitor, nipecotic acid. Nipecotic acid also directly enhanced stimulus-induced inhibition in certain cases. The action of exogenously applied GABA was blocked by the GABA receptor antagonist, bicuculline. Binaural ihibition could also be blocked by the application of bicuculline in doses which did not affect the monaural response. These data taken with the neurochemical and release data support a role for GABA as an important inhibitory neurotransmitter in the inferior colliculus.

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73.5 RESPONSES OF INFERIOR COLLICULUS NEURONS TO HIGH- AND LOW- FREQUENCY NOISE BANDS IN C57BL/6 MICE WITH AGE-RELATED HEARING LOSS. J.F. Willott and K.P. Hunter*. Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115.

During the first year of life, C57BL/6 mice undergo progressive peripheral hearing loss, predominantly for high frequencies. Extracellular single-unit responses were obtained from the central nucleus of the inferior colliculus (ICC) of anesthetized mice of 3 age groups: 1-mo.-old (optimal sensitivity), 7-mo.-old (moderate hearing loss), and 12-mo.-old (severe hearing loss). Recordings were obtained at approximate 200μm dorsoventral intervals, sampling the low- to high-frequency tonotopic axis. Two noise bands, 12kHz low pass (LP) and 12kHz high pass (HP) were used as stimuli (duration 200ms, 2.5Hz rate). Post-stimulus time histograms were obtained at 10 dB intensity steps from a maximum of 80 dB SPL to below unit threshold.

Response properties of ICC neurons changed across age, but the changes depended on the stimulus employed (HP vs. LP) and the dorsoventral depth of the neurons.

(1) In 1-mo.-olds, the shortest latencies were evoked by HP stimuli. Latencies tended to be shorter ventrally than dorsally. With aging, there was little change in the latencies of responses to LP stimuli, but latencies to HP stimuli tended to increase. The increase in HP latencies was more pronounced in the ventral ICC.

(2) The incidence of neurons with nonmonotonic intensity functions (presumably an indication of inhibition) decreased with aging. In 1-mo.-olds, there were more neurons with nonmonotonic intensity functions dorsally than ventrally, so the age-related decrease in nonmonotonic functions was most pronounced in the dorsal ICC. The stimulus also influenced the effects of aging on nonmonotonicity: In 1-mo.-olds, more nonmonotonic functions were associated with HP stimuli than with LP stimuli; in 7-mo.-olds, HP and LP stimuli were about equally capable of producing nonmonotonic functions; by 12 months, more nonmonotonic intensity functions were produced by LP stimuli than by HP stimuli.

(3) In 1-mo.-olds, the incidence of spontaneously active neurons was greatest in the ventral ICC. With aging, the incidence of spontaneously active neurons increased in the dorsal ICC, but not in the ventral ICC.

In summary, the effects of aging and/or hearing loss on the responses of ICC neurons are different in high frequency regions (ventral) and low frequency regions (dorsal) and depend on whether high or low frequency stimuli are used. A full understanding of the effects of peripheral hearing loss on central neuronal responses must take both factors into account.

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73.6 BINAURAL RESPONSES OF SINGLE NEURONS IN THE INFERIOR COLLICULUS OF THE UNANESTHETIZED RABBIT. T.R. Stanford*, R. Batra and S.Kuwada (SPON:G.Maxwell). Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

Most electrophysiological studies of the auditory system have used an anesthetized preparation. Since anesthetics have a powerful effect on synaptic transmission, we felt it essential to examine the responses of auditory neurons in an unanesthetized animal. Of the few studies that used an unanesthetized preparation, most employed free-field stimuli. Free-field stimuli are useful for determining functional features of neuronal responses, but, they do not provide the stimulus control necessary to study neuronal mechanisms, especially those involved in binaural interactions. We chose the rabbit because of its willingness to remain still with minimal restraint. To achieve stimulus control we used earmolds which allowed acoustic stimuli to be presented to each ear independently. We report preliminary results on the responses of single neurons in the inferior colliculus (IC) of the unanesthetized rabbit.

Under general anesthesia the skull was exposed and a steel rod cemented to the bone. A hole was drilled for electrode entry and capped with Silastic. Prior to recording, the rabbit was placed in a stocking and its head immobilized by clamping the steel rod. The Silastic was removed, dura swabbed with lidocaine, and an electrode placed using approximate stereotaxic coordinates.

Although studies have found changes in spontaneous rate and tuning characteristics, little is known of the effects of anesthesia on binaural interactions. We examined the responses of binaural neurons to changes in interaural phase, interaural time and interaural intensity differences. Some low frequency neurons showed interaural phase sensitivity. This was demonstrated by a discharge rate that varied cyclically as a function of interaural delay with a period corresponding to the stimulating frequency and also by a cyclic response to sinusoidal changes in interaural phase created by delivering slightly different frequencies to each ear. Some high frequency neurons were sensitive to changes in interaural intensity. These cells were contralaterally excited and ipsilaterally inhibited, and their discharge was dependent upon the relative intensities of the stimuli at each ear.

Our preliminary findings indicate that IC neurons in this preparation show a sensitivity to interaural phase and intensity similar to that seen in anesthetized mammals. However, our sample is too small to draw definitive conclusions. The unanesthetized rabbit avoids potential confounding effects of anesthesia. It also permits repeated sampling of neuronal responses within a nucleus as well as comparison of the responses of neurons at different stages of sensory processing within the same animal.

This work was supported by NIH grant NS18027 to S. Kuwada.

73.7 RESPONSE LATENCIES IN THE INFERIOR COLLICULUS OF THE CAT: ANOTHER EVIDENCE FOR AN AUDITORY PERIODICITY MECHANISMS. G. Langner and C. Schreiner*.
Zoological Institut, Technical University, 61 Darmstadt, FRG and Coleman Lab., University of California, San Francisco, CA 94143

Neurons in the auditory midbrain nucleus MLD of the Guinea fowl are involved in periodicity analysis (Langner, G.,Exp.Brain Res. 44:450, 1981, and 52: 333, 1983). Using sinusoidal amplitude modulations as simple models of complex periodic signals, many units in the MLD were found to be tuned to certain best modulation frequencies (BMF < 1000Hz), related to neuronal oscillations triggered by the AM cycles, and influenced to a certain degree by the carriers (in the range of phase coupling below 5kHz). These observations resulted in a temporal model for periodicity processing suitable also for the explanation of certain pitch effects in human subjects. The model is supported by additional results obtained in the IC of the cat (Schreiner, C. and Langner G., Soc. Neurosci. Abstr. Vol. 10, Part 1, p. 395, 1984). Further evidence for central periodicity analysis in vertebrates now comes from the observation of AM tuning of units in the midbrain of frogs (Rose, G. and Capranica, R.R. Science 219: 1087, 1983).

According to the periodicity model AM tuning of units in the midbrain may be explained by coincidence mechanisms involved in a cross correlation analysis between envelope and fine structure of periodic signals, resp. in an auto correlation of the envelope for carrier frequencies above 5kHz. As demonstrated for the central nucleus of the IC of the cat such AM tuned units are arranged on each isofrequency lamina in a concentric BMF map.

Additional evidence for temporal processing of periodicity comes from the measurement of response latencies together with CF and BMF in 637 recordings. Latencies were measured using tone bursts with frequencies at CF, 2ms rise time, and 60dB above the thresholds of the units. Temporal mechanisms are connected with delays and were consequently expected to have some influence on the neuronal latencies. In the present investigation latencies varied typically between 4 and 25ms. When BMF as a neuronal parameter was neglected the averaged latency appeared to depend strongly on CF. This is explained by the fact, that BMF averaged over all units with the same CF increased with CF. On the other hand using the periods $\tau_{CF} = 1 / CF$ and $\tau_{BMF} = 1 / BMF$ as variables a multiple lineal regression analysis resulted in a significant approximation ($r = 0.46$, $p = 0.05$, $n = 637$):

Latency $= (7.1+0.9)ms + (1.2+0.2) * \tau_{CF} + (0.16+0.03) * \tau_{BMF}$.

The contribution of BMF was much greater than that of CF, because on the average τ_{BMF} was 60 times greater than τ_{CF}. The contribution of CF may be a good approximation of the delay introduced by the cochlear mechanics. (Supported by the Heisenberg program of the DFG, NIH Grant NS-10414, the Coleman Fund and HRI.)

73.8 EFFECTS OF FRICATIVE AMPLITUDE ON CAT MEDIAL GENICULATE BODY (MGB) NEURON RESPONSES TO PLOSIVES IN FRICATIVE-PLOSIVE SEQUENCES. J.V.Urbas* and H.Spenner* (SPON: K.Albus). Dept. Neurobiology, Max-Planck-Inst. for Biophysical Chemistry, 3400 Göttingen, FRG.

Perception of stop consonants (plosives) depends on a preceding silent interval corresponding to articulatory closure (Dorman et al., J.Acoust.Soc.Am. 65:1518, 1979). If the silent interval between sibilant (fricative) and following plosive is removed, the plosive is no longer heard (e.g. spin is heard as sin). This has been interpreted as evidence for a phonetic stage in speech perception correlated with articulatory processes (Dorman et al., 1979). We previously reported that cat medial geniculate body (MGB) neuron responses to plosives in fricative-plosive (FP) sequences depend on the silent interval length (SIL) in a way similar to plosive perception by humans (Spenner and Urbas, J. Physiol. 328:39P, 1982).

The present report is based on 34 MGB neurons recorded from sodium pentobarbital anesthetized cats. All cells were located in either the laminated lateral ventral part or its dorsal cap. The signals were naturally spoken, digitally stored German words with FP sequences. The stored words were modified to have various SILs following the sibilant and also various sibilant sound pressure levels (SPLs). Thus, we copied two important parameter variations used in the paradigm of psychoacoustical forward masking.

The results show that for changes in preceding SIL and sibilant SPL, the neuron responses to plosives: 1) correspond well with results measured using simple tonal signals; 2) show good analogy to psychoacoustical results using the same word stimuli; 3) can be interpreted as evidence for involvement of subcortical auditory mechanisms in speech perception phenomena.

We conclude that special phonetic processes in speech perception are not necessary to account for plosive suppression by a preceding sibilant. Instead, evidence for the role of subcortical auditory mechanisms, analogous to those responsible for the perceptual phenomenon of forward masking, has been strengthened by our data.

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73.9 TIME DOMAIN ANALYSIS OF COMPLEX SOUNDS IN THE THALAMUS OF THE NORTHERN LEOPARD FROG (Rana pipiens pipiens). J. C. Hall* and A. S. Feng. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The mating calls of anurans show species differences in both the frequency and time domain. Females utilize these differences in their selection of conspecific males as mates. The neural correlates of this behavior apparently reside within the auditory pathway and the neural analysis of spectral and temporal acoustic cues provided by the mating calls.

The neural analysis of spectral information has been examined in detail throughout the auditory pathway. A recent study of R. p. pipiens (Fuzessery and Feng, J.Comp.Physiol., 150:333, 1983) implicated the posterior nucleus of the thalamus as an important center for call recognition in the frequency domain. On the other hand, the neural analysis of temporal information has been examined only up to the level of the auditory midbrain (Wilczynski and Capranica, Prog. in Neurobiol. 22:1, 1984). In this study, we have utilized single unit recordings and horseradish peroxidase (HRP) transport techniques to examine the physiological and anatomical substrates of temporal processing in the anuran thalamus.

The results of our study show neurons in the thalamus which respond selectively to two temporal parameters of complex sounds: the pulse repetition rate and the pulse duration. The latter is of particular interest as short duration pulses (< 10msec), approximating those of the mating call, may elicit a sustained discharge of 1-2sec from some neurons. This phenomenon represents a divergence from the classical concepts of duration coding in the central nervous system and indicates the need for their reevaluation. In addition,it also suggests that pulse duration, like pulse repetition rate, may be a critical feature for recognition. Histological verification of the recording site indicated that all of the neurons were located within the central nucleus. This is in contrast with findings (see above) demonstrating the presence of spectrally selective neurons in the posterior nucleus. Thus, temporal and spectral information may be processed in parallel by different subsets of neurons within the thalamus. This hypothesis is supported by anatomical data showing that the two nuclei have distinct afferent and efferent innervation patterns. (Supported by NSF grant BNS 82-04160 to A.S.F.).

73.10 NEONATAL DEAFENING ALTERS THE BRANCHING STRUCTURE OF NONPYRAMIDAL NEURONS IN RABBIT AUDITORY CORTEX. N.T. McMullen, B. Goldberger*, C. Suter* and E.M. Glaser. Dept. of Physiology, Univ. of Maryland School of Medicine and Dept. of Surgery, Univ. of Maryland Hospital, Baltimore, MD. 21201

The large spine-free nonpyramidal neuron is the major interneuron in lamina III/IV of the primary auditory cortex of the rabbit (McMullen, et. al., 1984). Computer microscope analyses of these cells have revealed a prominent vertical orientation of dendrites along the pia-white matter axis. In the present study, we investigated the effects of neonatal deafening on the branching pattern of these neurons in the auditory cortex. NZW rabbits (n=11), anesthetized with ether, were unilaterally deafened at postnatal day 1 (birth = day 0) by surgical removal of the incus and stapes ossicles and Kanamycin injection (330mg/ml) into the oval window. Litter mate control pups (N=5) received a sham-operation. All pups were allowed to mature until 60 days of age at which time auditory brain stem response tests (ABER) were conducted. All experimental animals exhibited the complete absence of an ABER on the side which received the neonatal surgery. Following ABER tests, the brains of both experimental and control rabbits were removed following exsanguination and processed according to Golgi Cox procedures. Alternate thick (300-400 um) and thin (75-100 um) sections through the auditory cortex contralateral to the deafened ear were processed and Nissl counterstained. Lamina III/IV spine free non-pyramidal neurons in the auditory cortex of the deafened and control rabbits were digitized at a magnification of 800x using oil immersion optics with our Image Combining Computer Microscope. Results from the deafened rabbits were compared to data from litter mate controls and normal adult rabbits. Early deafening affected the dendritic system of auditory cortical spine-free NP cells in two major ways : (1) their dendrites systems are larger and (2) there are a greater number of dendritic branches oriented parallel to the pial surface. Total dendritic length/cell in deaf animals ($\bar{X}$=4653 um) is elevated 42-50% in comparison to litter mate controls (3275 um) and normal adult rabbits (3087 um). A "fan-in" (Glaser & McMullen, 1984) analysis revealed a significant increase in the number of dendrites oriented parallel to the pial surface resulting in a 70% increase in dendritic length oriented within 30 degrees of the tangential (parallel to the pia) plane. Thus, early cochlear damage results in reorganization of the auditory pathway which extends to the cerebral cortex. (Supported by a grant from the Deafness Research Foundation and NIH Grant NS17861)

73.11 PATTERNS OF INHIBITION IN AUDITORY CORTICAL CELLS OF AWAKE SQUIRREL MONKEYS. D.Symmes and S.Shamma*.Lab.of Comparative Ethology, NICHD, Bethesda, MD 20205

We have studied inhibition in primary auditory cortical cells of awake squirrel monkeys, employing the method of single cell recording. Single tone bursts, simultaneous double tone bursts, and broad-band noise stimuli were presented under program control in a design which sampled low rate cells adequately and minimized the effect of short term response fluctuations. Inhibition was inferred from reductions in rate of extracellular spikes below background level, below excitatory response level, or both. Type A units(29%) exhibited low background activity, sharply tuned non-monotonic excitatory responses to tones, and weak responses to noise. Profound lateral or two-tone inhibition was demonstrable, which could appear both above and below BF, or asymmetrically. Type B units (26%) had high background rates and inhibitory responses to single tones. Two-tone testing was not attempted, but noise evoked excitatory activity was observed. We classified cells which had excitatory responses to single tones but no demonstrable lateral inhibition (i.e., summated excitatory responses to double tones up to saturation) as Type C (18%). A final class in this scheme includes cells with phasic single tone responses which produced temporal inhibition to any subsequent stimulus (Type D, 22%).

We propose that in the general sense inhibition in cortical cells has received insufficient attention experimentally, and may offer if understood some insights into the complex behavior of proposed feature detecting cells. We propose specifically in light of these data a network model which would generate similar response patterns.

73.12 THREE-TONE SEQUENCE SELECTIVITY IN CAT'S PRIMARY AUDITORY CORTEX (AI) IS SHOWN BY CROSSCORRELATION AMONG ITS SPIKE TRAINS. I.E.Espinosa* and G.L.Gerstein, (SPON: M.J.Bloom) University of Pennsylvania, Dept. of Physiology/G4, Philadelphia, PA 19104.

We report continued cross-correlation analysis of our separable multi-neuron recording data from cat's primary auditory cortex (AI) in response to three-tone sequences (1,2).

Crosscorrelation of spike trains from neuron pairs showed the usual signatures of direct and/or shared input. These appeared individually or in combination and for most pairs were present in spontaneous conditions. However, in stimulated conditions these spontaneous interactions were strongly enhanced. The analysis detected differences in neuronal interaction during presentation of different tones. Similar differences in neuronal interaction occurred during the presentation of any single particular stimulus if there was a history of different immediately previous tones. Controlled modifications in the auditory stimulation modified the crosscorrelations. When data from individual neuron pairs were put together to form a functional connectivity diagram among all recorded neurons, they turned out as different diagrams for different stimulus conditions. If the crosscorrelations are compared according to the class of stimuli, they show a statistically significant selectivity for some specific sequence of three-tones which is independent of the corresponding increase in the neurons' firing rate, that is, due only to neural interactions and not to stimulus coordination (3).

Variations in the connectivity diagram due to detection of previous tone-burst history and to three-tone sequence selectivity may represent different mechanisms. The former seems to depend on the interplay of excitatory-inhibitory influences due to poststimulus effects and could be responsible for a short-memory system. The latter, possibly using that system, differentiates sequences according to the correlated firing they evoke. Of 35 neuron pairs analyzed in detail, all presented the short-memory effect by increasing or decreasing their correlated firing rates and 16 pairs showed three-tone sequence selectivity. These mechanisms do not seem to depend on the geometry of the bundle microlectrode, nor on the type of interaction, nor on the recorded neurons localization in AI.

(1) Espinosa, I. and G.L.Gerstein, Soc. Neurosci. Abstr. 10, part 1:245,1984.
(2) Gerstein, G.L., et al.,IEEE Trans.SMC-13:668,1983.
(3) Dickson, J. and G.L.Gerstein, J.Neurophysiol. 37:1239,1974.

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73.13 COMMISSURAL TERMINAL FIELDS IN CAT PRIMARY AUDITORY CORTEX (AI): LAMINAR DISTRIBUTION AND AFFERENT-EFFERENT RECIPROCITY. R.A. Code and J.A. Winer. Department of Physiology-Anatomy, University of California, Berkeley, California 94720.

We studied the commissural cells of origin and terminal fields in cat primary auditory cortex (AI) with combined injections of horseradish peroxidase (HRP) and tritiated proline in the middle ectosylvian gyrus. Unilateral pressure injections of 0.3-2.0 µl of HRP (20-30%) and 30-200 µCi of tritiated proline (50-100 µCi/µl) were made with a Hamilton syringe. After a 24-72 hour survival, the contralateral AI and ipsilateral medial geniculate body were frozen sectioned in the frontal plane in two adjacent series: 60 µm thick for tetramethylbenzidine (TMB) histochemistry and 30 µm thick for autoradiography. The pattern of anterograde-retrograde labeling in the ipsilateral medial geniculate body was a control for the locus of injection; only experiments in which the ventral nucleus was the primary target are included here. The densest concentration of silver grains was in the contralateral layer III, followed by layers V, VI, and I. The labeling in layer I was heaviest in its deeper portion, layer Ib. Why layer Ib should receive such a heavy commissural innervation is curious, since it has no commissural cells of origin (Code, R.A., Winer, J.A., Proc. Soc. Neurosci., 1983, 9:953; Code, R.A., Winer, J.A., J. Comp. Neurol., 1985, submitted) and thus represents a striking exception to the principle of afferent-efferent reciprocity. Layer IV had the least labeling, followed by layer II. The labeling was always greatest over the neuropil and lightest over neuronal perikarya in all layers. Commissural terminal fields formed radial patches oriented perpendicularly to the pia, averaging about 500 µm wide (range: 330-1280 µm). The number of silver grains in a patch was consistently three times greater than that in an inter-patch area. However, the number of silver grains in an inter-patch area was always significantly above background, suggesting a commissural projection to those areas as well. Complete reciprocity between the commissural cells of origin and terminal fields was not found, although the correspondence was usually high. Often, patches of terminal fields were free of retrogradely labeled cells, and there were patches of labeled cells without an overlying commissural terminal field. The commissural terminal fields form bands oriented in a caudoventral-to-rostrodorsal direction across AI, similar to the orientation of EE (excitatory-excitatory) and EI (excitatory-inhibitory) binaural bands (Middlebrooks, J.C., Dykes, R.W., Merzenich, M.M., Brain Res., 1980, 181:31-48). Commissural terminal fields connect homotopic regions of the contralateral AI, and no region of AI lacks commissural innervation. These data suggest that commissural afferents might connect corresponding tonotopic and homotopic binaural portions of AI. This research was supported by USPHS grant R01 NS16832.

73.14 MINIMUM AUDIBLE ANGLES FOR MIDLINE AND LATERAL FIELD SOUND LOCALIZATION IN THE ALBINO RAT: IMPLICATIONS FOR STUDIES OF CORTICAL FUNCTION. G. L. Kavanagh* and J. B. Kelly. Dept. of Psychology, Carleton Univ., Ottawa, Canada K1S 5B6.

The ability to localize sounds in space has been shown in a number of mammalian species to depend upon the auditory cortex. Monkeys, cats, dogs, and ferrets, all suffer severe impairments in sound localization following bilateral auditory cortical ablations. Also, more recently it has been shown that the effects of cortical lesions are especially evident in the lateral fields of auditory space. The effects of unilateral lesions, not seen in tests of midline localization, are readily manifested in tests of sound localization within the contralateral field. Even with relatively small lesions restricted to the primary auditory cortex, impairments are found primarily in the quadrant of space opposite the side of the lesion. Also, in the ferret small bilateral lesions result in impairments in both left and right lateral fields, but have only limited effects on left vs. right localization around midline.

Studies of the effects of auditory cortical lesions in the rat have failed to show severe impairments in midline sound localization. Tests of lateral field localization were introduced, with the intention of revealing deficits which might have escaped our notice in previous studies. Animals were trained in a semicircular apparatus to localize clicks presented from loudspeakers around the perimeter of the apparatus. Three tests were given: a) left hemifield, with speakers at -90° and -30°, b) midline, with speakers at -30° and +30°, and c) right hemifield, with speakers at +30° and +90° azimuth. The animals had no difficulty localizing a single click in the midline test and were subsequently shown to have small minimum audible angles. Performance in both the left and right lateral fields, however, was very low and did not exceed the level expected by chance in most cases. Additional studies have shown that lateral field performance is also poor with white noise bursts provided that the stimulus duration is brief. These results preclude further study of the effects of cortical ablation on lateral field localization. We suggest that the albino rat is normally lacking an ability which is dependent on the integrity of the auditory cortex in a number of other mammals.

This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

73.15 EFFECT OF AUDITORY CORTEX LESIONS ON ABSOLUTE THRESHOLDS IN MACAQUES. H. E. Heffner, R. S. Heffner, and W. E. Porter*. Lab. of Comparative Hearing, Bureau of Child Research, Univ. of Kansas, Parsons, KS 67357.

Hearing thresholds of five Japanese macaques (Macaca fuscata) were assessed following two-stage bilateral lesions of auditory cortex including most or all of the superior temporal gyrus. The thresholds of two additional macaques were assessed before and after unilateral ablation of auditory cortex. Thresholds were determined for tones ranging from 63 Hz to 32 kHz using a conditioned avoidance procedure.

Bilateral ablation initially resulted in a complete inability to respond to sound with the first signs of hearing coming 2 to 11 weeks after surgery. Hearing sensitivity improved gradually with maximal recovery reached 24 to 35 weeks after surgery. Recovery was most pronounced for low frequencies which generally returned to normal or near-normal levels. However, the monkeys appear to have suffered a permanent hearing loss of up to 50 dB in their mid-frequency range around 8 kHz.

Unilateral ablation resulted in a hearing loss in the contralateral ear while thresholds in the ipsilateral ear remained normal. Hearing losses varied with frequency and initially ranged up to 66 dB with the largest losses generally occurring from 8 kHz to 25 kHz. The animals showed gradual recovery, particularly at the lower frequencies, but still had losses of 46 dB or more in the midfrequency range 6 weeks after surgery.

Though the possibility of a cortical hearing loss in monkeys was raised some time ago (Ferrier, Philos. Trans. Roy. Soc., 165:433, 1876), it is widely believed that such a deficit does not exist. The failure of others to note a hearing loss is probably due to the partial recovery of hearing, especially at low frequencies, as well as to the fact that few studies have ever determined thresholds. However, a review of the literature reveals reports of "postoperative amnesia" and "loss of learned habit" following bilateral auditory cortex lesions which may well have been due to initial deafness followed by partial recovery.

73.16 PITCH PERCEPTION IN NON-HUMAN PRIMATES R.W.W Tomlinson*, D.W.F Schwarz*, Otoneurology Lab, Acute Care Unit FF-153, University of British Columbia, Vancouver, B.C. Canada, V6T 2B5.

The psychoacoustics of the pitch of pure and complex tones has been well studied in humans. It is known that the pitch of a harmonic series is identical with that of the fundamental (lowest component), even though the fundamental may not be physically present. Some workers believe that the pitch of the missing fundamental may be perceived only by humans as an epiphenomenon of speech processing. The purpose of this study was to examine if non-human primates can also perceive the pitch of the lacking fundamental. Monkeys were trained to push a button when two tones presented in sequence had the same fundamental frequency. The intensities were randomly varied over 25 dB to eliminate frequency dependent intensity cues. The complex waveform was constructed digitally at a rate of 32 kHz, then converted to analogue, allowing complete control of frequency and intensity of each component. The monkey received sound from an overhead speaker in a sound attenuating room. The pitch of an unknown tone was found by changing the fundamental of a second complete harmonic series until a match was made by the monkey. Rich, 5-component harmonic complexes were used to match 5-component complex tones missing one, two or three of the lowest harmonics. Fundamentals of 200 to 600 Hz were used for the unknown tones. Under these conditions the monkeys consistently made matches to the pitch of the missing fundamental, though the error rate increased as the timbres became more different. It was concluded that non-human primates can perceive pitch in the synthetic mode (Terhardt, J. Acoustical. Soc. Am 71:679-688, 1982).

(Supported by the Pacific Otolaryngology Foundation and MRC, Canada.)

73.17 AFFERENT INPUT TO AUDITORY ASSOCIATION CORTEX OF THE CAT. C. Shipley (SPON: M. Chase). Mental Retardation Research Center, UCLA School of Medicine. Los Angeles, CA 90024.

Auditory responses can be recorded in a number of polysensory association areas of the cortex in awake or chloralose anesthetized cats. Several of these areas respond similarly after auditory stimulation and may share a common thalamic input. In the present study, afferents to three polysensory cortical areas in which auditory responses are present, peri-cruciate cortex, anterior lateral gyrus, and suprasylvian gyrus, were studied through the use of retrograde axonal transport of cortically injected markers. Double-labelling techniques were used in order to visualize the topographic relationship of thalamic fields projecting to different cortical areas and to determine if individual neurons projected to more than one of these areas.

Animals were anesthetized with alpha-chloralose and click evoked potential maps were made in two of the three cortical association areas. On the basis of these maps, an injection of wheatgerm lectin-bound horseradish peroxidase (HRP) was placed in a site responsive to auditory stimulation in one of the areas and nuclear yellow (NY) was placed in a responsive site in the second. In some cats evoked potential waveforms and latencies were nearly identical at the two injection sites while in others they differed greatly. Cats were terminated with pentobarbital 22-48 hours after the injections, perfused transcardially with paraformaldehyde and processed with tetramethylbenzidine for HRP.

Thalamic labelling was present in a number of nuclei including ventralis anterior (VA), ventralis lateralis (VL), centralis lateralis (CL), centromedian, the ventrobasal complex, lateralis dorsalis, and the lateralis posterior-pulvinar complex (LP-PUL). Dense labelling of both HRP and NY in adjacent or overlapping fields was found in all animals and these fields generally ran for a few millimeters rostro-caudally, crossing one or more borders of the traditionally defined thalamic nuclei. Depending on the injection sites, dense labelling of both HRP and NY was seen in VA, VL, CL, and LP-PUL. These results suggest that a major portion of the input to the polysensory areas studied originates in topographically related fields of the thalamus that may comprise a functional column extending across boundaries of several thalamic nuclei. Double-labeled cells were present but they represented far less than one percent of the labeled neurons.

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73.18 HUMAN AUDITORY EVOKED POTENTIAL INDICES OF TEMPORAL PROCESSING. E. O'Malley[1]*, C. Ollo[2]*, N.K. Squires[1]*, and A. Cutler[1]* (SPON: T. Baker[2]). Dept. of Psychology[1], SUNY Stony Brook, Dept. of Psychiatry[2], Sch. Med., Stony Brook, NY 11794.

Rapid temporal processing of auditory stimuli was evaluated using ERPs in a passive task in which a 600 msec noise burst was interrupted by a brief "gap", or silent interval. In the first experiment, binaural trains of noise bursts were presented to seven normal hearing subjects (18-22 yrs); the subjects ignored the stimuli and read a book during the experiment. Gap duration was varied across runs using gaps of 10 msec, 5 msec, 3.5 msec, 2.5 msec, or 1 msec duration. ERPs were recorded from Fz and Cz and referenced to the neck (band pass .1 to 100 Hz) and averaged on-line. The latency of the N100 component of the auditory evoked response was measured to stimulus onset and remained stable across gap size. Also, a well-defined component was identified that indexed the occurrence of the gap. The latency of this gap component increased with decreasing gap size ($p < .009$).

In experiment II, ear advantage was examined using monaural and binaural noise bursts with a gap interval of 10 msec. The latency of the onset N100 and the latency of the gap component were significantly faster in the binaural than in either monaural condition ($p < .009$ and $p < .014$, respectively). This finding cannot be attributed to an intensity increase due to binaural stimulation as N100 latency is relatively invariant for intensity increases of 10-20 dB at presentation levels above 60 dB SPL. Post-hoc analysis of the onset N100 latency revealed that left ear stimulation produced significantly faster responses than with right ear presentation. No significant ear effect was noted for the gap component.

Experiment III was conducted to clarify these findings. In this experiment only monaural presentations were used to determine if the previous relationship between gap component latency and gap size were due to the binaural stimulation. The results verified the findings of Exp. I showing that the latency of the onset N100 is constant while the gap component varies inversely with gap size ($p < .0001$). Analysis of ear of presentation data indicated that the gap component latency for left ear presentations was faster than for right ear stimulation($p < .03$). These data taken together suggest that the gap component may have a different physiological basis than the onset N100 and as such is a unique electrophysiological measure of rapid temporal processing of auditory stimuli. Behavioral correlates of this phenomena are being studied.

74.1 INCREASED NEURONAL SURVIVAL AND REGENERATION FOLLOWING POLYAMINE TREATMENT. M. Dornay*, V.H. Gilad* and G.M. Gilad. The Center for Neurosciences and Behavioural Research and The Isotope Department, The Weizmann Institute of Science, 76100 Rehovot, Israel.

After injury of their axon damaged neurons shift their metabolic activity into a reparative mode aimed at survival and recovery by axonal regeneration or, alternatively they undergo degenerative changes and die. We have recently demonstrated that at the initial stages of the response to axonal injury polyamines (PA) are essential for neuronal survival. When increased demand for PA arises, not only does PA biosynthesis increase, but also the capacity to take up extracellular PA is greatly enhanced. In the present study, therefore, we sought to establish if treatment of rats with PA can enhance survival and regeneration of neurons. Regeneration of crushed pre- or postganglionic sympathetic nerves of the superior cervical ganglion (SCG) was accelerated by daily intraperitoneal injections of spermine (10 mg/kg) or of a combination of putrescine, spermidine and spermine (10 mg/kg each) for 7d starting at the time of operation. This was adjudged by accelerated reappearance of choline acetyltransferase activity in the SCG, and return of [^{3}H]norepinephrine uptake in the pineal and iris, respectively. The functional recovery from ptosis of the eyelid was also accelerated after PA treatment. In newborn rats, injection of PA (10 mg/kg) subcutaneously from 2d to 9d after birth, resulted, at 35d of age, in a 40 to 50% increase in both nerve cell number and tyrosine hydroxylase activity in the SCG. PA had no effect on the diameters of neuronal cell bodies (22.6±0.3 μm) or nuclei (12.5±0.3 μm). The number of satellite glia cells surrounding each neuron (5.3±0.6) were also unaffected. We conclude: a) treatment with exogenous PA accelerates regeneration of crushed sympathetic nerves, and b) treatment of newborn rats with PA leads to an increased number of neurons in the adult SCG. The data imply that the beneficial effect of PA may be through enhancement of neuron survival during development and after axon injury. - Supported by grants from the Muscular Dystrophy Association and the American Paralysis Association.

74.2 VITAMIN A TREATMENT ALTERS PATTERN FORMATION IN REGENERATING EMBRYONIC RETINA OF XENOPUS LAEVIS. C.H. Holder* and C.F. Ide (SPON: F.E. Dudek) Dept. of Biology, Tulane Univ., New Orleans, LA 70118.

Vitamin A treatment has been shown to alter pattern formation during regeneration in vertebrate limbs. It causes clumping of cells near the wound edge as well as the production of serial and mirror pattern duplication of limb structures during subsequent regeneration. To test if vitamin A affects pattern formation during embryonic retinal regeneration, we removed 1/3 of the embryonic eyebud of stage 32 embryos. The resulting 2/3 size fragments not treated with vitamin A showed low incidence of external clumping of cells in the region of the ablation outside of the main fragment body (8/182 embryos, 4.3%). In preliminary studies they also formed relatively normal retinal ganglion cell projections to the midbrain optic tectum (assayed electrophysiologically). Fragments treated with retinal palmitate (1.5 IU/ml) for 24 hours after surgery and assayed at 24, 48, and 72 hours after surgery showed an increased incidence of clumping of cells in the region of the ablation (68/200, 25.3%); treated fragments also formed some retino-tectal projections with duplicated visual field points.

In both control and retinoid treatment fragments, clumped cells found in the region of the ablation were apparently derived from cell movements into the vacant area. In control embryos, these external cells organized into retinal layers and fused with the main fragment within 48 hours post-surgery. In retinoid treated embryos, the external cells remained rounded and undifferentiated as late as 72 hours post-surgery. Thus, vitamin A treatment may interfere with normal cell-cell recognition by altering cellular adhesion and/or other properties involved in the determination of normal retinal geometry and the formation of an orderly single projection to the midbrain optic tectum. Supported by NSF Grant PCM-8316142.

74.3 GANGLIOSIDE ADMINISTRATION REDUCES BEHAVIORIAL DEFICITS AFTER BILATERAL ENTORHINAL CORTEX LESIONS IN RATS. J. J. Ramirez*, S. Karpiak, T. Kilfoil*, B. Henschel*, & W. Grones* (Spon: G-Y. Wen). Dept. of Psychology, College of St. Benedict/St. John's University, Collegeville, Minn., and Div. Neurosience, NYSPI, Dept. of Psychiatry, Columbia U. Medical Center, NY, NY.

Administration of gangliosides facilitates recovery of learned spatial alternation behavior after unilateral entorhinal cortex (EC) lesions in rats [1]. This enhacement of recovery had been postulated to result from facilitated lesion-induced sprouting of the crossed-temporodentate projection to the denate gyrus. However the behavioral recovery occured well before lesion-induced sprouting takes place. Further studies showed that lesion-induced sprouting in the dentate gyrus was decreased after unilateral EC lesions in rats treated with gangliosides [2]. We have now examined the effects of ganglioside treatment in a paradigm where recovery is believed to occur independent of sprouting. We studied the behavioral recovery of rats subjected to bilateral EC lesions. This recovery is reported not to be associated with hippocampal afferent sprouting [3]. Sprague-Dawley male rats were randomly assigned to one of three experimental conditions and were trained in a Y maze to criterion. Two groups were subjected to bilateral EC lesions and the remaining group was sham-operated. One of the EC damaged groups and the sham controls were injected with total ganglioside peparation (i.m. 50mg/kg). The remaining group was injected with saline. Injections began one day preoperatively and were continued for five consecutive days, and every other day thereafter. The rats were tested to a maximum of 32 days post-EC lesions. Preliminary results indicate that the two lesioned groups were indistinguishable with respect to the number of errors committed or the number of days required to attain criterion. However the lesioned rats injected with ganglioside committed significantly fewer perseverative responses than saline controls (N=15, $p<0.01$). Although gangliosides did not reduce the lesion-induced memory deficit as indicated by the total number of errors committed, the ganglisoide treatments did reduce the disruption of response inhibition as measured by perseverative errors. The impairment of response inhibition is frequently associated with hippocampal afferent formation damage. It has been reported that ganglioside treatment reduces CNS edema, therefore limiting the extent of cell damage [4]. Therefore, the decrease in perseverative responses after ganglioside treatment may reflect a "protection" of neuronal plasma membranes (e.g. decreased dendritic degeneration).

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74.4 ENHANCEMENT OF HINDLIMB REFLEXES IN ADULT RAT WITH TRANSPLANTS OF EMBRYONIC CNS TISSUE. J. T. Buchanan*, H. O. Nornes (Sponsor J. E. Rash). Department of Anatomy, Colorado State Univ., Fort Collins, CO 80523

Transplants of embryonic CNS tissue into the adult brain of rodents may restore functional deficits due to chemical lesions, congenital abnormalities or aging. The purpose of this study was to determine if transplants of embryonic CNS neurons have an effect on spinal cord function in the adult spinal cord of rat.

Cell suspensions of catecholamine (CA) neurons were injected into the lumbar level (Sprague-Dawley, 180-200 gm. females). The anlagen of locus coeruleus was dissected from 8.5-11.0 mm (crown-rump) rat embryos and the tissue pieces were mechanically dissociated in glucose-saline solution (0.9% NaCl, 0.6% glucose). Five μls of suspension were injected into 3 different sites from L_3-S_1 in rats whose descending catecholamine fibers had been eliminated with intracisternal injections of 6-hydroxydopamine. Up to 1000 CA containing cells were found two and four months post-implantation. Reinnervation of CA-containing fibers was observable in animals with more than 100 CA-containing neurons.

The force of the hind leg reflex (which is enhanced by catecholamines) was used as a functional assay to determine whether the embryonic CA-containing neurons formed functional connections. After spinal transections, animals with suspensions had significantly stronger reflexes (about 150 percent) than those in the control animals. The reflexes were significantly reduced after i.p. injections of the alpha-adrenergic blocker, phenoxybenzamine, indicating that some of the enhancement was due to the release of CA from the transplanted CA neurons. Further, animals with 300 or more CA containing neurons had a mean reflex force of 25 percent stronger than those with less than 300 CA containing neurons.

Suspensions of CA neurons survived, formed a normal appearing fiber plexus in the grey matter of the cord, and enhanced hind leg reflexes. This study raises the possibility of restoring deficits in the spinal cord with intraspinal injections of cell suspensions of embryonic neurons.
(Supported by Spinal Cord Society and NIH grant 1F32NS07314-01).

74.5 REINNERVATION OF FOREIGN TISSUE TRANSPLANTS IN BABOONS UNDER CYCLOSPORIN A IMMUNOSUPPRESSION. D.D. Samulack, R.W. Dykes, R.K. Daniel*, E.P. Egerszegi* and S.E. Skanes*. Microsurgical Research Laboratories, McGill University, Montreal, Canada. H3A 1A1

Immunosuppression by Cyclosporin A (CyA; Sandoz Ltd) interferes with the production of antibodies against foreign tissues and has provided unique opportunities to study allogeneic (intra-species) tissue transplantation. In this study, neurovascular free flaps were allografted between unrelated individual baboons to investigate the degree to which allogeneically-transplanted tissues would re-innervate in a primate species (Papio anubis), in the presence of CyA immunosuppression.

Two surgical models were used. The first, an index finger model, involved the isolation and transplantation of the soft tissue coverage of the second digit, complete with its neurovascular pedicle. The second model involved the transplantation of a whole hand, at a level 4 cm proximal to the wrist. Transplantation success was achieved in five index finger free flaps and two hand transplants, with viable longterm survival ranging from 5 to 10 months, at which time their state of reinnervation was assessed.

Electrophysiological recordings from a sample of more than 300 single afferent fibers in median and radial nerves showed that sensory axons had reinnervated the transplanted skin. Well-defined, low threshold receptive fields were observed. Both slowly (SA) and rapidly adapting (RA) responses could be identified. In those cases which had undergone a period of rejection, the thresholds tended to be elevated with an excess of RA fibers observed. In contrast, in a flap that had not undergone any rejection, the ratio of SA to RA was reversed to favor the SA responses. The most striking abnormality was a reduced conduction velocity for those axons ensheathed by foreign Schwann cells. Reinnervation of donor muscle was also demonstrated through motor unit recruitment in step-wise fashion, following electrical stimulation of the recipient's median and ulnar nerves. Afferent fibers serving the donor's joints and muscle spindles were also observed.

Comparisons of the results from transplants that underwent a serious rejection episode with those experiencing only minor, or no episodes of rejection, suggest that (except for conduction velocities) the quality of neural function may be related to this variable, rather than to some intrinsic inability of the recipient axons to reinnervate foreign skin receptors.

(Supported by the Medical Research Council of Canada)

74.6 PRESYNAPTIC ELEMENTS ON IMPLANTED POLYLYSINE COATED BEADS. Richard W. Burry and Diane M. Hayes*, Department of Anatomy and the Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210

Previous studies with cell cultures of neonatal rat cerebellum have shown that neurites in contact with a polylysine coated bead can form a presynaptic element with the bead in the position of a postsynaptic element. Results reported here show that neonatal rats with implanted polylysine coated beads can also form presynaptic elements on beads.

Presynaptic elements on coated beads were identified in the electron microscope as neuronal swellings that contain both clusters of small diameter vesicles at the site of contact with the bead and a slight thickening of the plasma membrane at the site of contact (Burry, Brain Res., 1980, 184:85-98; 1982, 247:1-16). In addition, the small diameter vesicles in the swelling have been shown to stain with antibodies to synaptic vesicle antigens Synapsin I and SV48 (Burry, Neurosci. Abst., 1984, 10:578). The number of presynaptic elements on beads reaches a maximum at 7 days incubation and then shows a dramatic decline. Results from experiments in culture with antimitotic drugs that stop glial cell proliferation, have shown that the resulting decrease in number of glial cells correlates with an increase in the number of presynaptic elements on beads (Burry, Neurosci. Abst., 1983, 9:690). These studies have indicated that formation and loss of presynaptic elements on polylysine coated beads may serve as a model for regeneration and formation of synapses after lesion.

Experiments reported here show that axons of neonatal rat cerebellar neurons can also grow and form presynaptic elements on polylysine coated implanted beads. The number of beads with presynaptic elements was highest at 3 and 7 days after implantation and dropped to almost zero at 14 and 21 days after implantation. Measurements of the size of the presynaptic element and the number of vesicles at 3 and 7 days after implantation showed an increase, while these parameters decreased at 14 and 21 days after implantation. Additional data showed axons that formed presynaptic elements grew into the implant to a distance of several hundred micrometers.

These results show that presynaptic elements can form on implanted beads in neonatal cerebellum, and that the presynaptic elements show a decline in number after 7 days similar to that seen in culture. It is possible that the loss of presynaptic elements after 7 days may be related to the glial cells.

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74.7 IMPLANTATION OF DISSOCIATED FETAL SPINAL CORD CELLS INTO ADULT RAT SPINAL CORD. U. Vaidya and M.R. Wells., Neurochemistry Research Laboratory, V.A. Hospital, Washington, D.C. 20422.

Pieces of whole fetal rat spinal cord can be introduced into the injured or uninjured spinal cord of adult hosts using a variety of implantation techniques. The use of tissue of appropriate age can result in the growth and development of the implant within the host (Patel, U. and Wells, M.R., Soc. Neurosci. Abstr. 10:1023, 1984). The injection of dissociated fetal spinal cord as cells offers a relatively atraumatic and accurate means of introducing fetal tissues into host animals. Timed pregnant female rats were injected with 5 µCi./g. body weight of [^{3}H]thymidine 24 hours prior to use. E13 fetal spinal cords were collected from embryos, dissected free of meninges and adhering tissue and dissociated by trituration in culture media (3 spinal cords/ml media). Viability of dissociated cells and small aggregates were assessed by trypan blue exclusion. The dissociated spinal tissue (1 µl) was injected into the uninjured spinal cord of anesthesized adult hosts with a 10 µl syringe. At time periods of 1-6 weeks, anesthesized host animals were perfused with 4% parformaldehyde, the spinal cords embedded in paraffin, sectioned at 6 u, and processed for autoradiography with NTB-3 emulsion. After a 4 week exposure, slides were developed and stained. Over all time periods examined, cells with [^{3}H]thymidine labeled nuclei could be identified in the host. Many appeared as differentiated neuronal cells. Most labeled cells were located near the site of injection, but some cells were found on the surface of the host spinal cord in aggregates. The data demonstrate that dissociated fetal spinal cord can survive and develop after injection into adult host spinal cord similar to whole tissue implants. The close contact of dissociated cells with the host tissue after injection appears to be an advantage to the viability of the implant. Survival and development of similar implants in injured host spinal cord is presently under investigation.

Supported by the Veterans Administration.

74.8 SCIATIC NERVE AUTOGRAFTS (SNAs) INTO THE RAT HYPOTHALAMUS: NEUROPHYSIN IMMUNOHISTOCHEMISTRY AND FINE STRUCTURE. H.-D. Dellmann*, L.-F. Lue*, S. I. Bellin* and A. J. Cosimini* (SPON: C. Jacobson). Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

As part of an ongoing investigation of the capability of peripheral and central nervous tissues to induce regeneration of transected neurosecretory axons in the hypothalamus, several small pieces (0.25 mm^2) of intact or degenerated (14-40 days) sciatic nerve were autografted stereotactically into the region situated between the supraoptic and paraventricular nuclei. Animals were sacrificed 5, 10 and 20 days post operationem. Alternate 50 µm vibratome-cut horizontal sections were processed for neurophysin immunohistochemistry and conventional electron microscopy, respectively.

There is an increased degree of innervation of SNAs by neurophysin-positive axons with time: A few short axons penetrate the graft periphery at 5 days, more and longer axons with numerous varicosities are present at 10 days and abundant axons permeate the graft at 20 days. There are differential patterns and magnitudes of neurophysin-positive axon innervation between intact and degenerated SNAs. Areas of intact SNAs that contain numerous macrophages have no or only sparse innervation while degenerated SNAs and areas of intact SNAs without macrophages contain a dense plexus of beaded axons. In degenerated SNAs, the innervation appears to be more orderly with axons often coursing in parallel. Axons are more numerous in degenerated than in intact SNAs. Furthermore, within and in the vicinity of SNAs, blood vessels (arterioles, venules) are surrounded by a light plexus of neurophysin-positive axons at 10 days and by a heavy one at 20 days. These findings are confirmed at the fine structural level. SNAs are composed of varying proportions of fibroblasts, collagen fibers, neurolemmocytes, macrophages and axons; typical peptidergic neurosecretory axons as well as non-neurosecretory axons are partially or totally invested by neurolemmocyte processes. Blood vessels are surrounded by axon profiles located within the perivascular connective tissue space. Many of these axons are in direct contact with the basal lamina and have characteristics of typical neurosecretory axon terminals, i.e., neurosecretory granulated vesicles, vacuoles and microvesicles clustered frequently at the membrane facing the basal lamina.

SNAs obviously promote neurosecretory (and other) axonal regeneration or sprouting, not only into the graft but also into adjacent hypothalamic perivascular connective tissue spaces, an interesting phenomenon which is currently under further investigation. Supported by NSF grant BNS-84-04183 and by Basic Research Grant No. 1-900 from the March of Dimes Birth Defects Foundation.

74.9 DELAYED TRANSPLANTATION OF EMBRYONIC SPINAL CORD INTO THE INJURED SPINAL CORD OF THE ADULT RAT. J. D. Houle and P. J. Reier. Depts. of Neurological Surgery and Neuroscience, Univ.of Florida College of Medicine, Gainesville, FL. 32610.

We and others have recently demonstrated that embryonic CNS tissues can be successfully grafted into acute lesions of the neonatal and adult rat spinal cord. While some anatomical continuity could be restored in most cases, the degree of host and donor tissue approximation was nonetheless variable due to the presence of small cysts and focal infiltrations of connective tissue. In the present study, we investigated whether better fusion of host and donor tissue could be obtained by using a delayed grafting approach. This strategy was suggested by previous studies showing that delayed intraspinal grafts of peripheral nerve tissue yielded better host-graft appositions than when implants were made immediately after injury.

Subtotal lesions of the lumbar spinal cord were made in adult rats by aspirating tissue to form a 2-3 mm long cavity. E14 spinal cord (FSC) tissue was subsequently implanted against vascular-rich surfaces either immediately after the lesion or 6-14 days post-lesion. Animals were perfused 30 days after transplantation, and tissue was prepared for either conventional light microscopy, electron microscopy or immunocytochemical visualization of astrocytes using antibody to GFAP (Glial Fibrillary Acidic Protein).

The survival (i.e., ~80%) and differentiation of FSC tissue were equivalent under both acute and delayed conditions; however, delayed grafts exhibited a better apposition with the host spinal cord such that fewer cystic cavities and mesenchymal infiltrations were present at host-graft interfaces. Comparable GFAP-like immunoreactivity was also observed in both conditions within the transplant proper as well as at the interfaces between the adjacent tissues. On the other hand, an extensive astroglial reaction was observed in lesioned spinal cords which did not receive grafts. These observations demonstrate that embryonic CNS tissue can be grafted into the injured spinal cord following a post-injury delay of at least two weeks. Under this condition, extensive apposition of host and donor tissue can be achieved, and the delayed grafts can fuse with the host spinal cord without formation of any appreciable glial interfaces. Therefore, the delayed grafting approach may offer some practical advantages to embryonic neural tissue transplantation and spinal cord repair. Studies are in progress to determine whether similar grafting results can be obtained in chronic spinal cord lesions (≥1 month) and to determine whether axonal interactions between donor and host can be achieved under these conditions. (Supported by NIH grant NS-22316)

74.10 TRANSPLANTS OF SEPTAL EXPLANT CULTURES PROVIDE A CHOLINERGIC REINNERVATION TO ADULT RAT HIPPOCAMPUS. Elizabeth B. Ezerman and Lawrence F. Kromer. Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

In vitro explant preparations provide a convenient method for exposing developing CNS tissue to various trophic, neurotoxic, or labeling agents. Thus, this procedure was combined with the tissue transplantation technique in order to determine whether culturing embryonic septal-basal forebrain cells prior to transplantation altered their ability to provide a cholinergic reinnervation to denervated host hippocampi. For these studies E15-16 septal-basal forebrain tissue was maintained in explant culture for 1 to 4 days prior to transplantation into the hippocampus of adult rats with fornix-fimbria transections. The morphology of the transplants, survival of cholinergic neurons, and reinnervation of the host hippocampus by cholinergic fibers from the explant-transplant after one and three weeks survival in cerebro were assessed with AChE histochemistry, ChAT immunocytochemistry, and cresyl violet (CV) staining. Comparable explants were studied at 1, 4, and 8 days postexplantation.

At 1 day in vitro, the explants contain circumferential germinal zones containing small mitotic cells. By 4 days some cell bodies and short processes demonstrated light staining for AChE comparable to that observed at an equivalent in vivo stage (E19-20). Eight day explants also show AChE reactive cell bodies and processes which extend for considerable distances within the explant. In addition, the explants containa variety of AChE-negative neurons of various sizes. After 1 week in cerebro, the 1 day explant-transplants exhibit robust growth, contain clusters of AChE+ cells and fibers,and possess a similar degree of intrinsic organization to the 8 day explants. In addition, a dense plexus of AChE+ fibers is present at the transplant-host hippocampal interface, with some axonal growth into the host hippocampal formation. Septal explants in culture for 4 days prior to transplantation also have AChE+ cell bodies and some fibers penetrating the host hippocampus at a 1 week survival. By three weeks posttransplantation specimens of both 1 and 4 day explant-transplants contain ChAT+ neurons and possess an extensive intrinsic plexus of AChE+ fibers. In cases with a good host-transplant interface, there also is an extensive cholinergic reinnervation of the host hippocampus. This study demonstrates that short-term explants of embryonic septal-basal forebrain tissue survive transplantation and provide cholinergic reinnervation to denervated adult hippocampi. The combined explant-transplant experimental approach thus is a potentially valuable method to assess factors important for cholinergic cell survival and growth. (Supported by PHS Grants #NS-18126 and NS-07289.)

74.11 **EFFECTS OF NEURAL TUBE IMPLANTS OBTAINED FROM DIFFERENT STAGES UPON THE RESPONSES OF THE HOST NERVOUS SYSTEM.** **B.E. Sisken, J. Fowler and E. Bell.** Wenner Gren Res.Lab. and Dept. of Anatomy, Univ. of Kentucky, Lexington, KY 40506.

The right wing buds of stage 24 chick embryos were amputated at the future elbow region. Experimental embryos had a segment of stage 15, 24, 28 or 34 neural tube implanted longitudinally into the limb stump to induce regeneration (Fowler and Sisken, JEZ, 221, 1982). Stumps of control limbs were implanted with stage 30 heart, or were not implanted. To analyze effects of the neural implant upon the relations of the peripheral load to the host neural components, quantitative determinations were made of the peripheral load, lateral motor column of the spinal cord (LMC) and dorsal root ganglia (DRG) of one brachial spinal cord segment.

The peripheral load was estimated by determining the area of the skeletal elements of the amputated and unamputated limb of each embryo. The LMC was determined by counting the number of neurons on both operated and unoperated sides of a single spinal cord segment, the DRG by making area measurements of the ganglia in the same spinal cord segment.

The peripheral load was significantly greater in amputated limbs that received a neural tube implant, indicating a stimulation of growth even in limbs in which epimorphic regeneration did not occur. The area of the dorsal root ganglia showed a direct relationship to the size of the peripheral load indicating that cell death of sensory neurons was correlated with reduction of sensory targets by limb amputation. The number of neurons in the LMC was significantly greater in embryos with a neural implant than was expected based upon the peripheral load. This suggests that the neural implant may produce a neuronal survival factor that protects motor neurons from amputation-induced cell death but, affords no protection for sensory neurons. Supported by NIH NS197898-02.

74.12 REJECTION OF RETRANSPLANTED NERVE ALLOGRAFTS. A. A. Zalewski, A. K. Gulati*, Y. Kadota*, and N. A. Azzam. Lab. of Neurobiology, NINCDS, NIH, Bethesda, MD 20205 and Dept. of Anat., Georgetown Univ. Sch. of Med., Washington, D. C. 20007.

Nerve grafts, 5 cm long, were taken from adult male rats and transplanted into the thighs of immunologically deficient nude rats. After postoperative periods of one to five months, the ACI nerves were retransplanted into allogeneic Fischer (FR) rats. The purpose of this study was to determine whether the ACI allogeneic cells responsible for initiating nerve allograft rejection would die or become altered in an intermediate host rendering these ACI grafts non-antigenic. As a control procedure, some ACI nerves from nude rats were regrafted into isogeneic ACI hosts. All ACI nerves survived after transplantation into nude rats. The one month grafts were in the process of Wallerian degeneration whereas the five month grafts contained regenerated nude rat axons that were remyelinated throughout the entire graft length by ACI Schwann cells. In addition, all one and five month ACI grafts had a patent vasculature, a perineurium and no detectable cellular immune reaction. After retransplantation into FR hosts, all the nude rat passed ACI nerves were rejected. At five months all that remained of the ACI grafts was a short 5 mm nerve segment that contained a few regenerated and remyelinated axons. On the other hand, retransplanted ACI nerves survived in ACI hosts. These grafts retained their length, and they bore many remyelinated nerve fibers. They also contained a patent vasculature, a perineurium, and were free of any immune reaction. In another study, a similar rejection occurred in FR rats that received ACI nerves that were first passed through FR rats treated with the immunosuppressive agent cyclosporin-A. These results indicate that the antigenic cell type(s) responsible for nerve allograft rejection are long-lived and not readily eliminated by physiological cell turnover of one to five months. Accordingly, it is now important to identify the antigenic cell in a nerve allograft, alter or eliminate it, and determine what effect this has on the capacity of the nerve graft to support host axonal regeneration through it.

74.13 VIABILITY AND MORPHOLOGY OF TRANSPLANTED SUPERIOR CERVICAL GANGLIA IN PERIPHERAL NERVE OF ADULT RAT. D. W. Hoovler. Dept. of Physiol., George Washington Univ. Sch. of Med., Washington, D.C. 20037 and the Veterans Administration Med. Ctr., Washington, D.C.

Spinal cord injury causes the loss of functional connection and trophic influence to peripherally innervated structures below the site of injury leading to paralysis and atrophy of muscle tissue. Successful transplantation of viable central or ganglionic neurons into peripheral nerve and the subsequent afferent and efferent connection of the transplanted neurons with viable host tissue has the potential of maintaining the integrity of, or possibly restoring function to, functionally denervated tissues. The first step of this procedure involves the identification of tissues capable of surviving transplantation, being innervated by host fibers and elaborating axons that have the ability to innervate target tissue. Mature rat superior cervical ganglion (SCG) was chosen as the transplant tissue because it survived in other transplant studies, it is a relatively simple neural tissue that has the ability to change its neurotransmitter substance in vitro, it has the potential of being innervated by regenerating cholinergic peripheral axons or other types of fibers and its fiber outgrowth can be evaluated by fluorescence microscopy of catecholamines. SCG were removed from anesthetized adult male Sprague-Dawley rats and placed in ice cold Tyrode Ringer solution, desheathed and divided into 0.5 mm cubes. These were inserted into the nerve to the biceps femoris muscle of 60 adult male Sprague-Dawley rats through a small slit in the epineurium of the nerve at a site where the nerve had been crushed. In half the animals, the sciatic nerve proximal to the branching of the biceps femoris nerve was transected, creating 2 types of transplants; those in degenerating nerve and those in regenerating nerve. At 14, 30, 60, 90 or 120 days postoperative (DPO) host rats were anesthetized and the transplant site exposed and stimulated with a bipolar electrode. Tissue was processed for light and electron microscopy and for glyoxylic acid induced fluorescence of catecholamines. The allografts of mature rat SCG survived up to 120 days following transplantation. The transplants contained the constituents of normal mature SCG including postganglionic sympathetic neurons, Schwann cells, satellite cells, small intensely fluorescent (SIF) cells, blood vessels and catecholaminergic containing fibers. Morphological changes were present in the transplanted SCG neurons and signs of cellular necrosis were more prevalent in specimens by 90 or 120 DPO. There were indications of catecholaminergic fiber outgrowth from the transplants, however, no muscle response could be elicited by electrical stimulation of the implanted nerve where the transplanted mature SCG neurons were the only source of nerve fibers. (Supported by the American Paralysis Association).

74.14 ELONGATION OF AXONS FROM ADULT RAT MOTOR CORTEX INTO PNS GRAFTS. J.C. Horvat and A.J. Aguayo. Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec, H3G 1A4 Canada. In 24 Sprague-Dawley female rats (200-250 gms) a 1.5-2 cm of autologous sciatic nerve (PNS) was excised from the leg and used to test the regenerative response of motor cortex neurons injured near their somata. One end of the PNS segment was implanted 0.5 to 2 mm deep into the cortex, in the region known to contain the cells of origin of the corticospinal tract (Sanderson K.J. et al., Brain Res, 292:251-260, 1984) while the free outer end of the graft was sutured to the temporalis muscle. From 41 to 71 days later the peripheral tip of the PNS graft was transected and a 20% solution of HRP was applied to its cut end. Rats were perfused with fixative 48 hrs later and their brains processed by light microscopic histochemistry. In 17 animals we found a total of 393 HRP labeled neurons . The laminar distribution of the majority of these cells could be determined with considerable accuracy, as follows: layer II=3, layer III=13, layer IV=72, layer V= 251, layer VI= 17; 37 other cells could not be as accurately localized. The shape and size of many of the neurons in layer V indicate that the largest pyramidal cells (Betz) also extended their axons into the grafts. Thus, although PNS grafts inserted into the mid-thoracic spinal cord of rats have not been proven to be invaded by axons of the corticospinal tract (Richardson et al, Brain Res. 237:147-162, 1982), the cells of origin of this tract nevertheless regenerate processes into PNS grafts inserted near their somata. These findings and those reported for other descending and ascending spinal axons (Richardson et al, J.Neurocytol. 13:165-182, 1984), as well as for retinal (So and Aguayo, Brain Res. 328:349-354, 1985) and olfactory (Friedman and Aguayo, J.Neurosci. in press, 1985) projections indicate that the regenerative response of these central neurons is strongly influenced by the proximity of the lesion and graft to the cell body.

74.15 AXONAL REGENERATION FROM THE RAT RETINA AFTER TOTAL REPLACEMENT OF THE OPTIC NERVE BY A PNS GRAFT. M. Vidal-Sanz*, M. Villegas-Pérez*, P. Cochard* and A.J. Aguayo (SPON: M. Rasminsky). Neurosciences Unit, The Montreal General Hospital and McGill University. Montreal. Quebec. Canada H3G 1A4.

Retinal ganglion cells regenerate cut axons along peripheral nerve (PN) grafts inserted directly into discrete portions of the retina (So & Aguayo, Brain Res. 328: 349,1985). Here we describe in adult rats an extensive axonal regeneration from retinal ganglion cells after complete substitution of the optic nerve (ON) by a peripheral nerve graft.

In female Sprague-Dawley rats, weighing between 200 and 250 gms, a 3.5 cm segment of sciatic nerve was dissected and removed from the leg. By a superior temporal approach the left ON was exposed within the orbit and transected at its origin from the retina. The proximal stump of the autologous PN graft was attached to the optic disk and secured to the sclera with 10-0 sutures while the rest of the peripheral nerve segment was either laid subcutaneously over the skull or connected to the superior colliculus. Axonal regeneration from the retina into the PN grafts was examined from 6-8 weeks after ON nerve substitution by the application of HRP (40%) to the distal end of the PN segment. 48 hours later, the animals were perfused through the heart with saline, followed by a 4% solution of paraformaldehyde. Grafted eyes were dissected, placed in phosphate buffer and the retinae prepared as flat wholemounts, rinsed in phosphate buffer and reacted according to a modified Hanker-Yates method. Some of the retinae were also examined by fluorescence microscopy after incubation with antibodies to neurofilaments.

Grafted eyes did not show obvious intraocular residual damage. It was documented that ganglion cells of different sizes, located throughout the entire retina, had regenerated axons along the length of the PN grafts for distances equivalent to those of the normal retino-tectal projection.

74.16 RESPONSES TO VISUAL STIMULI OF RAT RETINAL GANGLION CELLS REGENERATING AXONS INTO PERIPHERAL NERVE GRAFTS. S.A. Keirstead, M. Vidal-Sanz*, M. Rasminsky, A.J. Aguayo, M. Levesque* and K.-F. So. Neurosciences Unit, Montreal General Hospital, Montreal, Quebec, Canada H3G 1A4.

As is the case for neurons in several parts of the adult mammalian central nervous system, axotomized retinal ganglion cells regenerate axons into peripheral nerve (PN) grafts inserted into the retina (So & Aguayo, Brain Res. 328:349,1985). We have now recorded electrical activity in single axons teased from such grafts 1 to 2 cm from the retina 9 to 48 weeks after the insertion of one tip of a 3-4 cm long autologous PN segment into the superior temporal retina, close to the optic disc. The other end of the graft was tied off and left unconnected under the scalp overlying the occiput.

In 47 of the 626 units recorded in 14 rats, the discharge frequency was influenced by changes in illumination. 28 cells were "on" units, responding to the onset of light with increased firing; 12 cells were "off" units that responded to the onset of the light stimulus with decreased firing and to the cessation of the light stimulus with increased activity; 7 cells were "on-off" units with enhanced firing both at the onset and cessation of light stimulation. 22 units had circular receptive fields <1 to 8° in diameter. These fields were invariably located in the inferior visual field consistent with the placement of the PN grafts in the superior portion of the retina. An antagonistic surround was found in 6 units. These responses by ganglion cells with regenerated axons are similar to those of normal rat retinal ganglion cells (Brown & Rojas, J. Neurophysiol. 28:1073,1965).

The number of light-responsive units appeared to decline with time after implantation of the blind-ended grafts. We recorded 32 responsive units in 3 rats with 9-11 week grafts, 13 responsive units in 5 rats with 25-28 week grafts and only 2 responsive units in 6 rats with 44-48 week grafts. The many units that did not respond to light could represent either retinal ganglion cells with altered membrane properties and/or altered afferent connectivity, or non-retinal neurons which extended axons into the grafts.

These experiments indicate that axotomized retinal ganglion cells regenerating axons into PN grafts can retain or regain a complement of synaptic input adequate to permit normal responses to light stimuli but that this responsiveness does not persist indefinitely. It is possible that the conditions in the PN grafts which promote the regeneration of retinal ganglion cell axons cannot assure the long term functional viability of cells lacking synaptic connections with appropriate targets.

74.17 HOW DOES PERIPHERAL NERVE INJURY TRIGGER ENHANCED CENTRAL REGENERATION OF PRIMARY SENSORY NEURONS? P.M.Richardson, and V.M.K.Verge*. Division of Neurosurgery, McGill University and Montreal General Hospital, Montreal, Canada H3G1A4.

In earlier studies, injury of the peripheral processes of bipolar sensory neurons was shown to enhance the regeneration of corresponding axons in the dorsal columns (Nature 309:791,1984). Axons ascending from the fourth and fifth dorsal root ganglia (L4 & L5 DRG) are one hundredfold more likely to regenerate into a peripheral nerve graft to the dorsal spinal cord if the sciatic nerve is cut rather than uninjured. This preparation has now been used to study the nature of the signal from injured peripheral axons that triggers an enhanced propensity to regenerate in sensory neurons.

Crushing of the sciatic nerve or single intraneural injection of colchicine (5ul, 25mM) each caused severe Wallerian degeneration yet was only one tenth as effective as nerve section in evoking central regeneration. However, four injections of colchicine to block retrograde axonal transport for two weeks mimicked more closely (42%) the inductive effects of nerve transection. A working hypothesis compatible with these observations is that sensory neurons are activated to regenerate when their cell bodies are deprived of a neurotrophic molecule normally supplied by peripheral glial cells along distal nerve segments.

Preliminary results of experiments with continuous infusion of nerve growth factor (NGF) to the proximal stump of the cut sciatic nerve indicate that NGF can mitigate this inductive phenomenon and thus may be implicated in the regeneration of sensory neurons.

74.18 REGENERATION OF MEDIAL FOREBRAIN BUNDLE (MFB) AXONS INTO RAT PERIPHERAL NERVE GRAFTS. R.P. Dum and C.G. Salame*, Dept. Neurosurgery, SUNY at Upstate Med. Ctr., Syracuse, New York 13210.

Many different types of CNS neurons regrow axons into implanted nerve grafts (Benfry et al, Nature 296: 150, 1982), but the neuronal population is usually heterogeneous and located quite close to the implant site. In this study, we attempted to induce a more specific population of neurons to grow into a peripheral nerve graft located remotely from the cell bodies.

Under aseptic conditions, autologous sciatic nerve grafts (25mm long) were implanted vertically through the cortex to intercept the MFB in anesthetized female rats (200gm). The distal end of the graft was implanted in muscle. Up to 1 year later, the cut end of the graft was dipped for 1 hour in a fluorescent dye, granular blue (GB) or Evans blue (EB). After perfusion with 10% formalin, the brains of GB rats were cut frozen at 30um. EB rats were pretreated with Nialamide (400mg/kg). Their brains were removed whole and frozen. Cryostat sections cut at 8um were reacted by the glyoxylic acid method to produce catecholamine (CA) histofluorescence.

In each case, the nerve graft interrupted the MFB at the posterior hypothalamus. The dense accumulation of CA histofluorescence on the experimental versus the control side was consistent with the interruption and blockage of CA fibers within the MFB.

In those rats with healthy grafts, large numbers of labeled neurons (mean 750±311; range 272-1084) were found in brainstem nuclei, but not in the cortex or thalamus. The location of labeled neurons always followed a consistent pattern, labeling brainstem nuclei which project rostrally in the MFB (Takagi et al, Brain Res. 193: 315, 1980). The heaviest labeling was found in the dorsal raphe nucleus which contained at least 50% of the total labeled cells. Both locus coeruleus and medial raphe nuclei were densely labeled while the raphe pontis and caudal linear nuclei were sparsely labeled. Infrequent labeling occurred in the following nuclei: A2, A4, parabrachial, lateral and ventral tegmental, interpositus, pontine gray, hypothalamus and the reticular formation.

CA containing neurons grew into the graft as revealed by the large numbers of CA fluorescent fibers within the graft and the high percentage of EB labeled neurons in locus coeruleus which also showed CA fluorescence.

The pattern of labeling was not due to spillage of dye directly on the cortex since many neurons were found in the cortex and thalamus following direct application of dye to the cortex in control rats.

Our results show that monoaminergic containing nuclei which project in the MFB have a high capability to grow into peripheral nerve grafts. Since the nerve graft is located remotely from the cell bodies, the physiological function of these MFB nuclei is more likely to be normal. Additionally, the epineurium provides a potent barrier which limits axonal access to the graft's cut end.

74.19 THE USE OF BASAL LAMINA TUBES FROM EVACUATED MUSCLE AS A PERIPHERAL NERVE GRAFT. J.W.Fawcett* and R.J.Keynes* (SPON: C.Asanuma). Salk Institute, La Jolla, CA 92037, and Dept. Anatomy, Cambridge University, England.

Severed peripheral nerves often cannot be rejoined without the use of a graft to bridge the gap between their proximal and distal stumps. Current clinical practice is to use sural nerve autografts for this purpose, although these have a number of disadvantages. We have investigated the use of basal lamina (BL) grafts derived from muscle as a substitute for these nerve autografts.

To prepare the grafts, either rat soleus or rabbit adductor magnus muscles were removed from recently killed animals. Myoplasm was evacuated from strips of muscle either by repeated freezing and thawing in liquid N_2 and buffer, followed by osmotic and mechanical evacuation, or by the technique of Wallis et. al (Biochim. Biophys. Acta 599, 505). This procedure leaves the BL essentially intact, but effectively eliminates most of the muscle components. The grafts were stored at -70°C until use.

The sciatic nerves of a series of rats were exposed and divided proximal to their bifurcation. A 0.5 cm BL graft was then stitched into the gap. We saw signs of nervous function returning to the leg about 1 month after surgery, and in cases with successful grafts, good functional recovery within about 2 months. Histological examination showed that many axons had regenerated through the grafts and into the distal stump within 10 days of operation. After 3 months the distal part of the nerve was almost as large as the proximal one, and there were large numbers of thick, myelinated axons in both the graft and the distal stump of the nerve. Although we have not yet stained specifically for Schwann cells, it looks as if these migrate into the graft with the front of ingrowing axons. Control sciatic nerve autografts were as successful as basal lamina grafts, but frozen sciatic nerve grafts were less satisfactory.

In our rabbit experiments a 4cm graft derived from rabbit adductor magnus was sutured into the divided nerve. In animals killed 3 weeks after surgery there were many axons that had grown through the graft, and into the distal stump; this corresponds to a growth rate of more than 2mm/day. After 3 months the animals showed significant but incomplete functional recovery, and had thick and healthy nerves distal to the repair.

In order to see if grafts from one species could be used in animals of a different species, we grafted rabbit derived BL grafts into rat sciatic nerves. These nerve repairs seem to be as satisfactory as those done with rat derived grafts.

Muscle BL grafts are a potential source of nerve grafts which have the advantages that they can be made in almost any size or shape, can be stored indefinitely, and do not have to be surgically removed from elsewhere in the body.

74.20 CHANGES IN GLIAL REACTIVITY TO IMPANTATION OF NITROCELLULOSE BRIDGES INTO ACALLOSAL ANIMALS AT VARIOUS AGES AND THE EFFECT ON REGENERATING CALLOSAL AXONS. G.M. Smith*, R.H. Miller*, and J. Silver (SPON: M. Singer). Dept. of Developmental Genetics and Anatomy., Case Western Res. Univ., Cleve., OH 44106.

When the "sling-like" glial structure that guides developing callosal axons is surgically severed in normal mice the commissural axons form large neuromas lateral to the midline, producing an acallosal animal (Silver et al. J.C.N. 210,1982). If a specially designed piece of Millipore filter is implanted into the would-be callosal pathway of acallosal neonates, axons can be induced to extend out of the neuromas and across the midline along astrocytes that, in essence, recreate the embryonic enviornment as they attach to the filter's surface (Silver and Ogawa, Science 220, 1983). Since callosal neuromas persist into adulthood, we asked whether a critical period does exist, and we have documented a variety of changes in the reactive gliosis that, in part, may lead to the axon growth refractory state. In acallosal postnates given untreated implants prior to day 10, GFAP + stellate shaped astrocytes rapidly attached to the filter by inserting foot processes into the filter's pores. This form of gliotic response established an axon growth promoting substratum within 48 hours after implantation. During this pre-critical stage there was no evidence of typical CNS scar formation or necrosis at or around the implant surface. However, when acallosal mice were implanted later than P 10, extensive tissue degeneration occured, and 5 to 7 days later a mixed population of astrocytes and fibroblasts invaded the surface of the implant producing a dense, basal lamina that permeated the scar. Reactive astrocytes within the scar had a flattened rather than stellate morphology, and did not promote axonal outgrowth.

It is important to determine which of the various changes in reactive gliosis contribute to an inhibition of axon growth during the post-critical period. In order to determine if the physical change in the shape of the astrocyte, from stellate to flat, influences axon outgrowth, untreated implants were partially crushed midsagittally prior to implantation into P 2 (pre-critical period) acallosal animals. Crushing, reduces the size of the filter's pores and forces the astrocytes to flatten along that portion of the surface. In such animals axons were observed growing among the stellate astrocytes (uncrushed region) but, turn abruptly at the crush/noncrush interface and did not extend on flat astrocytes.

In conclusion, our studies suggest that when controlled, gliosis during pre-critical stages can be a benefical process that can promote the reconstruction of malformed axon pathways. In older animals a variety of changes in the reactive glia may work together to hinder axon regeneration during the post-critical period. Supported by NSF (BNS8218700)

75.1 **LIPID PEROXIDATION-INDUCED CALCIUM UPTAKE BY BRAIN SYNAPTOSOMES AND CULTURED SPINAL CORD NEURONS.** J.M.Braughler, L.A.Duncan* and R.L.Chase*, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Ischemic or traumatic injury to the CNS is associated with accumulation of intracellular Ca^{++} that is hypothesized to play a role in the development of irreversible cell damage (Siesjo, J. Neurosurg. 60:883, 1984). Lipid peroxidation (LP) also has been postulated to cause cellular damage following trauma or ischemia (ibid). Previously we have examined the interactive membrane-damaging effects of LP and Ca^{++} in CNS and found them to be synergistic (submitted) in agreement with studies by Barsacchi et al. (BBA, 762:241, 1983) in heart. In the present study, the ability of LP to induce a large and rapid movement of Ca^{++} ions across CNS membranes is reported.

Brain synaptosomes (BS) were isolated from male Sprague-Dawley rats (Braughler, J. Neurochem. 44:1282, 1985) and cultures of murine spinal cord neurons (SCN) were prepared (Ransom et al., J. Neurophys. 40:1132, 1977). Ca^{++} uptake into BS was measured by incubating BS at 37°C in Hepes-Krebs buffer, pH 7.4 (KB) containing 1.5mM 45-$CaCl_2$. Uptake was terminated by rapidly diluting, filtering, and washing incubated BS in ice cold Ca^{++}-free KB containing 1mM $LaCl_3$. Filters were transferred to scintillation vials. Ca^{++} uptake by SCN was determined similarly, except that SCN were washed with La^{+++}-KB, then solubilized with 1% SDS for counting. LP of BS was initiated with 500μM ADP, 100μM H_2O_2, and 200μM $FeCl_2$, and followed continuously using a Clarke-style O_2 electrode. Malonyldialdehyde (MDA) was determined by TBA reaction, and conjugated dienes (CD) were monitored in Lubrol-dispersed BS by Abs, 232nm. Loss of arachidonic acid (AA) from SCN was followed by measuring release of 14-C-AA from SCN preloaded with 14-C-AA.

Within 30 sec after Fe^{++} initiation of LP in BS, Ca^{++} uptake had risen 10-20 fold, and by 6 min had increased 30-40 fold. The initial 30 sec burst of Ca^{++} uptake was coincident with a sharp increase in O_2 consumption and CD formation; MDA formation was minimal at this early time. In the absence of Fe^{++} a 3-5-fold increase in Ca^{++} uptake occurred that apparently corresponded to breakdown of pre-existing lipid hydroperoxides in BS membranes. Fe^{++}-induced Ca^{++} uptake and LP were partially blocked by methylprednisolone sodium succinate (100μM) and completely by desferrioxamine (500μM). The Ca^{++} channel blockers verapamil, nifedipine, and cinnarizine partially inhibited Ca^{++} uptake by BS in the absence of Fe^{++} but did not prevent Fe^{++}-induced Ca^{++} uptake or LP. LP of SCN caused a 3-9-fold increase in Ca^{++} uptake that was associated with AA release. The rate of AA release was half that seen with Ca^{++} ionophore, A23187 (10μM).

These findings suggest that perturbation of neuronal membranes by LP can result in the rapid influx of Ca^{++} which may mediate further membrane damage, perhaps via phospholipase activation. Observations such as these suggest an interactive role between LP and membrane permeability to Ca^{++} that may be important in the pathophysiology of CNS trauma and ischemia.

75.2 NEURONAL CELL DEATH AFTER DENDRITE AMPUTATION. J. H. Lucas*, G. W. Gross, C. R. Gardner* and J. B. Kirkpatrick††
Department of Biology, The Texas Woman's University, Denton, TX 76204 and ††Department of Pathology, Baylor College of Medicine, Houston, TX 76030.

Little is known about the reactions of neurons to dendritic disruption. Dendrites may ramify as far as 1000 μm from somata (Shepherd, G. M., The Synaptic Organization of the Brain, p. 83, 1976) and dendritic damage undoubtedly is an important part of both diffuse and localized CNS injury. Using UV laser microbeam techniques (Gross et al., J. Neurosci., 3: 1979, 1983), we have amputated dendrites of mouse spinal neurons in culture (3-5 wks post seeding). Dendritic processes (2-5 μm in diameter) were amputated at distances of 50, 100 or 150 μm from the perikarya. A total of 60 cells was operated at each distance. Cell survival values at 24 hrs were 31 ± 3 %, 53 ± 6 % and 71 ± 5 % respectively at the three lesion distances. No further cell death was observed at 48 and 72 hrs.

Previous investigations have demonstrated that percent loss of resting potential 20 min following neurite amputation is an exponential function of lesion distance (Lucas et al., Neurosci. Abst. 10: 1178, 1984). However, injury potentials are surprisingly persistent. Complete recovery of resting potentials has not been observed in a one hr period. Preliminary data indicate that even at 24 hrs potentials are still slightly below unoperated control cell resting potential values.

Measurements of physical parameters of the operated cells at a fixed lesion distance (100 μm) reveal a definite inverse correlation between percent cell survival and diameter of the transected neurite at the lesion. A direct correlation between the combined membrane areas of the soma and the proximal segment with cell survival is also indicated. These parameters probably reflect the magnitude of the injury current through the lesion and the compensating effects of ionic pumps in the membrane. Cell survival may ultimately depend upon the ability of the cell to buffer these ionic influxes especially the calcium component. The effect of low calcium on cell survival is presently under investigation.

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75.3 VERY EARLY CHANGES IN CHICK DORSAL ROOT GANGLIA FOLLOWING PARTIAL ABLATION OF THE NEURAL CREST. V.McM. Carr. Dept. Biochem., Molec. and Cell Biol., Northwestern Univ., Evanston, IL 60201.

Responses of dorsal root ganglia (DRG) to partial ablation of the brachial neural crest were examined in chick embryos. Ablation was carried out at the level of DRG 15-17 as described previously (Carr, J.Neurosci.4:2434,1984). In those previous studies embryos were examined at H. and H. stage 35, near the end of the period of naturally occurring neuronal cell death in the brachial DRG (Hamburger, et al., J.Neurosci.1:60,1981). Significant changes were found in the size of the ganglia examined as well as in neuronal nuclear size, neuronal numbers, and degenerative activity. A most surprising observation was that compensatory hypertrophies were not confined to brachial DRG (DRG 13-17) but were found in cervical ganglia several segments anterior to the site of lesion.

The current experiments examine responses of the DRG during early stages of neuronal cell death. Studies at stage 27, the time of onset of naturally occurring cell death (Hamburger, et al. op. cit.), show that ganglionic volume was reduced at the site of lesion as expected. However, compensatory enlargements of cervical DRG occurred as far anterior as DRG 10, the rostral limit of the investigation. These hypertrophies were highly significant in all cases (p .005) and thus agree with the cervical DRG hypertrophies seen at stage 35. To date changes in cell numbers have been tabulated in DRG 13. These changes correlate with the ganglionic hypertrophy: cellular hyperplasias of 42 to 45% were found in whole ganglia (p .005) as well as in lateroventral and mediodorsal regions of the ganglia. However, no changes in degenerative activities were found. These observations would suggest that the ganglia may in some way be able to respond to reduction in brachial ganglionic material several segments away prior to the onset of neuronal cell death. Further investigations are being carried out.

75.4 ACCELERATED MOTONEURON CELL DEATH IN THE BRACHIAL LATERAL MOTOR COLUMN (LMC) OF WINGLESS (WI) MUTANT CHICK EMBRYOS. M.E. Lanser*, C.J. Flanary* and J.F. Fallon* (SPON: A.W. Clark). Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Cell death is an integral part of the development of the nervous system. While there are many theories to account for this phenomenon, little is known about its true nature. In the chick embryo spinal cord, the LMC undergoes a predictable degeneration. Cell loss occurs after cellular proliferation and migration, begins on embryonic day 6 (D.6) when contact with the target limb musculature is established, and eliminates about half of the initial population of motoneurons. Because of the temporal relation between cell death and target innervation, it has been proposed that the dying motoneurons are those that fail to acquire some kind of trophic support from the target. Since limiting the amount of available target results in proportionately fewer motoneurons surviving the cell death period, it has been proposed that motoneurons normally compete with each other for a limited amount of trophic support. However, these experimental data can only be taken to indicate the normal occurrence of simple competition if the time course of cell loss is the same in each case. In previous work on limbless chick embryos, we demonstrated that cell death was accelerated in the brachial LMC in the absence of a periphery (J. Neurosci. 4(8):2043-50). In order to confirm this observation, we examined the pattern of cell death in the wi brachial LMC. Wi is an autosomal recessive mutation that results in no wing development and variable leg development in homozygotes. We counted the number of living motoneurons in the brachial LMC of wi embryos and their normal sibs on D.6 through D.12. The results are:

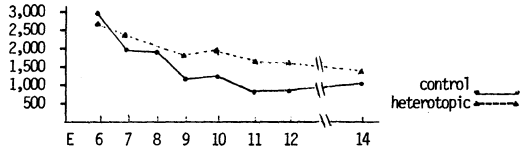
	D.6	D.8	D.10	D.12
Normal:	14,774 (5) +/- 2,292	13,150 (2) +/- 704	10,396 (5) +/- 750	8,462 (5) +/- 1,649
Wingless:	10,830 (2) +/- 1,974	3,942 (2) +/- 79	2,264 (5) +/- 968	2,879 (5) +/- 1,195

This shows that: (1) there are already fewer motoneurons in the wi brachial LMC on D.6 when target contact is normally first established; and (2) cell death is accelerated in the wi brachial LMC compared to normal. Because of this altered time course, these results do not necessarily indicate or support a role for simple competition in regulating naturally occurring neuronal death. These results do suggest that embryonic motoneurons require an interaction with the developing limb to stabilize them through an early critical period in their development. The failure to establish this interaction results in rapid destabilization and death. These data do not address the question of whether the required interaction is chemical, mechanical or functional in nature. (Supported by NIH-T32AM07389 and NSF-PCM8406338)

75.5 GROWTH AND DIFFERENTIATION OF THORACIC NEURAL TUBE TRANSPLANTED TO THE LUMBAR REGION IN THE CHICK EMBRYO. M. O'Brien*, R. W. Oppenheim and D. Prevette*. Dept. of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

In order to study the growth, differentiation and survival of neurons located in a foreign environment, heterotopic transplantation of several (2-6) segments of thoracic neural tube to the lumbar region (T-L) was carried out on embryonic day (E)2, shortly after neural tube closure. Control operations included homotopic T-T and L-L transplants. Embryos were sacrificed at different stages between E5 and E14, processed histologically and examined in the light microscope. Prior to sacrifice the movements and reflexes of hindlimbs were recorded. Hindlimbs innervated wholly or partially by thoracic spinal cord exhibited normal appearing movements and reflexes up to the oldest stages examined so far (E12-E14). Embryos retained for detailed histological examination exhibited normal differentiation of the heterotopic thoracic cord and in most cases the transplanted tissue had merged with host cord so that there was apparent anatomical continuity. Ongoing studies of the retrograde transport of HRP between donor and host cord will be able to substantiate this apparent anatomical continuity. The sympathetic preganglionic neurons (nucleus of Terni) that are characteristic of normal thoracic spinal cord were present in the heterotopically located thoracic tissue. Motoneurons (MNS) in the transplanted segments also appeared to differentiate normally. In fact, MN size (nuclear diameter) on E10 was larger in the transplanted vs control thoracic spinal cord (5.6 vs 5.9 μm, $p<0.01$). Cell counts between E6-E14 indicate that there are significantly more MNS in heterotopic vs control thoracic spinal cord (Fig. 1). Furthermore, in cases where 3-4 segments of transplanted thoracic cord were located in the central or rostral segments of the lumbar region the number of MNS in the lumbar lateral motor column caudal to the transplanted tissue was increased when compared to segment-matched control regions (L4-8) of the lumbar LMC (cont. = 1,800/seg., n=3; exp. = 2,400/seg., n=3). This suggests that the presence of fewer MNS in adjacent thoracic segments may have resulted in less cell death of contiguous lumbar MNS due to reduced competition for target-related survival factors. Studies are presently in progress on: (1) the hindlimb projection patterns of heterotopic thoracic MNS; (2) the projections of sympathetic preganglionic neurons from transplanted tissue; and (3) the development and differentiation of various properties of hindlimb muscles innervated by foreign MNS.

[Fig. 1]



75.6 LOSS OF ACETYLCHOLINE ESTERASE POSITIVE CELLS AND CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE SEPTAL AREA AND DIAGONAL BAND OF BROCA FOLLOWING FIMBRIA FORNIX TRANSECTION. Klas Wictorin*, Walter Fischer*, Lawrence R. Williams, Silvio Varon, Anders Björklund, and Fred H. Gage, Dept. of Histology, University of Lund, Dept. of Biology, UCSD, Dept. of Neurosciences, UCSD.

A variety of retrograde changes occur in central and peripheral nerve cell bodies following axotomy. In instances of minimal collateralization proximal to the axotomy, cells have been reported to die. In order to investigate the mechanisms involved in this cell death, and to develop potential therapies that may prevent the cell death, a model system is required. We, and others (Grady et al., SN, 1984), have chosen the septo-hippocampal system as a model system to investigate cell death in the CNS because of its well defined anatomical, and biochemical characteristics.

In the present study unilateral fimbria-fornix (FF) aspiration lesions were performed, which destroy a major cholinergic pathway, prior to extensive collateralization in the hippocampus. At 1 day and 1,2,4 and 6 weeks following aspiration, we measured the number and size of acetylcholine esterase(AChE)-positive cell bodies in the medial septum and vertical and horizontal limbs of the diagonal band of Broca. Animals were pretreated with diisopropylfluorophosphate (DFP), to facilitate the quantification of cell size and number. In a parallel set of animals we measured choline acetyltransferase (ChAT) activity in the septo-diagonal band region ipsilateral to the FF lesion and compared those values to the values obtained from the contralateral septo-diagonal band region.

A significant 31% loss of AChE-positive cells was observed in the medial septum already at 1 day following the FF lesion. The loss reached 64% by 1 week, but did not significantly change between 1 week and 6 weeks post-lesion. At all time points there was a greater decrease in cell number in the medial septum as compared to the vertical limb, and a greater decrease in cell number in the vertical limb relative to the horizontal limb. No significant difference in cell size (major axis of the measured AChE positive cells) was observed until a decrease in cell size was measured at 4 weeks post-lesion. This difference was also apparent at 6 weeks. No change in ChAT activity was observed between tissue taken contralateral to the lesion and tissue from unoperated controls. A significant decrease in ChAT was observed ipsilaterally 1 day following lesions, but this difference gradually returned to normal control levels over 6 weeks. A marked sprouting of AChE positive fibers in the dorsal lateral septum was observed in rats not treated with DFP, which could account for the gradual return of ChAT activity to normal levels. These results suggest that a cascade of events occur in the cholinergic septo-diagonal band region following axotomy, which include a dramatic loss of cell bodies, a decrease in cell size as well as a possible sprouting response of the remaining cholinergic neurons.

75.7 AN AUTORADIOGRAPHIC ANALYSIS OF ALTERATIONS IN CELL DEATH CONSEQUENT TO MONOCULAR ENUCLEATION IN THE HAMSTER RETINAL GANGLION CELL LAYER. J. I. Raabe and B. L. Finlay. Department of Psychology, Cornell University, Ithaca, NY 14853.

Monocular enucleation results in decreased cell death in the temporal retina of the remaining eye (Sengelaub and Finlay, Science, 213, 1981) and an enhanced ipsilateral projection. In normal development of the retinal ganglion cell layer, the rate of cell loss is highest for the first generated cohorts (those cells generated on embryonic days 10 and 11), and lower for later cohorts (E12-15) (Finlay, Sengelaub and Dolan, Neurosci. Abs., 10, 1984), and it is possible that the earliest generated cells might serve as pioneers in the early establishment of projection laterality. In addition, cells of different embryonic cohorts may be of different types, such as x-, y- or w-like cells, or "displaced" amacrine cells (Drager, Neurosci. Abs., 10, 1984; Finlay, et al., Neurosci. Abs., 10, 1984). To determine if the increased cell survival seen in the temporal retina following monocular enucleation results from a specific saving of the early generated cohorts, and if enucleation differentially alters survival of particular cell types, we examined the relative loss of cells in particular embryonic cohorts in enucleated and normal animals using tritiated thymidine.

Pregnant hamsters were injected with ^{3}H thymidine (5μCi/gm body weight; specific activity 20Ci/mM) on one of embryonic days 10, 12, or 14 (E15 = day of birth). On the day of birth, half of the pups in each litter were monocularly enucleated. Pups were killed on either postnatal day 4 or 5, the days of maximal reduction in cell loss in the enucleate relative to normal. After autoradiographic processing, counts of labeled normal and degenerating cells in the retinal ganglion cell layer were made from horizontal retinal sections.

In all the unoperated controls, almost all of the labeled degenerating cells observed were generated on E10 and E12, 5 times the number observed from E14. This pattern remained the same after enucleation, and is further evidence that the E14 cohort consists primarily of "displaced" amacrine cells. Rates of neuronal loss (number of labeled degenerating cells per 1000 labeled normal cells) for the E10 (early) and E12 (later) cohort were reduced in the enucleates with respect to normal, but equal to each other. The effect was greatest in the temporal retina, where cell death rates were reduced by half. Since enucleation reduced cell death in both the early and later generated retinal ganglion cell cohorts equally, it is unlikely that the earliest generated cohort serves as pioneer neurons and competitive processes must affect both cohorts equally.

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75.8 SPATIAL GRADIENTS IN BOTH NEURONAL GENERATION AND DEGENERATION PRODUCE THE RETINOTOPIC AND LAMINAR ORGANIZATION OF THE HAMSTER SUPERIOR COLLICULUS. K. C. Wikler, D. R. Sengelaub, M. H. Kane*, and B. L. Finlay. Department of Psychology, Cornell University, Ithaca, NY 14853.

Neuronal degeneration in the early development of the hamster superior colliculus has two striking spatial inhomogeneities (Finlay, et al., J. Comp. Neurol., 204, 1982). First, neuronal death is elevated in the intermediate and deep gray laminae relative to the superficial laminae, and second, neuronal death is elevated in the peripheral margins of the superficial laminae relative to the center. In order to assess the relative contributions of neuronal proliferation and neuronal death to the final composition of the adult superior colliculus in terms of its laminar and retinotopic organization, we traced the spatial distribution and pattern of degeneration of those cells produced on the peak day of neurogenesis in hamster (Crossland and Uchwat, Devel. Brain Res., 5, 1982), through the period of peak neuronal death to adulthood.

Pregnant hamsters were injected with ^{3}H thymidine (5μCi/g. body weight, specific activity 20 Ci/mM) on embryonic day 11, with the 24 hours following mating designated as E0. Pups were killed on postnatal day 4 (prior to the peak of degeneration), P6 (after the degeneration peak), or at adulthood. After processing for autoradiography, 3 equally-spaced coronal sections of the superior colliculus were charted for labeled and unlabeled degenerating and normal cell profiles.

Rates of degeneration for all cells (degenerating cells per 1000 normal cells) on both P4 and P6 replicated the spatial differences found in the previous study. For the E11 cohort alone, however, unlike the pattern for all cells in the superficial laminae, cell death rates were uniform throughout (center = 14.3 ± 6.6, periphery = 13.2 ± 9.6). Therefore, earlier cohorts must produce the center/periphery disparity in cell death rates, a pattern which we have observed previously in the creation of center/periphery disparity in cell density in the hamster retinal ganglion cell layer.

As was the case for all cells, degeneration rates for the E11 cohort were elevated in the deeper laminae (P6, superficial mean rate = 13.5 ± 8.1; deep = 34.0 ± 12.7). Prior to cell death, the number of cell labeled in the intermediate and deep gray laminae was twice (ratio = 2.18) that of the superficial laminae. However, by adulthood the proportion of labeled cells was nearly equal (r = 1.1), indicating that the number of cells in the lower laminae was differentially reduced, consistent with the cell degeneration pattern. Throughout development and to adulthood, the proportion of all cells in the superior colliculus from the E11 cohort remained constant, indicating that all cohorts must be subject to the same differential generation and loss of the deeper laminae. Initial spatial gradients or uniformity in patterns of cell generation observed early in development can thus be reversed or abolished by later differential cell loss.

Supported by NIH grants 1 K04 NS00783 and 1 R01 NS19245 to B. Finlay.

75.9 A REDUCTION IN MOTONEURON DEATH IN THE CHICK LATERAL MOTOR COLUMN IN VIVO BY EXTRACTS OF EMBRYONIC SKELETAL MUSCLE.
GUY J. PETRUZZELLI*and W. F. HUGHES* (SPON: S.K. JACOB).
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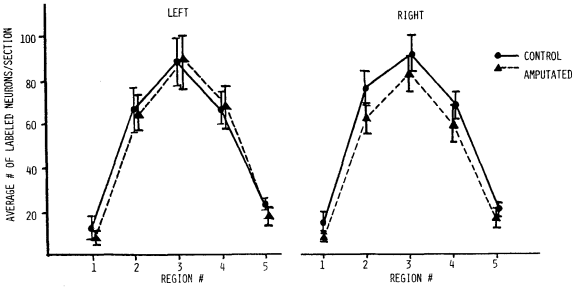
Factors present in the soluble fractions of muscle cell homogenates have been shown to enhance neuron survival, neurite outgrowth, and choline-acetyl transferase activity in vitro (Nurcombe,V., Brain Res., 291:19, 1984; Smith, G., Science,219: 1079,1983). The present study attempted to determine if crude extracts applied to the vascularized chorioallantoic membrane (CAM) could modify the in vivo developmental program in the lumbar lateral motor column (LMC). In our experiments, skeletal muscle extracts were prepared according to previously reported methods. The total protein concentration of our extract was determined to be 3.266 mg/ml using the dye binding assay of Bradford. Extracts (0.5 mls.) were applied to the CAM of embryonic chicks on alternate days between embryonic days 5 to 12 (E 5,7,9,11). During this period of normal development, a 40 % loss of somatic motor neurons is observed in the LMC. In day 12 extract treated embryos, counts of neurons in the lumbar LMC were increased 11.4 % over values for aged-matched normal embryos. The mean of the untreated LMC populations was consistant with values in the literature. re: 12,636 neurons ($\pm$ 238.70, n=8) Embryos receiving extracts had a mean LMC population of 14,077 neurons ($\pm$ 272.09, n=9). The student's t-test indicated significance at a $P < 0.002$ level. Control embryos receiving heat inactivated extract showed intermediate increases at the 12 day period. The results suggest a possible role for a diffusible muscle derived motoneuron growth factor in the regulation of the normal pattern of neuron survival in the lumbat LMC. A reduction in naturally occurring cell death has similarly been demonstrated in the pre-ganglionic sympathetic motor column using purified beta-nerve growth factor applied to the chorioallantoic membrane. Our results also suggest the utility of this model as an in vivo assay system in which purified muscle fractions or other motoneuron survival modulating factors can be tested.
(This work was supported by Basic Sceinces Research Grant No. 37242 awarded to G.J.P.)

75.10 THE EFFECTS OF AMPUTATION ON MOTOR NEURON POOLS.
E.R. Feringa and H.L. Vahlsing*.Univ. Calif. and VAMC, San Diego, Ca. 92161, and Medical College of Ga. and VAMC, Augusta, Ga. 30910.

It has been suggested that the loss of corticospinal neurons (CSNs) in amyotrophic lateral sclerosis may be a secondary phenomenon following loss of lower motor neurons (LMNs). Such trans-synaptic degeneration of neurons has been demonstrated in the visual system of man and other mammals. Degeneration of geniculate cells after loss of an eye occurs in adult or young animals. Retrograde trans-synaptic degeneration in the retina and optic nerve after occipital cortex lesions is established in young animals and some evidence suggests it may also occur in adults. In this experiment the number of LMNs in the L-5 gray matter and the number and distribution of CSN somata remaining 25 weeks after amputation of the right hind limb of the rat were compared with unoperated controls.

Twenty five weeks after right hind limb amputation, the number of large neurons in the area of L-5 spinal gray which contains those cells projecting to the hind limbs is dramatically decreased on both sides when compared to exactly matched isogeneic controls. In control rats $2.92 \pm .22$ large neurons were identified per 7µm section on the left side and $3.14 \pm .20$ on the right side. Twenty five weeks after right hind limb amputation $1.60 \pm .21$ such cells were found on the left and $1.72 \pm .21$ on the right. The decrease in large neurons was significant on both sides ($p<0.001$).

The same experimental rats showed 5726 ± 652 CSNs on the right and 6169 ± 778 CSNs on the left. Controls showed 6862 ± 673 CSNs on the right and 6441 ± 831 CSNs on the left. The number and distribution of demonstrated CSNs 25 weeks after right hind limb amputation is not significantly different from that found in controls.



HRP Labeled Neurons Found In Sensory Motor Cortex After T_3/T_4 Insertion Of HRP 25 Weeks After Right Hind Limb Amputation

75.11 MOTOR NEURON SURVIVAL FOLLOWING REDUCTION IN THE SIZE OF NEURON POOL.
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Loss of large numbers of motor neurons during the course of embryonic development appears to be an integral part of ontogeny of some neural centers. It is commonly supposed that motor neurons compete for some aspect of the periphery. The competition at the periphery is thought to be for postsynaptic sites and/or trophic substances essential for neuron survival. The motor neurons which die during normal development are thought to be those which have failed in this competition. In the present study an attempt was made to test the competition hypothesis by reducing the size of the initial neuronal pool, before motor neurons extend their axons into the periphery.

The trochlear nucleus of duck embryos attains a maximum of about 2400 neurons and after the period of cell death about 1300 neurons. The maximum number of neurons in the trochlear nucleus of Japanese quail embryos is about 1250 and after the period of neuron drop out only 750 survive. The midbrain vesicle of quail was transplanted in place of the midbrain of duck embryo on day 3 of incubation by established microsurgical procedures. The grafted neurons were verified by taking advantage of the structural difference in the interphase nucleus of these embryos. Only the quail nuclei exhibit a large heterochromatic mass associated with the nucleolar RNA. The peripheral connections of the grafted motor neurons were confirmed by retrograde transport of HRP injected into the superior oblique muscle, the sole target of innervation of the trochlear motor neurons.

Our preliminary counts on motor neuron survival indicate an average of about 700 neurons in the trochlear nucleus of the experimental embryos. In other words cell death of normal magnitude occurred despite increased periphery or reduced peripheral competition. According to the competition hypothesis none of the neurons should have degenerated. That is, the experimental embryos should have had about 1250 neurons in the nucleus. These observations suggest that the trochlear motor neurons die for reasons other than competition at the periphery.

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75.12 LIMB REGULATION OF MOTOR NEURON NUMBER DURING THE PERIOD OF PROFOUND CELL LOSS.
E.D. POLLACK, N.A. KETT* and C.A. BIALY* . Inst. Study Develop. Disabil., Dept. Biol. Sci., Comm. Neurosci., Univ. Illinois at Chicago, Chicago, IL 60680.

The presence of the limb during development has been long recognized as a necessity for the ongoing survival of lateral motor column (LMC) neurons. As the target for the spinal motor axons, limb absence invariably results in the eventual depletion of LMC neurons. Less obvious is the requirement for an intact limb in order that the natural occurrence of motor neuron loss can proceed according to a developmental timetable. We have been examining the influence that the limb exerts on the number of retained motor neurons during the period when cell loss is normally at its maximum in the lumbosacral LMC of the frog tadpole (stages IX through XIII). When the developing hindlimb was amputated at the usual time of onset of profound neuronal loss in the LMC, the process of reduction in cell number was inhibited so that the LMC retained throughout this period nearly all the neurons that were initially present. Some neuronal retention also occurred in the contralateral LMC. A series of control experiments that included the traumatization of the limb without its removal, but with accompanying axotomy, demonstrated that intact motor neuron-limb communication is required for the initiation of maximal cell loss. Absence of the limb or axonal transection presumably prevents this interaction, and thus interferes with the flow of information that is required for the selective events of neuronal loss and survival.

Developmental limb asynchrony produced by unilateral, partial limb amputation at an early larval stage (stage V), followed by regeneration, resulted in a paradoxical LMC cell count during the profound cell loss phase. This was manifested by equal numbers of neurons on both sides of the spinal cord, even though one limb was morphologically younger than the other. Once again, an effect appeared in the LMC contralateral to the manipulated limb in which case the neuronal count was representative of a stage younger than that indicated by the unoperated limb. Analysis of neuronal distributions in the LMC reflected the differentiated configuration of the regenerate in which its proximal portion was appropriate to the stage of the animal, and the distal region was appropriate to the delayed development of a regenerating limb.

It is clear that the extent of LMC cell reduction is strongly dependent on the developmental events related to the limb target. The data from each of these limb manipulations can be explained in terms of the availability of a putative motor neuron survival factor originating from the "least differentiated" or "dedifferentiated" tissue of the limb.

75.13 THE EFFECT OF T3 AND METHIMAZOLE ADMINISTRATION ON MOTONEURON CELL DEATH IN THE SPINAL CORD OF THE CHICK EMBRYO. C. Williams*, C. Wohlenberg* and M.J. O'Donovan. (Spon: M. Dekin) Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242

Thyrotropin releasing hormone has recently been shown to influence motoneuron cell death in the lumbosacral spinal cord of the chick embryo (Weill, Neurosci. Abstr. 10:641,1983) an effect that could be mediated by altered levels of thyroid hormone. The purpose of the present study was to investigate this hypothesis by determining the effects of thyroid status on motoneuron cell death in the lumbosacral spinal cord of the chick embryo. Chick embryos received daily injections of triiodo-L-thyronine (L-T3) or methimazole (NMI) in concentrations previously reported to induce hyper- or hypothyroidism (L-T3: 1-3 µg/100 g body wt; NMI: 5mgm/100 body wt). Control embryos received daily injections of either saline or a biologically inactive isomer of T3, triiodo-D-thyronine (D-T3). T3 injections were begun after motoneuron proliferation and encompassed the cell death period (day 6-13). Methimazole injections spanned day 2-13 and were started earlier to ensure low T3 levels during motoneuron cell death. The embryos were sacrificed at the end of the cell death period (day 13 or later), processed histologically and the total number of surviving lumbosacral motoneurons determined. All counts with the exception of the methimazole series were performed "blind" so that the individual counting the sections was unaware whether they were from experimental or control embryos. The results are summarized in Table 1.

L-T3	D-T3	MMI	CONTROL
11,582±1484	12,082±1408	12,753±2081	12,469±1408
(N=9)	(N=13)	(N=8)	(N=19)

TABLE 1. Uncorrected motoneuron counts (mean±sd) in control and experimental embryos.

The results show that neither T3 nor methimazole injections lead to significant changes in the extent of motoneuron cell death, indicating that thyroid hormone probably does not regulate this aspect of motoneuron development. Therefore, the effect of TRH on motoneuron cell numbers is presumably mediated through a mechanism other than altered levels of T3. (Supported by MDA).

75.14 THE ENHANCEMENT OF NATURAL MOTONEURON CELL DEATH BY GLUCOCORTICOIDS. C.L. Weill. Depts. of Neurology and Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Binding of (^{3}H)corticosterone has been localized to spinal cord motoneurons (Duncan and Stumpf, Brain Res. 307:321,1984). Our unpublished studies demonstrate the presence of glucocorticoid receptors in motoneurons on embryonic day 6. This study was undertaken to determine the effect of exogenously added glucocorticoids on natural motoneuron cell death.

Steroids dissolved in sesame seed oil were administered to chick embryos (Harco X Partmer red) by injecting 25 ul into the yolk sac on embryonic days 5-9. Embryos were sacrificed on day 10, staged, and weighed. The lumbar cord was dissected, fixed, stained, embedded in paraffin, and serially sectioned at 10 um. For every 10th section all large, dark-staining cells of the lateral motor column (LMC) containing at least one nucleolus were counted at 400x.

During normal development a peak of 22,838 +/- 731 (mean +/- S.E.M., n=6, uncorrected) motoneurons per LMC is observed on embryonic day 6. The number of cells declines by 33% to 15,260 +/- 403 (n=4) on day 10 in oil-injected control embryos. Injections of 0.197 ug of dexamethasone (Dex) per embryo resulted in a 28% decrease in survival yielding 10,913 +/- 813 (n=4) motoneurons per LMC. Reducing the dose 2-fold resulted in the survival of 11,263 +/- 1370 (n=3) cells, an increased loss of 26%. Equal molar injections of the glucocorticoid antagonist, methyl-hydroxyprogesterone (MHP), resulted in a statistically significant increase (29%) in motoneuron survival yielding 17,420 +/- 115 (n=4) cells. Treatment with the mineralocorticoid, aldosterone (Ald), yielded 16,757 +/- 652 cells (n=3), a value statistically indistinguishable from controls.

Body weight for controls was 2.545 +/- 0.108 g (n=8). Treatment with 0.197 ug Dex reduced mean body weight by 27% ($P<<0.01$) to 1.865 +/- 0.074 g (n=4). Treatment with MHP increased body weight by 16% ($P=0.001$) to 2.966 +/- 0.157 g (n=11). Serial reconstruction indicates that the LMC's of both controls and treated embryos traverse the same number of spinal segments. Qualitative examination of the cross sectional areas of the LMC's does not indicate significant differences between control and treated embryos.

These data demonstrate that the exogenous addition of glucocorticoids to developing chick embryos during the period of natural neuronal death enhances the loss of motoneurons from the spinal cord LMC. Addition of a glucocorticoid antagonist prevents motoneuron loss, while a mineralocorticoid had no effect. Thus these data support the suggestion that glucocorticoids may regulate natural neuronal death (Cowan et al., Sci. 225:1258,1984), but do not permit a distinction between a direct effect on motoneurons via an intraneuronal steroid-receptor system and an indirect effect via altered muscle trophic factor or synaptic site availability. Supported by NIH grant NS18642.

75.15 MINIMAL EFFECT OF INTRAOCULAR TETRODOTOXIN ON THE POSTNATAL REDUCTION IN THE NUMBER OF OPTIC NERVE AXONS IN THE ALBINO RAT. D.Crespo, D.D.M.O'Leary, J.W.Fawcett* and W.M.Cowan The Salk Institute, P.O. Box 85800, San Diego, CA. 92138

We have previously determined that about two-thirds of the axons found in the optic nerve at E20 are eliminated by about P10. About 90% of this loss occurs postnatally and reflects a comparable decline in the number of retinal ganglion cells. We have now examined the effect of blocking retinal ganglion cell (RGC) activity with the sodium channel blocker tetrodotoxin(TTX) on the postnatal reduction of the number of optic axons. The left eyes of albino rats were injected with 0.03 ul-0.06 ul (dose increased with age) of 0.1% TTX on alternate days, beginning with P0. The rats were perfused on P3, P7, P12 or P14 with saline followed by 1.5% paraformaldehyde, 3% glutaraldehyde, 0.6% acrolein and 0.6% DMSO in a 0.1% cacodylate buffer(pH 7.3). The left and right optic nerves were removed, postfixed, rinsed, osmicated, and embedded in Spurr's resin. Semithin and ultrathin sections were cut from the midpoint of each nerve. The ultrathin sections were photographed on a Zeiss 109 EM. From montages (2000X) the cross-sectional area of each nerve (excluding the area occupied by large glial processes, cell bodies and blood vessels) was determined. Micrographs were taken at 12000X from areas relatively free of large glial processes, and enlarged 3X. From these the mean density of nerve fibers was determined, and the total number of axons in each nerve was calculated. Three pairs of nerves were examined at each age. The means ± S.D. for both normal (from Crespo, O'Leary & Cowan, Dev. Brain Res., in press) and experimental rats are given below.

AGE	NORMAL	TTX TREATED(left)	CONTROL(right)
P0	273,740 ± 20,970	---	---
P3	216,600 ± 18690	238,360 ± 13,500	234,690 ± 9540
P7	167,760 ± 5900	161,250 ± 6370	160,120 ± 5960
P10	118,540 ± 15,410	124,600 ± 4890(P12)	136,250 ± 4240(P12)
P14	105,810 ± 5230	111,220 ± 15,000	129,300 ± 10,840

Our results indicate that both the magnitude and time course of the reduction in the number of axons in the TTX treated and control nerves are similar to that seen normally, and thus a near normal amount of RGC death occurs in both the TTX treated and control retinas. Preliminary results suggest that the slightly greater number of optic axons found in the P12 TTX treated rats can also be brought about by large systemic injections of TTX. We have previously shown (Fawcett, O'Leary & Cowan, PNAS 81:6131; Fawcett & O'Leary, TINS, Apr.'85) that intraocular injections of TTX prevents the preferential elimination of both ipsilaterally projecting and topographically incorrectly projecting RGCs. The present findings confirm that the overall magnitude of RGC death is affected little by TTX, although there may be a small systemic effect. Supported by NIH grants EY-03653 and FO5-TW3540.

73.16 SYNAPSE FORMATION AND DENDRITIC DEVELOPMENT OF SPINAL MOTONEURONS IN THE CHICK EMBRYO FOLLOWING CHRONIC CURARE TREATMENT. A. Okada*[1], S. Furber*[2], R. W. Oppenheim[2] and N. Okado[3] (SPON: K. O'Steen) [1]Dept. Anat., Nihon Univ. Sch. of Med., Tokyo 173, Japan, [2]Wake Forest Univ., Bowman Gray Sch. Med., Winston-Salem, NC 27103, USA and [3]Univ. Tsukuba, Inst. Basic Med. Sci., Ibaraki 305, Japan.

During normal development of chick spinal cord, there is a major loss of motoneurons in the lateral motor column (LMC) between embryonic day (E)5 and E12. Previous experiments in which neuromuscular activity was chronically blocked with curare during the cell death period have shown that motoneuronal death can be reduced or prevented. This result raises two questions: (1) whether motoneurons which are rescued from natural cell death by curare treatment can receive afferents; and (2) whether afferent inputs can form synaptic contacts on motoneurons which have lost functional interactions with their target.

Four embryos that received daily injections of curare (2 mg curare/0.2 ml saline) between E6 and E9 and four saline injected controls were used for electron microscopic analysis. The packing densities of synapses in the lumbar LMC (Seg. 26) were examined on E10 and E16. The number of synapses per unit area of the motor neuropil (packing density) was used for estimating axodendritic synapses; the ratio between the amount of somal membrane which is covered with active zones (synapses), vs the entire somal circumference (synaptic covering ratio) was used for estimating axosomatic synapses. On E16 packing densities of axosomatic (6.08 ± 2.75%; M ± SD) and axodendritic (0.0810 ± 0.0174 synapses/µm^2) synapses in the curare embryos were significantly lower than axosomatic (9.22 ± 4.09%) and axodendritic (0.1389 ± 0.0214) synapses of controls. By contrast, no significant differences were found for either type of synapse between E10 curare and saline groups. Because the total number of motoneurons in the LMC of curare embryos was approximately 1.5 times greater than controls, and because there were no significant differences in motoneuron size or total volume of LMC, the total number of axosomatic synapses probably does not differ between curare and controls on E16. By contrast, the decrease in axodendritic synapses in the curare embyros on E16 is probably considerably greater than the 40% reported above since the size of the motor neuropil in the curare group is reduced to an extent proportionate with the increment in motoneuron numbers. The reduction in axodendritic synapses does not appear to be due to abnormal dendritic development. Therefore, the number of afferent terminals may be directly affected by the curare treatment. Measures of dendrite length and branching from Golgi-impregnated motor neurons in curare embryos on E10-11 and E15-16 were similar to those found in control embryos on E10-11 and E15-16.

75.17 CHRONIC MORPHINE ADMINISTRATION DELAYS NORMAL CELL DEATH IN THE AVIAN CILIARY GANGLION. S.D. Meriney and G. Pilar. The Department of Physiology and Neurobiology, The University of Connecticut, Storrs, CT 06268.

Natural cell death in the ciliary ganglion occurs during the period of synapse formation with the target and results in an approximate 50% reduction in cell number (6260 ± 339 at E 8 to 3704 ± 241 at E 14). Previously, enkephalin-like immunoreactivity has been demonstrated in preganglionic terminals in vivo, and ciliary ganglion cells themselves in vitro (Crean,G. et al. Soc. Neuro. Abst. 10, 429, 1984). We have examined the possibility that opiates may play some role in regulating normal cell death in the ciliary ganglion. Drugs were administered daily to the chorioallantoic membrane on embryonic days 7 through 14. Experimental and control embryos were sacrificed on E 14 and the ciliary ganglia were fixed in Bouins, paraffin sectioned at 8 u, stained with hematoxylin/eosin orange, and counted at 400x. Daily treatment with an increasing dose of morphine (20-200ug/day) rescued most of the cells that would have died (5948 ± 345 at E 14), and this effect was reversed by naloxone. Daily treatment of naloxone alone did not significantly affect cell survival when administered during the normal cell death period. When morphine treatment was discontinued at E 14 and the animal was sacrificed at E 16, cell death increased toward control levels (4302 ± 509 at E 16). When morphine treatment was continued through hatching to 7 days-post-hatch (DPH) a slight increase in cell survival was still present. When the treatment was discontinued at 7 DPH and the animal was sacrificed a 14 DPH, cell death reached control values. Therefore, morphine treatment in increasing doses appears to delay cell death only until the treatment is discontinued. To determine if these effects are restricted to the ciliary ganglion, morphine's effect on cell death in the lumbosacral LMC of the spinal cord was examined. There was no significant effect of morphine on spinal motoneuron survival. Therefore, morphine's influence on cell survival does not seem to be a general effect on motoneurons, and may be restricted to cells with endogenous opiates.

The mechanism by which morphine influences cell survival in the ciliary ganglion is not known. Morphine's effect on iris contractions is not significant (measured pupilomorphically following electrical stimulation of the ciliary ganglion), but morphine can partially block synaptic transmission through the ganglion. Morphine's effect in the ciliary ganglion may be do to its influence on activity in this system, or a direct effect of the opiate on the ciliary ganglion cells themselves. Supported by BSN 8410581 and the U. CT Research Fdn.

75.18 HYPOGLYCEMIA-INDUCED NEURONAL NECROSIS IN THE RAT STRIATUM IS PREVENTED BY A N-METHYL-D-ASPARTATE-RECEPTOR ANTAGONIST. T. Wieloch* (SPON: W.H. Oldendorf), Laboratory for Experimental Brain Research, University of Lund, S-22185 Lund, Sweden

Hypoglycemia if severe enough to cause cessation of brain electrical activity (isoelectricity) lead to neuronal necrosis in discrete brain areas (1). Following 30 minutes of isoelectricity 50-70% of the neurons in the striatum are irreversibly damaged. Recent evidence have shown that excitatory amino acids may be important factors in the development of hypoglycemia-induced brain damage (2). Unilateral ablation of the frontal neocortex in rats subjected to 30 minutes of hypoglycemia-induced isoelectricity, protected the subjacent striatum against neuronal necrosis. Concomitantly, a 10% decrease in the striatal levels glutamate was observed in the lesioned hemisphere (3).

The present investigation was performed to establish whether glutamate receptors of the N-methyl-d-aspartate (NMDA) type are involved in the pathogenesis of neuronal necrosis due to hypoglycemia.

Hypoglycemia was induced by 8 IU insulin i.p. (1). Thirty to forty minutes prior to the onset of isoelectricity, the rats were injected either with 2 ul saline (n=4) or 40 ug 2-amino-7-phosphonoheptanoic acid (APH) (n=5) in 2 ul saline pH 7.0 , into the striatum over a period of 10 minutes. Following a hypoglycemic insult of 30 minutes and one week recovery, the rats were perfusion fixed with formalin, sectioned and stained with acid fuchsin and celestine blue. Neuronal necrosis in the striatum was assesed in an area (600x400 um) surrounding the injections site.

		number of neurons	
treatment	hemisphere	necrotic (%)	total
saline	injected	61 ±8 (57)	106 ±9
saline	contralateral	74 ±10 (61)	121 ±11
APH	injected	6 ±8 (5) p 0.01	110 ±10
APH	contralateral	81 ±7 (68)	118 ±9

The data demonstrate that APH prevents hypoglycemia-induced neuronal necrosis in the striatum. Furthermore the results indicate that activation of the NMDA receptors by aspartate or glutamate may be important factors in the pathogenesis of hypoglycemic neuronal damage.

1.R.N. Auer, T. Wieloch, Y. Olsson, B.K. Siesjö, Acta Neuropath (Berl.) 64:177-191.
2.T. Wieloch, Prog Brain Res (1985) in press
3.T. Wieloch, B. Engelsen, E. Westerberg, R.N. Auer, Neurosci Lett (1985) in press

75.19 RAPID DOWN REGULATION OF HIPPOCAMPAL ADENOSINE RECEPTORS FOLLOWING BRIEF ANOXIA. K.S. Lee and W. Tetzlaff. Dept. of Neuromorphology, Max Planck Institute, 8033 Planegg-Martinsried, West Germany.

The selective vulnerability of certain CNS neurons to brief periods of anoxia has been known for over a century. Rather than causing a uniform type of cellular damage, the interruption or reduction of the normal oxygen supply to the brain results in a variety of cellular responses which can vary from region to region or even within subregions of the same structure. Pyramidal cells in the CA1 region of the hippocampus are perhaps the most sensitive cells in the brain to transient periods of anoxia. Following a 5-15 minute period of global anoxia, these cells exhibit a relatively slow series of changes which ultimately result in their death after 1- 2 days. This "maturation" process is of particular clinical interest inasmuch as the elapsed time between the anoxic trauma and the resultant cell death is presumably long enough to allow for therapeutic intervention. In the present study we sought to examine whether a change in the strength of the inhibitory, neuromodulatory action of adenosine might contribute to the hyperexcitability and epileptiform activity which precedes cell death in the hippocampus. The regional density of high-affinity, A1 type of adenosine receptors is known to be one of the factors which control the strength of the inhibitory action of adenosine. Thus quantitative autoradiographic studies of 3H-CHA (which binds to A1 type adenosine receptors) binding were undertaken to examine whether changes in the number of adenosine receptors occur subsequent to a brief period (12 min) of global anoxia. In the neocortex and striatum, a transient but nonsignificant decrease in 3H-CHA binding was observed. These changes were small, less than 5%, and tended to recover within 4-22 hours post-clamping. In contrast, the stratum radiatum of CA1 in the hippocampus exhibited a rapid and significant reduction in 3H-CHA binding following anoxia. Within one hour, CA1 showed a reduction of 10-15% and by 2 hours post-clamping the amount of 3H-CHA binding was reduced ca. 20% from control levels. This loss of 3H-CHA binding persisted for at least 22 hours, the longest time period tested and a time at which CA1 pyramidal cells have begun to irreversibly cease firing. This effect was not due to some form of selective swelling as the width of the stratum radiatum was not changed. A Scatchard analysis of 3H-CHA binding to crude membrane preparations from the CA1 region showed that the reduction in binding was due to a loss of binding sites, with their apparent affinity being unchanged. In summary, the region of the brain most sensitive to transient anoxia, i.e. CA1 of the hippocampus, showed a rapid and selective loss of 3H-CHA binding sites following brief anoxia. This reflects a reduction in the number of A1 type adenosine receptors and may be responsible for the hyperexcitability and epileptiform activity seen in CA1 pyramidal cells following tranisient global anoxia.

75.20 EVIDENCE OF INCREASED CELL DEATH IN RAT CEREBRAL CORTEX FOLLOWING IN UTERO EXPOSURE TO HYPOXIA. Mang C. Yu. Dept. of Anatomy, New Jersey Medical School, Newark, N.J. 07103.

The vulnerability of the central nervous system to hypoxia during development was studied in rats. Dams (Sprague-Dawley strain) on the 12th day of pregnancy were exposed to hypoxia (10% 02; 90% N_2) for 7 consecutive days. After hypoxic exposure, the dams were returned to breathe normal air until they littered. At birth, the neonates were removed from their dams and cross-fostered by healthy lactating dams until weaning. For control, neonates from dams which were exposed to normal ambient air during pregnancy were used. The hypoxic rats and the control were killed at postnatal days 5, 12 and 22, respectively. Biochemical measurements of DNA of the entire cerebral cortex, as an index of cell number, and electron microscopic examination of morphological alteration in the parietal cerebral cortex revealed the following findings. The total DNA contents of the hypoxic rats were significantly less than the control, being 70, 85 and 65% of the control at days 5, 12 and 22, respectively. Morphologically, the number of developing nerve cells showing degenerative changes in the cortical plates, particularly at postnatal days 5 and 12 in the hypoxic rats, was much greater than the control group. This study suggests that the reduction in cell number in rats exposed to hypoxia in utero was due, in part, to increased cell death.

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75.21 IN VITRO ANOXIA SELECTIVELY DAMAGES STRIATAL AND HIPPOCAMPAL ACETYLCHOLINE AND CARBON DIOXIDE PRODUCTION. V. Mykytyn* and G.E. Gibson. Cornell Univ. Med. College, Burke Rehabilitation Center, White Plains, NY 10605.

An in vitro model of brain ischemia may facilitate an understanding of the molecular basis of selective vulnerability. Our previous studies with tissue slices (0.3 x 0.3 mm) from whole brain demonstrate that anoxic incubations in depolarizing concentrations of K^+ (treatment incubation) cause lasting metabolic changes in a subsequent incubation in the presence of 100% oxygen and depolarizing concentrations of K^+ (test incubation). Decreases are apparent in CO_2 production from glucose (-67%; a general measure of oxidative metabolism) and ACh synthesis (-63%; a marker of coupling of metabolism to function). Omission of calcium from the treatment incubation ameliorates these deficits, as determined in the test incubation. Since in vivo pathological studies of others suggest that ischemic damage may be post-synaptic or in the cell body, the consequences of this "in vitro ischemia" were examined with hippocampal slices, which contain cholinergic nerve terminals but not cell bodies, and with striatal slices which contain cholinergic cell bodies and cholinergic nerve terminals. An anoxic treatment incubation reduced the subsequent test incubation production of CO_2 similarly in hippocampus (-41%) and striatum (-36%). However, ACh synthesis was altered differently in the two regions; the prior ischemia reduced production by 48% in the striatum, whereas hippocampal ACh formation was unaffected. Addition of EGTA to the treatment incubation did not alter test incubation ACh synthesis by hippocampus, whereas it increased striatal synthesis by 80% (i.e. diminished damage of the treatment incubation). These results suggest that cell bodies are more damaged by anoxic insults than nerve terminals and that calcium is important in the production of these changes. To further test this possibility, synaptosomes were made anoxic in a treatment incubation; this did not alter test-incubation ACh synthesis or CO_2 production. Together, these results suggest that cell bodies (in this model, cholinergic ones) may be more sensitive to anoxic-induced damage than nerve endings. (Supported by grants NSO3346, AGO4171, and the Burke Relief Foundation.)

75.22 GOLGI STUDIES OF THE MATURATION OF CELL DAMAGE FOLLOWING REVERSIBLE FOREBRAIN ISCHEMIA. L.A. Paul*, J. Deshpande*, T. Wieloch* (SPON: S. Zornetzer). Laboratory for Experimental Brain Research, Lund University, S-22185 Lund, Sweden.

Neuronal necrosis in the hippocampus following cerebral ischemia takes time to develop, and maximal cell loss occurs many hours or even days after the ischemic insult. Rodent models allow the induction of reversible ischemia followed by recovery to full consciousness, both for varying amounts of time. The nature and distribution of cell death as seen with Nissl stains after ischemia followed by one week recovery have been described (Smith et al., 1984). This study details cell damage as seen with the Golgi stain in animals subjected to ischemia for 10 minutes and allowed to recover for several days.

Wistar-SPF rats (Møllegaards Avslab., Copenhagen) were anesthetized with halothane. Catheters were inserted in the jugular vein for subsequent withdrawal of blood, and in the tail artery for monitoring blood gases. Both common carotid arteries were exposed and loose sutures placed around them. Needle electrodes were placed into the temporalis muscle to monitor EEG. The rats were immobilized, artificially ventilated on N_2O:O_2, and the halothane discontinued. Ischemia was induced by the infusion of trimetaphan (Arfonad^R) and the withdrawal of blood until MABP reached 50mm Hg. Vascular clamps were then applied to both carotid arteries. Isoelectricity was observed within fifteen seconds in all cases. Ischemia lasted 10 minutes from the time the EEG became isoelectric. To end the ischemia, blood was reinfused, vascular clamps removed, and $NaHCO_3$ was given. Animals were extubated at 45-60 minutes, when they resumed spontaneous ventilation. They were placed in their cages with access to water and food, and allowed to recover varying times.

Following formalin perfusion, brains were removed and stained with a variant of the rapid Golgi technique and 90-micron coronal sections taken. Qualitative study in cortical regions of this material revealed no gross pathology such as denuded dendrites or distorted dendritic trees. However, in CA_1-subiculum of ischemic rats, an apparently spine-free cell with soma in s. pyramidale and large, regular swellings on its dendrites was seen. These swellings were more marked in the longer-recovered animals. It is likely from this cell's shape and location that it is a basket cell, supposed to play an inhibitory role in hippocampus.

Smith, M.-L., R.N. Auer, B.K.Siesjö. Acta Neuropath. (Berl.) 64:319-332, 1984.

75.23 INVESTIGATION OF A RAT MODEL FOR STUDYING THE EFFECTS OF DRUGS ON SURVIVAL DURING CEREBRAL ISCHEMIA. K. Viik* and B.R. Cooper, Dept. of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Certain barbiturates and related substances are known to have a protective effect on the brain during cerebral ischemia. One of these agents, phenobarbital, has occasionally been used clinically as an adjunct to brain surgery, or as a treatment of ischemia due to stroke or trauma. The CNS sedative effects of the barbiturates, however, are a major limitation in the use of barbiturates for stroke and certain other hypoxic conditions. Development of easily quantitated animal models of cerebral ischemia sensitive to the protective actions of standard drugs such as phenobarbital would facilitate research into CNS protective non-sedative antihypoxic agents. A number of "stroke" models using gerbils and middle cerebral artery ligation have been described in the literature (Levine, L. and H. Payan, Exp. Neurol. 16:255-262, 1966; Robinson, R. and F. Bloom, Biol. Psychiatry, 12:669-680, 1977). We will report in this abstract that measurement of survival time of 500+ gm male Long Evans rats after bilateral carotid artery occlusion provides another model sensitive to protective effects of phenobarbital and suitable for limited pharmacological screening. Male 500+ gm Long Evans rats were briefly anesthetized with fluothane and the carotid arteries occluded by cauterization. The wound was closed and phenobarbital, saline, or other drugs were injected i.p. five minutes after termination of fluothane anesthesia. Survival time of control animals was 101 ± 22 min., and 10 out of 11 died in the 5 hr observation period. Phenobarbital (30 mg/kg i.p.) doubled survival time in the 3/7 animals that died within the 5 hr observation to 205 ± 33 min., and 4/7 lived longer than 24 hrs. The effects of phenobarbital were less dramatic at higher doses that also had overt sedative and muscle relaxant effects. In addition to phenobarbital, pretreatment with intracisternal injection of 400 µg of the GABA-T inhibitor, BW 357U, naloxone (10 mg/kg i.p.), dilantin (30 mg/kg i.p.), diazepam (5 mg/kg i.p.), haloperidol (5 mg/kg i.p.), amitryptyline (10 mg/kg i.p.), muscimol (1 mg/kg i.p.), baclofen (5 mg/kg i.p.) and other drugs did not prolong survival time when given in this model. In conclusion, the bilateral carotid occlusion model of cerebral ischemia is sensitive to the protective effects of phenobarbital against brain ischemia, and suggests that elevation of GABA levels in brain may protect the brain from the lethality of cerebral ischemia as well.

75.24 LOCAL PROLIFERATION OF CAPILLARIES ASSOCIATED WITH GLIAL CELL ACTIVATION IN IBOTENIC ACID LESIONS OF THE PRIMARY SENSORY CORTEX IN RAT. D.J. Reis, C. Iadecola, S.P. Arneric, L.W. Tucker and H. Baker. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

The excitotoxin ibotenic acid (IBO), when locally microinjected into brain, destroys neuronal perikarya sparing fibers and is associated with glial proliferation (Schwarcz et al., 1979). It is not known, however, whether the neuronal loss and the concomitant glial growth is associated with changes in local capillary surface-area (CSA) and/or metabolic demands resulting in altered local cerebral blood flow (lCBF). In this study, local IBO lesions were produced in order to assess: (a) the time-course of the glial proliferation, (b) whether glial proliferation is associated with growth of capillaries and, if so (c) whether lCBF is modified with the lesion. IBO (10 ug in 1 ul) was microinjected unilaterally into the primary sensory cortex (SmI) of rats under halothane anesthesia. One, 3,5,7,15,30 days later, animals were were sacrificed and the brain sectioned and processed. Glial cells were counted on thionine-stained sections (20 um). Brain capillaries were visualized on adjacent sections by staining for alkaline phosphatase; CSA was determined using a vidicon-based image analysis system (Eyecom, PDP 11/45). lCBF was measured autoradiographically in anesthetized (chloralose), paralyzed rat, by the 14C-iodoantipyrine technique.

No perikarya were detectable in the lesioned SmI by 24h. By day 1, the number of glial cells within the lesion increased 2.8-fold (with respect to unlesioned controls), continued to rise up to 11.5-fold at day 7, and then fell to reach a steady 5.4-fold increase by 15 and 30 days ($p < 0.05$; analysis of variance; n=3-4 per group). In the lesioned SmI, CSA was unchanged at day 1, increased linearly from day 5, reaching a maximum of 3.2-fold at day 7 ($p < 0.05$) and stabilizing at a 2.7-fold increase at 15 and 30 days ($p < 0.05$). In the contralateral SmI and in the underlying caudate n., CSA did not change. Five to 7 days after IBO treatment, when the glial-capillary proliferation was maximal, lCBF in the lesion was dys-homogeneous with high-flow zones corresponding to areas of maximal glial proliferation and with low-flow zones corresponding to areas of hypocellularity. However the average lCBF in the lesion (117+36 ml/100 gr x min) did not differ from that of the contralateral SmI (123 + 8; $p > 0.05$; paired t-test; n=5). We conclude that IBO lesions of the SmI induce a massive glial proliferation paralleled by an increase in CSA. The increased CSA, most likely reflecting increased number of capillaries, it not associated with a proportional increase in local tissue perfusion, suggesting that not all the neoformed capillaries are patent and/or available for exchange. Elimination of local neurons in cortex does not result in reduced lCBF, most likely because the increased local metabolic activity resulting either from the concomitant glial growth or activity of innervating processes keeps lCBF in the normal range. Glial cells may be an important factor contributing to control local cerebral microcirculation.

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76.1 AUTORADIOGRAPHY OF [3H]-TRYPTAMINE RECEPTORS IN BRAIN. L. L. Martin*, A. M. Wasley*, M. R. Marien and C. A. Altar (SPON: R. Gerber). Neuroscience Res., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

[3H]-Tryptamine binds to brain homogenates with a high affinity and pharmacological selectivity indicative of functional receptors for the putative neurotransmitter tryptamine (C. Cascio and K. Kellar, Eur. J. Pharmacol. 95: 31, 1983). The present studies determined the capacity, affinity, pharmacology and distribution of [3H]-tryptamine binding sites in coronal and horizontal sections of rat brain.

[3H]-Tryptamine binding to brain sections was 45-58% displaced by 10 uM tryptamine, of high affinity (Kd values of 3.6-7.4 nM), and showed a 10-fold quantitative variation between brain regions. The concentration of sites was highest in the n. accumbens (Bmax values of 701-760 fmol/mg protein). Specific binding was also high in the claustrum, hippocampus, septum, olfactory tubercle, frontal cortex, cingulate cortex and caudate-putamen (Bmax values of 440 to 660 fmol/mg protein). [3H]-Tryptamine binding was displaced with high affinity (IC50 < 30 nM) by 5-methyltryptamine, harmaline and p-methoxyphenylpropylamine (PMPPA). Hill coefficients obtained with harmaline and PMPPA were less than one in most brain regions suggesting the presence of pharmacologically different populations of tryptamine sites. NE, 5-HT and spiperone occupied these sites with very low (≥ uM) affinity. Thus, [3H]-tryptamine binds to site(s) recognized by phenylalkylamines, B-carbolines, and tryptamine analogues but not to alpha- or beta-adrenergic, D_2 dopaminergic, or S_1 or S_2 serotonergic sites.

Our findings with autoradiography agree closely with studies with brain homogenates. Since the distribution of [3H]-tryptamine receptors in brain is similar to that of dopamine nerve terminals and correlates highly with the autoradiographic localization of [3H]-N-methyl-4-phenylpiperidine ([3H]-MPP+) binding (M. R. Marien et al., this volume), it is possible that tryptamine may modulate dopaminergic transmission in the brain.

76.2 A STUDY OF THE UNDERLYING MECHANISMS FOR ACUTE METHAMPHETAMINE DEPRESSION OF STRIATAL TRYPTOPHAN HYDROXYLASE. P.K. Sonsalla*, J.W. Gibb and G.R. Hanson, Dept. of Biochem. Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City, UT 84112

The administration of a single dose of methamphetamine (METH) to rats produces a very rapid, but transient depression of tryptophan hydroxylase (TPH) activity, the rate-limiting enzyme in the synthesis of 5-hydroxytryptamine (5HT) without altering tyrosine hydroxylase (TH) activity, the rate-limiting enzyme in dopamine (DA) synthesis (Knapp et al., 1974; Bakhit and Gibb, 1981). In contrast, multiple doses of METH produce long-lasting decreases in neurochemical parameters of both the 5HT and DA systems which can be prevented or attenuated by DA receptor blockade (Hotchkiss and Gibb, 1980; Schmidt et al., 1984; Sonsalla et al., 1985). These findings suggest 1) the 5HT system responds more rapidly and is more sensitive than the DA system to METH treatment, 2) DA receptor activation contributes to the actions of METH on both the DA and 5HT systems with multiple dose treatment, and 3) different mechanisms may be involved in the response of the 5HT system to acute vs repeated treatment with METH.

The present studies were undertaken to investigate possible mechanism(s) involved in the acute actions of METH on TPH activity. To determine if the METH-induced depression of TPH activity was mediated by either DA or 5HT receptors, the D1 antagonist, SCH 23390 (0.5 mg/kg/i.p.), the D2 antagonist, sulpiride (80 mg/kg/i.p.) or the 5HT2 antagonist, ritanserin (1 mg/kg/i.p.) were coadministered with METH (15 mg/kg/s.c.). The rats were killed 4 h after treatment. In addition, in vitro experiments were conducted to determine if this acute action of METH was mediated locally at the 5HT terminal sites. Striatal slices were incubated in a Kreb's buffer solution containing METH (10^{-3}M) for 1 or 3 h. The slices were then homogenized and TPH activities measured.

In the in vivo experiments, METH alone decreased TPH activity in the neostriatum to approximately 50% of control; none of the antagonists tested modified this response to METH. In the in vitro studies, incubation of striatal slices with METH did not depress TPH activity. These findings suggest the acute effect of METH on TPH activity 1) is not related to D1, D2, or 5HT2 receptor-mediated actions and 2) is not mediated locally within the neostriatum, but rather, appears to require an intact neuronal circuitry.

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76.3 CHARACTERIZATION OF HIGH- AND LOW-AFFINITY [^{3}H]IMIPRAMINE BINDING SITES IN RAT BRAIN HOMOGENATES. J.R. Ieni, K. Saidara, S.R. Zukin and H.M. van Praag. Depts. of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Analysis of [^{3}H]imipramine binding in rodent brain (Reith et al., 1983; Conway & Brunswick, 1983; Ieni et al., 1985), human brain (Cash et al., 1984) and human platelet membranes (Ieni et al., 1984) gives curvilinear Scatchard plots consistent with the existence of more than one class of binding sites. In our laboratory, computer-assisted analysis of [^{3}H]imipramine binding to whole rat brain homogenates yielded an apparent high-affinity component (K_d = 8 nM, B_{max} = 505 fmol/mg pr.) and an apparent low-affinity component (K_d = 805 nM, B_{max} = 10.9 pmol/mg pr.). Characterization of the high-affinity binding site suggests a relationship with the neuronal reuptake system for serotonin. The high-affinity component has been implicated as a possible biological marker for depressive illness, since some laboratories have demonstrated reduced densities of these sites on platelet membranes of depressed patients compared to controls. Determination of the pharmacological and physiological significance of the low-affinity binding component was the goal of the present study.

The binding of [^{3}H]imipramine to rat brain homogenates is influenced by Na^+ and K^+ ions. Removal of these ions from the incubation medium resulted in decreases in the number of high-affinity binding sites and increases in the number of low-affinity sites without altering the two K_d's. Incubation of the rat brain homogenates with 50 nM nonlabeled imipramine resulted in a linear Scatchard plot consistent with the low-affinity component of [^{3}H]imipramine binding under control conditions. Displacement of drugs from the low-affinity site labeled with 300 nM [^{3}H]imipramine yielded IC_{50}'s in the 1-10 uM range for tricyclic antidepressants (trimipramine, 2.6 uM; cyanoimipramine, 2.7 uM; chlorimipramine; 2.7 uM; protriptyline, 3.4 uM; imipramine, 4.1 uM; desipramine, 4.4 uM and amitriptyline, 4.9 uM. Zimelidine-Z gave an IC_{50} of 23.5 uM at the low-affinity site. Serotonin was unable to displace low-affinity [^{3}H]imipramine binding. These data suggest that the ratio of high- to low-affinity [^{3}H]imipramine binding sites may be regulated by Na^+ and K^+ ions. However, based on the displacement experiments, since the tricyclics all show similar potencies, the low-affinity site does not appear related to serotonin uptake. Further research is needed to identify and characterize this low-affinity site, its relationship to antidepressant drug action as well as affective disorders.

76.4 β-PHENYLETHYLAMINE AND DOPAMINE: CONCURRENT DEPLETION FOLLOWING 6-OHDA OR ELECTROLYTIC LESIONS OF THE SUBSTANTIA NIGRA. A.J. Greenshaw* and A.V. Juorio* (SPON: A.A. Boulton). Psychiat. Res. Div., Sask. Health, Univ, Sask., Saskatoon, Sask., Canada.

β-Phenylethylamine (PE) is a behaviourally active compound which occurs naturally in mammalian central nervous system (CNS) and which forms the basic carbon structure of a range of sympathomimetic amines. The status of PE as an amine associated with the intraneuronal compartment of CNS remains to be elucidated. To address this problem, the relationship between striatal PE levels and the depletion of striatal dopamine (DA) and 5-hydroxytryptamine (5-HT) has been investigated. Striatal DA depletion was accomplished with unilateral 6-OHDA (8 µg) or electrolytic lesions of the substantia nigra (SN). Striatal 5-HT depletion was accomplished with electrolytic lesions of the dorsal and median raphé nuclei. Concurrent depletion of PE (to 55% of controls) DA, DOPAC and of HVA (to 30%, 48% and 45% of controls) was observed 7 days after SN lesioning. These effects were evident with pretreatment with a monoamine oxidase inhibitor (l-deprenyl 2 mg/kg^{-1} 2 hr prior to assay).

In contrast to these results no striatal PE depletion was observed 7 days after electrolytic raphé lesions at which time striatal levels of 5-HT and 5-HIAA were reduced to 50% and 38% of their respective control values. The results demonstrate clearly an intraneuronal localisation of PE in the striatum. These observations indicate the possible existence of PE-containing cells in the nigro-striatal pathway or PE-containing neurons whose axons pass through the region in or around the SN. Although PE is possibly associated with catecholamine neurones rather than monoamine-containing neurones per se (in view of the lack of effect of 5-HT depletion), the previously reported lack of effect on PE levels of reserpine indicates the possible independence of PE and DA in this context.

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76.5 SIMILARITIES IN DORSAL RAPHE NEURONS IN BALB/c AND CBA MOUSE STRAINS. L.A. Mattiace, S.G. Speciale and D.C. German. Depts. of Physiol. and Psychiat., Univ. of Texas Health Sci. Cntr., Dallas, TX. 75235.

The BALB/c mouse has significantly more midbrain dopaminergic (DA) neurons than the CBA mouse but no difference in the number of locus coeruleus (LC) noradrenergic neurons (Ross et al., Nature, 264:654-656, 1976). The BALB/c mouse also exhibits more DA-related behaviors than the CBA (i.e. spontaneous locomotion, exploration and stimulant-induced locomotion). Although these behavioral differences may be related to DA system differences, raphe serotonin (5-HT) neurons can also influence the DA system and DA-related behaviors (Lucki & Harvey, Neuropharmacol., 18:243-249, 1979).

The purpose of the present experiment was to compare, in these two mouse strains; (1) dorsal raphe (DR) cell numbers using a tyrosine hydroxylase (TH) antibody, which recently has been reported to cross-react with DR serotonergic neurons (Towle et al., Neurosci. Abstr., 9:1056, 1983), and (2) 5-HT levels bilaterally in the caudate nuclei, a projection site of the DR. For the neuroanatomical studies, three male mice of each strain (20-30 gms) were perfused sequentially with: 0.1 M phosphate buffered saline (pH 7.4; 32°C) and 10% buffered formalin (pH 7.4; 32°C). Adjacent fifty micron sections from the midbrain at the level of the occulomotor complex, through the rostral hindbrain to a level immediately anterior to the appearance of the LC (1.5-1.7 mm) were stained with a TH-antibody using the PAP immunocytochemical procedure. For the biochemical studies six mice of each strain were sacrificed, the brains rapidly removed and micropunches (1.0 mm diameter) of the left and right caudate nuclei were removed and analyzed using HPLC-EC. The round/oval DR cells measured 10-14 μm in diameter and the CBA mice had an estimated 2600 DR cells. The CBA mice had 13% more cells than the BALB/c, however, this difference was not statistically significant. The CBA also had 12% more 5-HT in the caudate nucleus than did the BALB/c (5.39 ± 0.58 vs. 4.83 ± 0.43 ng/mg protein), and this difference was also not statistically significant. There was no left/right caudate differences in 5-HT levels in either strain. In conclusion, the BALB/c and CBA mice differ predominantly in DA neuronal numbers and not in serotonergic or noradrenergic cell numbers. Research supported by grant MH-30546.

76.6 DISTRIBUTION OF TYROSINE HYDROXYLASE POSITIVE NEURONS IN THE BRAIN OF THE GOLDFISH. S. C. Sharma*, Dept. of Ophthalmology, New York Medical College, Valhalla, N. Y.

The regional distribution of tyrosine hydroxylase (TH) positive cells and axonal branches was assessed in the goldfish brain using sheep anti TH antibodies and peroxidase anti peroxidase methods for light microscopy.

A large cell group in the area dorsalis telencephali pars centralis bordering the area dorsalis ventralis pars centralis was observed. Labelled cells were usually small in diameter (5-7μ) with finer branches extending laterally. Fine caliber axons were also labelled in the anterior commissure and in the pars medialis. Further caudally and ventrally, numerous cells in the preoptic recess were labelled. These cells apparently sent axons which innervated the paraventricular area of the hypothalamus. In the diencephalon, cells in the nucleus dorsalis lateralis and medialis showed a very high sensitivity to TH. The axons of these cells were also labelled and were traced to at least three distinct laminae in the ipsilateral optic tectum, e. g. stratum opticum, stratum fibrosum et griseum superficiale (SFGS) and stratum album centralae. These are the same areas in which optic axons terminate.

Axonal branching and fine varicosities were easily recognizable in the SFGS, which has the highest number of labelled axons. Optic axons were not labelled.

Dorsolateral to the medial longitudinalis fascicle, in the isthmal area of the tegmentum, were 10-12 large labelled cells which sent axons both rostrally and caudally. In addition, the mesencephalic and medial reticular formation contained large-size neurons which showed positive TH reaction.

Based upon the distribution pattern of TH labelled cells and comparing this pattern of labelling to that of serotonin in the goldfish brain, it is quite likely that TH labelled cells represent dopaminergic neurons. Supported by N.E.I. 01426.

76.7 INTERACTION OF tele-METHYLIMIDAZOLEACETIC ACID WITH BENZODIAZEPINE BINDING SITES IN RAT BRAIN MEMBRANES. P.Blandina*,S.Maayani and J.P.Green.Dept. of Pharmacology, Mt. Sinai School of Medicine, City University of New York,New York,NY 10029.

All evidence supports the hypothesis that histamine (HA) is a neurotransmitter in brain (Prell and Green,1986,Ann.Rev.Neurosci.,in press).The final product of HA turnover in brain is tele-methyl -imidazoleacetic acid (t-MIAA).Since imidazoleacetic acid (IAA),the peripheral metabolite of HA,acts like GABA on GABA sites,including those associated with benzodiazepine (BZ) binding sites,we examined the effect of t-MIAA on the binding of [3H] flunitrazepam ([3H]FLU) to washed membranes from different brain regions.The experiments were given further impetus by suggestive evidence that the GABAergic and histaminergic systems may be colocalized (Takeda et al.,Proc.Nat.Acad.Sci.USA,1984,81:7647-7650).

t-MIAA (10-100 μM) depressed the affinity of [3H]FLU for its binding sites,both in the presence (10 μM) and absence of GABA. Scatchard analysis showed that the effect of t-MIAA is due entirely to a shift in the affinity of the drug for its binding sites with no change in the number of binding sites.The effect of t-MIAA was observed in hypothalamic,cerebral cortical and cerebellar membranes. HA (100 μM), its immediate metabolite, tele-methyl- histamine (100 μM),IAA (100 μM) and pros-methylimidazoleacetic acid (100 μM) all failed to affect the binding of [3H]FLU.As had been observed by others IAA (100 μM) antagonized the effect of GABA (10 μM) on [3H]FLU binding.Bicuculline (10μM),the GABA antagonist, completely antagonized the effect of 10 μM GABA but failed to affect the action of t-MIAA on [3H]FLU binding.The GABA receptors are part of complex containing receptor sites for GABA,BZ and picrotoxin/barbiturates. Experiments showed that t-MIAA does not interact with picrotoxin sites. The effect of t-MIAA on [3H]FLU binding occurs at a site different from sites affected by bicuculline and picrotoxin.This work also implies that metabolites of transmitters may have functions, functions different from the transmitters.

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76.8 PHARMACOLOGICAL AND NEUROCHEMICAL ACTIONS OF ALPHA-FLUOROMETHYL-HISTAMINE. G.D.Prell, P.Blandina*, S.Maayani and J.P.Green. Department of Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York, N.Y. 10029.

In mammalian brain, histamine (HA) functions as a neurotransmitter. Its functions in the brain are obscure , although evidence suggests that it is likely to have direct (e.g. prolactin release) as well as modulatory (e.g. ACTH release) effects (Prell and Green, Ann. Rev. Neurosci. [in press]). The use of highly selective inhibitors (Kollonitsch et al., Nature 274: 906, 1978) of the HA synthesizing enzyme, histidine decarboxylase, could be important tools in exploring the functions of histamine. Alpha-fluoromethylhistidine (α-FMHis), an irreversible inhibitor, has been extensively used to deplete the brain and peripheral tissues of HA. Alpha-fluoromethylhistamine (α-FMHA), another selective irreversible inhibitor, may not penetrate the blood-brain barrier in vivo in analogy with HA. α-FMHis and α-FMHA could be useful tools to distinguish the central from the peripheral effects of endogenously synthesized (and released) HA. For these purposes, α-FMHA must be devoid of significant effects other than histidine decarboxylase inhibition.

We examined the effects of α-FMHA on brain HA receptors, whole brain HA levels and on HA methylation by histamine N-methyltransferase. α-FMHA was a partial agonist at the H_2 receptor linked to stimulation of adenylate cyclase in guinea pig hippocampal homogenates. Its intrinsic activity was approximately 0.3 relative to HA with a K_B about 100 μM . Administration of α-FMHis (400 μmol/kg,i.p.) to rats markedly reduced brain HA levels after five hours but the same dose of α-FMHA failed to cause significant changes. α-FMHis (up to 1 mM) had no effect on HA methylation by histamine N-methyltransferase, but α-FMHA was a substrate for this enzyme in vitro.

Although these results suggest that α-FMHA has actions in addition to histidine decarboxylase inhibition, the use of α-FMHA in parallel with α-FMHis to distinguish between central and peripheral actions of HA may remain a valid tool if α-FMHA is used at low concentrations. In addition, since histidine may be decarboxylated in vivo by an enzyme(s) other than histidine decarboxylase when given in large amounts (Prell et al., Fed. Proc. 44: 1246,1985), it is feasible that some α-FMHis may be converted to α-FMHA. Additional studies of the actions of α-FMHA and its possible formation from α-FMHis are warranted before these agents can be accepted as specific probes of the histaminergic system.

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76.9 HISTAMINE-IMMUNOREACTIVE SMALL CELLS IN SYMPATHETIC GANGLIA OF THE RAT. H. PÄIVÄRINTA, O. HÄPPÖLÄ, S. SOINILA AND P. PANULA. Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 A, SF-00170 Helsinki, Finland.

Biochemical, physiological and pharmacological studies have provided evidence that histamine may function as a neurotransmitter in the mammalian central nervous system. Immunohistochemical studies have demonstrated the presence of neuronal cell bodies showing histamine-immunoreactivity in the hypothalamic and premamillary areas of the rat brain. Biochemical studies also suggest that histamine is present in the preipheral nervous system, where it has been proposed to have a dual localization: in a mast cell pool and in a non-mast cell pool. Histamine has been shown to affect nervous transmission through sympathetic ganglia. The aim of the present study was to investigate the presence of histamine in the rat sympathetic ganglia by immunohistochemistry.

Adult rats were perfusion fixed with 4 % paraformaldehyde. Cryostat sections were cut and incubated with specific histamine antiserum. The indirect immunofluorescence method was employed.

Histamine-immunoreactive small cells were observed in the superior cervical ganglion and in the coeliac- superior mesenteric ganglion complex. Mast cells were also often detected in the ganglia but they were clearly distinguished from the small immunoreactive cells due to their large diameter and granular cytoplasmic immunoreaction. The small immunoreactive cells, 10-20 μm in diameter, were either solitary or, more often formed clusters. The immunoreactive cell clusters were often around, or in close contact with blood vessels. The principal nerve cells were distinguished as large cells, 30-60 μm in diameter, with no detectable immunoreaction. No histamine-immunoreactive nerve fibres were observed. The specific immunoreaction of the small cells and the mast cells was completely abolished by absorption with 50 μM histamine.

Due to close morphological similarities, small size and high nucleus/cytoplasm diameter ratio, it is concluded that the small histamine-immunoreactive cells represent small intensely fluorescent (SIF) cells first detected by formaldehyde-induced fluorescence method.

76.10 THE CENTRAL CARDIOVASCULAR ACTIONS AND BLOCKING EFFECTS OF TWO NEW H2 ANTAGONISTS: BMY-25405 and BMY-25368 IN THE CONSCIOUS FREELY MOVING RAT. J.J. Poulakos*, E.N. Gerges* and S.B. Gertner* (SPON: R.D. Howland).Dept.of Pharmacology, N.J. Medical School, UMDNJ, Newark, N.J. 07103

Histamine and cimetidine produce a dose dependent pressor response and bradycardia when administered intracerebroventricularly (ICV) to conscious freely moving rats. The experiments reported here were undertaken to study the cardiovascular actions of other H2 antagonists as well as their ability to block H2 receptors in the CNS. Two new H2 antagonists: BMY-25405 and BMY-25368 which are selective and long acting when tested on atrial muscle and gastric acid secretion, were administered ICV to conscious freely moving Sprague-Dawley rats. Graded doses of BMY-25405 and BMY-25368 were given ICV and challenged 15' later with 25ug ICV of the selective H2 agonist impromidine (IMP), to determine the cardiovascular H2 blocking activity of these compounds. Since centrally administered IMP causes desensitization of the pressor response upon repeated injection, (Poulakos & Gertner, Frontiers in Histamine Research Symposium, Jouy-en-Josas, France, 1984 P.67), control responses of IMP were obtained 24 hours after the first injection, when there is full recovery of the response. BMY-25405 produced dose dependent pressor responses with 50ug, 100ug and 200ug doses (11.2±4.7, 13.6±2.5 and 20.8±2.1 mmHg rise in mean arterial pressure (MAP)), which recovered in 10 minutes. The bradycardia induced by ICV BMY-25405 was also dose related but reached a plateau at the 100ug and 200ug doses (25.2±10.3, 61.5± 8.0 and 47.2±12.6 beats per minute (BPM)). More importantly, BMY-25405 totally blocked a challenge dose of 25ug IMP 15' after ICV administration of the 200ug dose. Unlike BMY25405, graded doses of BMY-25368 (10ug, 25ug and 50ug) did not produce graded pressor responses (34.6±2.5, 42.1±2.5 and 30.1±2.8 mmHg increases in MAP). Heart rate decreases were also equivalent (67.3±7.2, 77.6±9.6 and 73.0±7.0 BPM). These three doses of BMY-25368 failed to block a challenge dose of 25ug IMP given 15' later. These cardiovascular effects are of central origin since the intravenous administraion of 25ug, 100ug and 500ug doses of BMY-25405 or BMY-25368 produced a tachycardia and had no effect on MAP. The aforementioned results illustrate that BMY-25405 and BMY-25368, like the H2 antagonist cimetidine, cause a pressor response and bradycardia upon ICV administration. However, only BMY-25405 shows the ability to block the centrally elicited pressor response of the H2 agonist IMP and is therefore working by blocking the central cardiovascular H2 receptor.

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STRUCTURE AND FUNCTION OF IDENTIFIED CELLS I

77.1 EFFECTS OF AN ORGANIC SOLVENT ON NERVE CELLS IN VITRO AND IN VIVO. S. Jacobson, R. Auerbach*, D. Albertini*, Departments of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston, MA. 02111.

Organic solvents are one of the most common sources of pollutants in our environment and exposure to these compounds produce physiological and behavioral dysfunction. In order to determine the effects from exposure to these agents on the organelles and cytoskeleton of neurons we have exposed mammalian nerve cells to an organic solvent trichloroethylene (TCE).

Adult albino rats were exposed to TCE in an environmental chamber for 1-2 hrs for 5-10 days. Pregnant albino rats were also exposed to the TCE in the chamber. The levels of TCE in the chamber were 280-670 ppm.

The brains from the non-pregnant animals were removed after perfusion fixation and prepared with Nissl, H&E, Myelin, Acetylcholinesterase and Golgi Neuronal stains. The brains from the exposed fetuses (E14-17) were mechanically dissociated and placed in tissue cultures. The media consisted of DMEM supplemented with amino acids, vitamins, L-Glutamine, PS, Ara C, Sodium Pyruvate, and Fetal Calf Serum.

Brain cells from normal pregnant animals were isolated from fetuses (E14-18) and newborn pups and mechanically separated and exposed to TCE dissolved in DMSO at 100-500 or 1000 ppm. The tissue cultures were fixed and stained with monoclonal antibodies to identify neurons, glia and fibroblasts.

The exposure to TCE in the chamber produced a light to moderate anesthetic affect. All the animals that were exposed to TCE developed increased respiration and hypersensitivity. The neurons from pups exposed to TCE on E14-15 and their controls were placed in tissue culture. The cells from the exposed pups demonstrated more neuritic processes and did not aggregate. In order to more accurately document the formation of processes we are undertaking scanning electron micrographic analysis (SEM) of the cells in tissue culture. The brains from the adult animals exposed to TCE in the chamber are currently being examined.

The major affect we have noted on cells has been an increase in neuritic processes as a consequence of exposure to TCE in utero. The SEM in conjunction with the monoclonal antibody technique should permit determination of the cell types producing the neurites and other cell to cell interactions which are affected by TCE exposure. We are currently analyzing neurons with the other neurohistological methods to determine if there are other changes in the cell surface or in the organelles. (Supported by funds from The Center for Environmental Management Tufts University).

77.2 VESICULAR PROFILES IN RAPID-FROZEN, FREEZE-SUBSTITUTED FROG PERINEURIAL CELLS. C.H. Latker*, K.J. Lynch*, N.L. Shinowara and S.I. Rapoport (Spon: R. Burke) Lab. of Neurosciences, National Institutes on Aging, NIH. Bethesda, Maryland 20205.

The perineurial cells in the sciatic nerve of the frog contain numerous vesicular profiles throughout their cytoplasm. In order to determine if these vesicles are influenced by chemical fixation, we examined rapid-frozen, freeze-substituted, as well as glutaraldehyde-fixed frog perineurium. We also compared nerves that were rapid-frozen or fixed immediately after they were removed from the animal, with nerves kept in Ringers for several hours prior to fixing or freezing.

The frogs were divided into two groups. In group I, the nerves were rapid-frozen on a Polaron quick freeze unit either immediately after removal or after five hours of incubation. In group II, the nerves were chemically fixed either immediately after removal or after five hours of incubation. All tissues were processed for electron microscopy.

Vesicular profiles were present in the perineurial cells in all experimental groups. Some of the vesicles opened to the surface while others appeared to be isolated profiles within the cytoplasm. No difference was observed in the morphology of the individual vesicles in nerves frozen after incubation and in nerves frozen immediately. However, there was a characteristic rosette-like configuration of fused or linked vesicles observed in the incubated, rapid-frozen cells. These were not as frequently seen in the immediately frozen cells. In the chemically fixed tissues vesicles were always present, but rosettes were rare. Vesicular profiles were spherical, but more variable in shape than in rapid-frozen cells, and caveolae usually were connected to the surface membrane by longer neck regions than in the frozen perineurium.

Aldehyde fixation is slow and does not stabilized all membrane constituents at the same time. The ultrastructure of tissues that have been well frozen and freeze-substituted is presumed to be more representative of the in vivo configuration than is the ultrastructure of fixed tissues. The present study shows that vesicular profiles are prominent in frog perineurial cells in rapid-frozen as well as in chemically fixed tissues. It can be concluded, therefore, that vesicular profiles are a normal constituent of perineural cells and do not result from chemical fixation. However glutaraldehyde fixation may modify their size, shape and number.

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77.3 COMPARISON OF CYTOSKELETAL ELEMENTS IN RAT PHEOCHROMOCYTOMA CELLS (PC12) AND A VARIANT PC12 SUBLINE IN CULTURE. K.J. Daniels*, I. Kim*, N.J. Pantazis, and A. Sandra*. (SPON: A.K. Afifi) Dept. of Anatomy, Univ. of Iowa Medical College, Iowa City, IA 52242.

In comparison with primary cells in culture, transformed cells characteristically exhibit a less ordered cytoskeleton with fewer stress fibers, microfilaments, microtubules, and loss of adhesion plaques. Typically, therefore transformed cells are anchorage independent and grow quite well in suspension. Changes in the rates of synthesis of the major cytoskeletal proteins have also been reported for many cell lines compared to primary cells. Since substrate attachment may play a key role in the expression of cytoskeletal elements, the present study explores possible differences in cytoskeletal organization and/or protein composition in substrate-attached versus non-attached transformed cells.

The PC12 cell line, cloned from a rat pheochromocytoma (Greene, L.A. and Tischler, A.S., Proc. Natl. Sci. USA, 73:2424, 1976) grows well in suspension. In response to nerve growth factor, NGF, the PC12 cells will attach to a substratum. In addition, NGF will induce changes in various cytoskeletal elements including actin organization and neurite outgrowth. Recently, Kim and Pantazis (see abstract this meeting) observed that with time in culture, a substantial number of PC12 cells attached to the flask. The PC12 cells were therefore divided into two sublines PC12A which readily grew attached to the flask; and PC12F which, more typical of PC12 cells, did not attach and grew in suspension. Other variants of PC12 cells which also attach have been reported (Bothwell et al., Cell 21:857) but cytoskeletal changes have not been examined in any of these PC12 variants.

Homogenates of PC12A and PC12F cells were examined on two dimensional polyacrylamide gel electrophoresis using Coomassie blue staining. In the PC12A cells there was a qualitative increase in the amounts of α and β tubulin and isoactins compared to PC12F cells. Vimentin, an intermediate filament protein, was lacking in both cell types, a result which has been previously observed.

In conclusion, preliminary gel analysis indicates that there are significant cytoskeletal differences between the two PC12 cell sublines. Whether or not these cytoskeletal differences are involved in or a result of substrate attachment is unknown. Future work will examine the organization of the cytoskeleton using indirect immunofluorescence against the major cytoskeletal proteins: actin, tubulin and intermediate filament proteins. Rates of synthesis of cytoskeletal proteins will be determined by ^{35}S-methionine incorporation followed by two dimensional electrophoresis, autoradiography and scintillation spectrometry. (Supported by NIH grant GM28644 to N.J.P.).

77.4 A RAPID GOLGI METHOD FOR STAINING EXPLANT OR DISSOCIATED CELL CULTURES OF CEREBELLUM. A.P. Oliver and U. Vaidya. Neuropsychiatry Branch, NIMH, Intramural Research Program, Saint Elizabeths Hospital, Washington, D.C. 20032.

Morphological characterization of cells as they differentiate in culture is an important aspect of development and intercellular relationships. The Golgi technique is an excellent means for demonstrating the morphology of neurons, but usually involves extended times for impregnation. We have developed a rapid Golgi method requiring approximately 2.5 hours suitable for explant or dissociated cell cultures of cerebellum based on a standard Golgi technique (Gabbott and Somogyi, J. Neurosci. Meth. 11:221-230, 1984). Rat E20 cerebellum explants (100 μm thick) or dissociated cells were cultured on collagen coated dishes using culture techiques and media described by D.L. Gruol in 1983. At various times after culturing, up to 1 month, cultures were fixed in 4% gluteraldehyde and 2% paraformaldehyde mixture in 0.1 M phosphate buffer pH 7.3 followed by phosphate buffer rinse (15 min.). Cultures were then post fixed in 1% osmium in phosphate buffer for 5 min. and rinsed. Cultures were immersed in 2% potassium dichromate for 35-45 min., rinsed with distilled water and placed in 0.5% silver nitrate for 1-1.5 hours. Cultures were gold toned in 0.2% gold chloride (rinsed 5-10 sec.) and placed in phosphate buffer. Differentiated bipolar, multipolar and Purkinje like cells seemed to selectively stain although some neuroglia were also impregnated. Cell bodies, axons and dendrites with primary, secondary and tertiary processes could be clearly distinguished. The speed, convenience and simplicity of this techique make it an excellent method for examining the development of neurons in culture.

Supported by NIMH.

77.5 SELECTIVE UPTAKE BY PURKINJE NEURONS OF ANTIBODIES TO S-100 PROTEIN. S.E. Karpiak, M.M. Rapport & S.P. Mahadik. Div. of Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, Biochemistry & Molecular Biophysics, College of Physicians & Surgeons, Columbia U., 722 W 168th St., NY, NY. 10032.

A recent study describes the selective extraction of both large and small molecules by cerebellar Purkinje cells [1], and this process may account for the susceptibility of these neurons to various pathological agents [2]. In connection with our studies of the effects on CNS function of antibodies directed against neural antigens, we studied the localization of several rabbit antibodies in vivo. We found that affinity purified antibodies to S-100 protein localized in Purkinje cells whereas affinity-purified antibodies to goat-immunoglobulin G or to bovine serum albumin did not. Male Sprague-Dawley rats, anesthetized with ether, were injected (intaventricularly) unilaterally into the lateral ventricle with 5µg (in 20µl) of purified antibodies labled with [125]I (0.22µCi/ug protein). Forty-eight hours later, the rats were perfused with fixative transcardially. Brains were embedded in paraffin and coronal sections of 10um were cut selectively throughout the brain. Slides were examined by exposure to photographic nuclear emulsion for 3-4 mos.

In rats injected with antibody to S-100, intense localization was seen in Purkinje cells of the cerebellar vermis. The localization was primarily in the cell body, with further localization extending for a distance of 25-30µm into the axon. No localization was seen with antibodies either to BSA or goat immunoglobulin. No other (intracellular) localization was seen in other brain regions with any of the antibodies. Our study suggests that with antibodies Purkinje cell uptake is selective and probably antigen dependent. While the uptake by Purkinje neurons of molecules like propidium iodide [1] results in motor disturbances (tremor, ataxia & nystagmus), the uptake of antibodies directed against specific antigens may result either in less generalized or more subtle deficits. Although animals used in the localization studies were not studied for behavioral effects, we have shown that the intraventricular injection of antibodies to S-100 interferes with learning [3].

1. Borges et al. Science 228:346-348 (1985).
2. Pentney. Brain Research 249:397-408 (1982).
3. Karpiak et al. Brain Research 102:313-321 (1976).

77.6 PRODUCTION AND CHARACTERIZATION OF A MONOCLONAL ANTIBODY THAT SELECTIVELY LABELS MOSSY FIBER BOUTONS. B.D. Boss. The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

I recently reported success in generating a panel of mouse IgM monoclonal antibodies against cultured rat dentate granule cells using a simplified in vitro immunization procedure [Boss, B.D. (1984) Brain Res. 291:193-196]. Among this panel of monoclonal antibodies, one termed G2 has been found to selectively label mossy fiber terminal boutons both in vivo and in vitro. Immunoperoxidase localization of G2 reactivity in vivo closely resembles the reactivity of hippocampal tissue to Timm's silver sulfide stain. Interestingly, intracisternal injection of 48 µg colchicine 48 hours prior to tissue fixation appears to enhance the terminal bouton immunoreactivity of G2 (as compared to saline controls). In addition, G2 immunoreactivity is preserved by perfusion with Carnoy's solution, a fixative commonly used in conjunction with Timm's silver sulfide stain.

Since this monoclonal antibody reacts equally well with monkey and rat tissue, the G2 antigen appears to be highly conserved. Ideal fixation conditions for the rat antigen appear to be 3.5% paraformaldehyde pH 7.4 for 30 minutes with no postfixation or detergent treatment. While frozen sections of monkey brain react well with G2, freezing rat tissue greatly diminishes G2 immunoreactivity. Electron microscopic visualization of the reaction product indicates the G2 antigen surrounds the numerous synaptic vesicles found in mossy fiber terminals.

In culture, G2 immunoreactivity is confined to bouton-like structures located along nerve fibers and neuronal cell bodies, most probably reflecting the rearrangement of synapses known to occur in vivo when the normal target cells of granule neurons are removed. Growth of granule neurons in chemically-defined N2 medium [Bottenstein, J.E. and Sato, G.H. (1979) Proc. Natl. Acad. Sci. U.S.A. 76:514-517] enhances G2 immunoreactivity. It is not known whether this enhancement is an indirect consequence of the increased neuronal clustering seen in N2 medium. Electron microscopic localization of the immunoreactivity in vitro shows it to be identical to the in vivo reactivity (i.e., surrounding synaptic vesicles).

When rat hippocampal homogenates are separated by SDS-PAGE and blotted onto nitrocellulose, one band of G2 immunoreactivity stains heavily at approximately 70,000 Mr. Homogenates of rat cerebral cortex, olfactory bulb and cerebellum contain relatively smaller amounts of this protein, verifying the sparse to undetectable nature of G2 reactivity found immunohistochemically in these other areas of the brain.

The time course for appearance of G2 immunoreactivity in vivo appears to parallel the development of mossy fiber terminals, being present by postnatal day 6 (earliest tested) and increasing with age over the next few months.

77.7 A MONOCLONAL ANTIBODY THAT STAINS THE PERIMETER AND GOLGI APPARATUS OF SUBPOPULATIONS OF NEOCORTICAL AND CEREBELLAR NEURONS. P.E. Marshall*, L.M. Hemmendinger, A.M. Boyer*, M. Yamamoto & V.S. Caviness, Jr. Dept. of Neurol., Mass. Gen. Hosp., Boston, & E.K. Shriver Ctr., Waltham, Mass.

Monoclonal antibody 4F4 was prepared against whole cells from E15-17 rat cortex in Balb/c mice. The distribution of antibody staining in the cortex and cerebellum of adult mice was mapped on paraformaldehyde-fixed sections using direct and indirect labeling methods. Two patterns of intracellular staining can be distinguished. Some neurons demonstrate intense staining concentrated at the perimeter of the perikarya and dendrites, with punctate intracellular staining. Other cells show only diffuse or punctate intracellular staining.

At the electron microscopic level, the two patterns of cellular staining described above can be related to the intracellular distribution of the 4F4 antigen. In cells with intense staining around the perikaryon and dendrites, deposition of reaction product occurs just inside the plasma membrane as well as in the Golgi apparatus. Cells with diffuse or punctate staining have smaller amounts of reaction product associated with the Golgi apparatus only.

The majority of neurons in all neocortical regions contain the diffuse pattern of intracellular 4F4 staining. Neurons with intensely stained perimeters constitute subpopulations which vary in their distribution within the different cortical areas. In parietal fields these neurons are concentrated in two zones corresponding to layers III-IV and lower V-VIa. In contrast, the intensely stained neurons have a wider distribution in cingulate cortex and are found throughout layers II-VIa. In motor cortex and other frontal fields, intensely stained neurons are found primarily in lower V-VIa. This distribution continues throughout occipital and temporal fields, but fewer intensely stained neurons are found.

In the cerebellum, intense staining is seen in Golgi II neurons and in the deep cerebellar nuclei. All Purkinje cells and some stellate cells demonstrate diffuse staining only. Basket and granule cells are not stained.

The 4F4 antigens have been identified by thin layer chromatographic and Western blotting techniques as a glycolipid and glycoprotein in the embryonic brain (Schwarting et al., unpublished) and as a 150-250 kD glycoprotein in adult cortex. The structure of the carbohydrate epitope has been identified by comparison with a standard glycolipid isolated from human sciatic nerve (Chou et al. Biochem. Biophys. Res. Comm. 128, 383-388, 1985).

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77.8 MONOCLONAL ANTIBODIES TO NEURAL ANTIGENS IN ASCARIS GENERATED AFTER SPECIFIC IMMUNOSUPPRESSION. Paisarn Sithigorngul*, Carl D. Johnson*& Antony O.W. Stretton
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We have used the immunosuppression technique pioneered by Matthew and Patterson (1983, CSHSQB XLVIII,635) to increase the yield of monoclonal antibodies against neural antigens. Pure neural tissue from Ascaris is hard to obtain because of the close association of the nervous system with other tissues, especially hypodermis and muscle. We therefore immunized mice with homogenates of dissected muscle and/or hypodermis and after two days injected 0.8 mg cyclophosphamide to suppress the cells stimulated by the primary injection. The mice were then immunized with dissected nerve cords (which included about 80% muscle, 10% nerve cord, and 10% hypodermis) or with nerve cord obtained after removal of muscle with collagenase (50% nerve cord and 50% hypodermis). After boosting with the same antigen hybridomas were generated and screened using ELISA, frozen sections and whole mount preparations from collagenase-treated worms. Compared with our previous experiments in which nerve cords dissected from collagenased worms were used as immunogens (Soc. Neuroscience Abstr. 9, 302) the number of clones producing antibodies that bound to hypodermis was much reduced; furthermore the antibodies that bound to neurons show much lower background binding than those we described earlier.

We obtained a number of antibodies which label all neuronal cell bodies and have virtually no reactivity toward hypodermal antigens. Other antibodies react with the surfaces of all neurons. These antibodies have been used to locate and identify all of the neurons in adult female Ascaris. They have been particularly useful for analyzing the anatomy of neurons located in the tail (30 cells in the female) and for neurons whose cell bodies are in the lateral lines in the body (7 bilaterally symmetrical pairs of cells distributed over a distance of 20-30cm).

Other interesting antibodies isolated from these fusions react with subsets of neurons. These include one which labels only a single bilaterally symmetrical pair of neurons in the anterior lateral ganglion and several antibodies which label the nuclei of subsets of neurons.

Supported by NIH grant AI 20355

77.9 ULTRASTRUCTURAL DISTRIBUTION OF CALCIUM WITHIN NEURONS: AN OXALATE PYROANTIMONATE STUDY. M. Mata, J. Staple*and D.J. Fink. Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105

Calcium appears to play a critical role in a number of normal neuronal cell functions as well as in pathologic studies. In order to define the role of calcium, we undertook to determine the ultrastructural distribution of calcium within neurons of the rat sciatic nerve using the oxalate-pyroantimonate technique. In this method oxalate is used to chelate the calcium while other cations are rinsed out prior to precipitation with pyroantimonate. Both stained and unstained grid sections were examined in a JEOL 100S electron microscope.

We used 2 controls to determine that the precipitates represent calcium pyroantimonate. In the absence of pyroantimonate treatment no precipitates occurred, and washing the grids with 10mM EGTA for 1 hour removed precipitates. X-ray microanalysis using a KEVEX energy dispersive spectrometer with deconvolution of the spectrum obtained showed a calcium peak. Analysis by electron spectroscopy imaging with a Zeiss 902 EM confirmed the calcium distribution.

In cell bodies of the dorsal root ganglia, calcium precipitate was found in the nucleus but not in the nucleolus, in the golgi, mitochondria and large vesicles but not lysosomes, and prominently absent from regions of rough endoplasmic reticulum and ribosomes. A similar distribution was found in non-neuronal cells.

At the neuromuscular synapse in the gastrocnemius muscle calcium was also seen in mitochondria, and a dense precipitate was found in every synaptic vesicle. While the synaptic cleft itself appeared free of calcium, there was prominent precipitate in the muscle just underneath the post-junctional folds.

Within the axon itself precipitate was also found sequestered in mitochondria and smooth endoplasmic reticulum. In addition to precipitate sequestered in organelles, precipitate appeared diffusely through the axoplasm with a gradient of distribution. Precipitate appeared uniform in unmyelinated fibers but in myelinated fibers the amount of precipitate decreased predictably in the axoplasm beneath the Schmidt-Lantermann clefts and in the paranodal regions at the nodes of Ranvier. This correlated with the presence of dense precipitate in the Schmidt-Lanterman clefts themselves and in the paranodal loops of myelin.

Intracytoplasmic ionic calcium is maintained at 10^{-7} M by balanced processes of influx, sequestration and extrusion of calcium. The irregular distribution of calcium precipitate in the axoplasm of myelinated fibers suggests that there may be specific regions of preferential efflux across the axolemma. The relationship of these results to previous results using methods that measure calcium binding sites or calcium sequestration will be discussed.

77.10 X-RAY MICROANALYSIS OF INTRACELLULAR CALCIUM IN RAT PERITONEAL MAST CELLS. A. Angel*, R.M. Kriebel, and J.F. Fiekers (SPON: S. Robison). Dept. of Anatomy and Neurobiology, Univ. of Vermont Col. of Med., Burlington, VT, 05405.

It is well known that calcium has a major role in the initiation, regulation and control of numerous cell functions. However, the ultrastructural localization of intracellular stores of calcium and their involvement in cell function is not well understood. X-ray microanalysis has been used in these studies to examine intracellular calcium location within mast cells. Peritoneal rat mast cells were obtained by abdominal lavage with Locke's solution and purified by density gradient centrifugation through 24% metrizamide. After washing, the pellets containing >90% mast cells were fixed in 2.5% phosphate buffered glutaraldehyde. The cells were washed in buffer, postfixed in 2% osmium tetroxide, rapidly dehydrated and embedded in epon. Thin sections were mounted on copper or aluminum grids and studied in a JEOL 100CXII STEM equipped with a Tracor Northern energy dispersive spectrometer. Energy spectra were collected by either spot analysis or rastering the beam over defined regions of cells. In control preparations, microanalysis of intracellular granules within mast cells yielded energy spectra demonstrating prominent peaks for calcium. In addition, peaks of sulfur corresponding to mucopolysaccharides within granules were also shown. Compound elemental mapping for calcium and sulfur verified their association with granules. Analyzed areas of mast cell nuclei as well as eosinophils generated spectra with insignificant or reduced peak intensity for calcium. Pretreatment of purified cells with EGTA in zero calcium Locke solution was used to produce "chelated mast cells". Significant decreases in calcium peak intensity were observed following this perturbation. To explore the potential role of calcium in receptor-mediated events, cell suspensions similarily prepared were exposed to either 48/80 or substance P prior to fixation. It has been shown that chemical fixation is problematic in the maintenance of intracellular elemental content and interpretation of such studies needs to be made with caution. Physical fixation procedures have been used to optimize conditions for minimal extraction and translocation of substances. The chemical and microanalytical methodologies applied in these studies, without the expense of cryo-preparative equipment, have provided a successful preliminary assessment of intracellular calcium distribution and provide the basis for future comparison with results obtained under similar conditions on quick frozen preparations. (supported by PHS-5429-23-13).

77.11 ULTRASTRUCTURAL LOCALIZATION OF A CALCIUM-ACTIVATED PROTEASE (CALPAIN) IN RAT CNS: ASSOCIATION WITH MICROTUBULES, MITOCHONDRIA, AND SYNAPTIC ELEMENTS. L.S. Perlmutter, C. Gall, R. Siman and G. Lynch. Depts of Psychobiology and Anatomy, Univ. of Calif., Irvine, CA 92717.

The importance of calcium-dependent proteases as modulators of both normal and pathological cellular metabolism has recently received much attention (Trends in Biochem. Sci., 8:167, 1983). Calpain (the family of proteases) has been implicated in such diverse phenomena as the degradation of neurofilament, myelin and myofibrillar proteins, the aggregation of platelets, and shape changes in erythrocytes and dendritic spines. Research on the enzyme has thus far been principally biochemical; little work has been done on its actual anatomical distribution and subcellular localization. To maximize our understanding of its ultrastructural localization, both monoclonal and polyclonal anti-calpain I, as well as pre-embed and post-embed immuno-electron microscopic techniques, were employed. Regions of the spinal cord, olfactory bulb, and the hippocampal formation were chosen for study.

Young adult rats were perfused with 2-4% paraformaldehyde, 0.1-0.4% glutaraldehyde (pre-embed) or 1-2% paraformaldehyde, 0.2-2.5% glutaraldehyde (post-embed). For pre-embed, sections were prepared on a freezing microtome or vibratome and incubated in either monoclonal or polyclonal anti-calpain I. For post-embed, tissue was first embedded in LR White Resin and the immunocytochemistry carried out directly on the thin sections. The biotin-avidin-peroxidase system (Vectastain) was used throughout. Both types of antibody and both methods yielded virtually identical results, which were seen across brain regions. Reaction product was found to decorate both axonal and dendritic microtubules, the outer surface of mitochondria, many postsynaptic densities, and, occasionally, synaptic vesicles and presynaptic terminals. The apparent association of calpain with the microtubules suggests a role for this enzyme in intracellular transport; it also suggests that the protease may be involved in the cytoskeletal disarray seen in degenerative diseases such as Alzheimer's. The sporadic occurrence of immunoreactive presynaptic terminals and postsynaptic densities may reflect either the vagaries of the technique (although this possibility is lessened due to the use of both pre- and post-embed methodologies), the very specific localization of this antigen to these regions, or, more plausibly, the blocking of antigenic sites due to interactions with other molecules (Neurosci, 10:449, 1983). Thus, calpain immunoreactivity may be a reflection of activational state. If this is correct, it suggests a role for calpain in neurotransmission and perhaps in the synaptic plasticity known to occur in some of these regions. (Supported by MH19793-12 (GL) and BNS8200319 (CG))

77.12 CO-LOCALIZATION OF A CALCIUM ACTIVATED PROTEASE (CALPAIN) AND ONE OF ITS SUBSTRATES (FODRIN) IN THE RAT CENTRAL NERVOUS SYSTEM. M.B. Passani*, C. Gall, R. Siman, L. Pearlmutter and G. Lynch. Depts. of Psychobiology and Anatomy, Univ. of Calif., Irvine, Ca., 92717.

Recent studies have shown that fodrin or brain spectrin, a high molecular weight protein that cross-links cytoskeletal elements, is an excellent substrate for calcium activated proteases (calpains) (Proc.Nat.Acad.Sci.,1984,81). This provides a mechanism through which transient changes in intracellular calcium levels could produce structural modifications of neurons as well as of the organization of membrane surface proteins (Science,1984,224). In support of this, evidence has been reported that degradation of fodrin by calpain in synaptic membrane fractions results in an increase in the number of binding sites for a putative neurotransmitter (Nature,1985,313). Given the evidence that both calpain (J. Neurochem.,1983,41) and fodrin(Siman et al,unpublished) exist in multiple pools,it seems likely that their interaction is involved in a number of cellular processes. In considering this question it will be useful to have information on the extent to which the enzyme and substrate covary in concentration across cell types and brain regions. The present experiments were directed at this question.

Young adult rats were intracardially perfused with 4% paraformaldehyde and 30 um brain sections were incubated for 48 h at 4^0 C in both monoclonal anti-calpain I and polyclonal anti-fodrin. FITC-conjugated and rhodamin-conjugated secondary antibodies were used. Immunoreactivity of both antibodies was visualized on the same section.

Calpain-like and fodrin-like immunoreactivity were co-localized in cell bodies and dendrites of neurons in many brain regions including cerebellar Purkinje cells, deep cortical pyramidal cells, substantia nigra pars compacta, subiculum and spinal motor neurons. Certain cell groups including the granule cells of the cerebellar cortex exhibited only very weak staining to either antibody. Finally, some cell groups contained intense fodrin-like but little calpain-like immunoreactivity (i.e. hippocampal and certain cortical pyramidal cells.)

Biochemical experiments have demonstrated the existence of both soluble and membrane-associated calpain in the brain (J.Neurochem. 1983, 41); it is possible that the surprising discrepancy in calpain versus fodrin concentration found in some cell types is due to the absence of one of these pools. It has been suggested that the soluble (and larger) fraction of neuronal calpain is involved in the turnover of cytoskeletal elements; if this idea were correct, then the present results might indicate that significant differences exist between neurons in the rate at which skeletal elements are broken down and replaced.
(Supported by NIMH Grant MH19793-13 to G.Lynch.)

NEUROETHOLOGY I

78.1 A QUANTITATIVE STUDY OF THE VARIANTS OF CRAWLING IN THE LEECH. W. Stern-Tomlinson, M.P. Nusbaum, L.E. Perez* and W.B. Kristan, Jr. Dept. of Biology, Univ. of Ca. San Diego, La Jolla, Ca. 92093.

We have done a parametric study of crawling in leeches, as a prelude to an investigation of the neural basis for this behavior, including the neural mechanisms underlying behavioral modifiability. By using this data as a control, we will then be able to specify the behavioral abnormalities induced by tethering, reduction, and dissection in the neurophysiological preparation.

The leech, Hirudo medicinalis, is an amphibious, segmented worm which can crawl in two related ways: vermiform crawling and inchworm crawling (looping). The active components of either variant are rostrocaudal body extension and fixation of the anterior sucker, followed by rostrocaudal body shortening and fixation of the posterior sucker. There are also two quiescent components: a post-extension pause and a post-shortening pause. These four components comprise a cycle. The qualitative difference between the two variants is the final position of the posterior sucker; in inchworm crawling, the sucker is fixed at a position close to the head, resulting in arching of the body, whereas in vermiform crawling, this sucker is placed at some distance from the head, and the body remains parallel to the substrate.

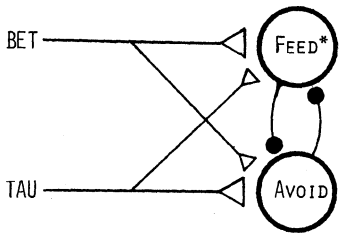
From videotaped behavior, we measured at intervals the absolute position of the head and tail, and the lengths of five body sections delineated by small beads sewn along the lateral aspects of the body. Subsequent analyses were done from this data. The results of comparison between the two crawl variants show some differences:

1. The duration of a cycle (the period) and the distance traveled per cycle are greater for inchworm crawl than for vermiform crawl. (The velocity along the substrate is the same for both.)
2. For both variants, the durations of the crawl components usually co-vary with the period, the exception common to both being the duration of the post-extension pause in the two most posterior body sections. The difference between the crawl types is that the duration of extension in the most posterior body section is independent of period for inchworm crawl, and not for vermiform crawl.
3. The onsets of post-extension pause, shortening, and post-shortening pause occur earlier in the cycle for inchworm crawl in the most posterior body section. Post-shortening pause occurs earlier for inchworm crawl in the two most anterior body sections as well.

We are now testing the influence of sensory input on crawling by doing similar analyses on data from animals in which part of the peripheral innervation has been eliminated.
Supported by the Whitehall Foundation, PHS NS14410, and PHS 5-T34-GM08035.

78.2 MIXED SIGNALS IN CHEMOSENSORY REGULATION OF FEEDING BEHAVIOR: MOTIVATION TIPS THE BALANCE IN PLEUROBRANCHAEA. R.-C.Huang and R.Gillette. (SPON: F. Delcomyn). Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801

We found that betaine (trimethylglycine; BET) was the most effective amino acid stimulant of feeding behavior in P. californica (10^{-6}-10^{-3}M) followed by cysteine, then glycine. Surprisingly, taurine (TAU: 10^{-5}-10^{-3}M) elicited strong aversive behavior. Both amino acids are present in high concentrations (>10 mM) in many animals acceptable as prey. In their behavioral responses to TAU, animals fell in two groups. One group showed aversive responses alone and was associated with high feeding thresholds to BET; the second showed initial transient feeding responses to TAU followed by avoidance, and had low BET thresholds. When animals were tested with mixtures of various dilutions of BET + 1 mM TAU, feeding thresholds for BET were raised significantly. In these experiments BET did not suppress the aversive response to TAU unless active and overt feeding was elicited; in this situation, avoidance always followed the cessation of feeding. That is, feeding behavior appears to suppress avoidance and vice versa. Satiation was found to raise the feeding threshold to BET, but did not effect the TAU threshold for aversion. BET alone can elicit avoidance in some satiated animals. These findings suggest a neural model for the organization of feeding behavior in which sensory pathways for BET strongly excite a central pattern generator (CPG) for feeding and also excite the CPG for avoidance. TAU pathways may weakly excite the feeding CPG and strongly excite that for avoidance. Feeding and avoidance CPGs are mutually inhibitory, and the normal expression of the two is governed by a motivational factor setting the arousal level in the feeding CPG. Our findings depict a type of structural organization of behavior likely to be widespread among animals.



*Arousal

78.3 DESCRIPTION OF A NEWLY-DISCOVERED SPONTANEOUS BEHAVIOR, "RESPIRATORY PUMPING SEIZURE", IN APLYSIA. J. E. KANZ, W. D. QUAST*. Marine Biology Department, Texas A&M University at Galveston, Galveston, TX. 77553.

We report the discovery of a previously unreported stereotyped neuronal/behavioral event in the Gastropod Mollusc, Aplysia californica, termed "respiratory pumping seizure" (RPS). Each RPS consists of a long series of single respiratory pumping (RP) responses (totally 20-60) with an initially very short inter-response interval ranging from 10-15 seconds (measured onset-to-onset) that increases, in a regular fashion, to 5-10 minutes as the seizure progresses. The seizure's duration, number of RP responses and progression of inter-response intervals were reasonably stereotyped for a given animal.

RP seizures occur infrequently (0-3 per 24 hrs.) under ambient normoxia, and are usually initiated while the animal is quiescent. Long-term videotape and in vivo extracellular electrophysiological monitoring of intact freely-behaving Aplysia also revealed other forms of spontaneously occurrring RP activity including 1) RP doublets and triplets (i.e., 2 and 3 RP responses, respectively, with inter-response intervals of $\leq$ 10 seconds) 2) abbreviated RP seizures (i.e., a temporal sequence of RP responses identical to that of an RPS but shorter in duration and with $\leq$ 10 individual RP responses), and 3) a double RPS (i.e., 2 full RP seizures, one after the other).

Long-term ambient hypoxia (1.2 - 2.7 ppm O_2) depressed all forms of RP except for an increase in the rate of single RP responses as hypoxia was first developing; the rate of single RP responses decreased following this initial elevated rate. Therefore, there was no correlation between the degree of respiratory stress (dissolved O_2 level) and a hierarchial display of RP activity (i.e., from single RP responses to RP doublets/triplets, to abbreviated seizures to single and double RP seizures).

In one in vitro experiment involving an isolated abdominal ganglion, an RPS was recorded. Thus, under certain circumstances, the RP cellular network(s) appears sufficient to generate RP seizures without peripheral initiation. Two potential triggering and maintenance mechanisms for RPS's are : 1) a stimulus(i) extrinsic to the RP cellular networks elicits a series of RP bursts from the network(s) in a stimulus-concentration dependent fashion or 2) an extrinsic stimulus(i) elicits a series of RP bursts from the network(s), however, the number of bursts in the series is independent of the initiating stimulus, but dependent on the synaptic efficacy between cells within and across each RP network.

Clearly, the Asplysia behavior known as respiratory pumping is more complicated than previously supposed. And any behavioral/physiological adaptation ascribed to it must account for the selective occurrence of all forms of RP activity.

78.4 INFLUENCE OF ABDOMINAL NERVE CORD LESIONS ON THE DIRECTION AND AMPLITUDE OF WIND ELICITED ESCAPE TURNING MOVEMENTS IN THE COCKROACH. J. P. DOWD* and C. COMER. Dept. Biological Sciences, Univ. of Illinois at Chicago, Chicago, IL 60680.

Cockroaches (P. americana) usually respond to abrupt wind puffs by turning away from the source of wind. This turn is truly directional in that turn amplitude (angle) is related to stimulus position, i.e. puffs from the rear elicit small angle contraversive turns, while those which originate more frontally elicit larger angle contraversive turns. Previous work has suggested that turn direction, at least, might be related to a bilateral comparison of wind information at the level of sensory input since covering one of the cerci (paired abdominal appendages containing sensory hairs) frequently causes turns to be incorrectly directed toward wind on the side of the covered cercus (Camhi & Tom, J. Comp. Physiol. 128:193, 1978).

Cercal receptors primarily activate wind sensory giant interneurons on the ipsilateral side of the abdominal nerve cord. We have therefore tested the possibility that a bilateral comparison of wind information at the interneuronal level determines the orientation of turns. Transmission was blocked on one side of the nerve cord by cutting all the axons in one abdominal connective. The behavior of these hemisected animals was compared with the behavior of surgical controls and animals with one cercus removed. Nine adult male cockroaches have so far been studied. Their responses to standard puffs of wind were recorded with a high-speed video system.

Both hemisection and unilateral cercal removal caused animals to respond less often to standard wind stimuli, but the pattern of turning behavior was different in the two groups. Unilateral cercal removal increased the number of misdirected turns as compared with prelesion levels. The increase in incorrect turns was specific to frontal winds on the side of the missing cercus (3% before versus 47% after removal). Hemisection of the cord also increased the number of incorrect turns made in response to winds from the side of the lesion, but in this case 100% of turns to both frontal and rear winds were incorrect. Analysis of turn amplitude as a function of wind stimulus angle along the side of the lesion showed that the incorrect turns were not directional. For the correct turns made in response to winds from the unlesioned side, however, turns were directional as in normal animals and controls (amplitude was correlated with wind stimulus angle).

These results are consistent with the idea that a bilateral comparison of wind information in the CNS is necessary for the proper specification of turn direction, but wind information within one connective is sufficient for the determination of turn amplitude, at least for ipsilateral stimuli.

(Supported by NSF grant #BNS 83-11980 to C.C.)

78.5 PROCESSING BEYOND THE RETINAL MAP IN THE VISUAL SYSTEM OF A JUMPING SPIDER (SALTICIDAE: PHIDIPPUS JOHNSONII). D. W. Sivertsen* (SPON: J. Allman), Division of Biology, Caltech, Pasadena, CA 91125.

Jumping spiders have a highly specialized visual system for the detection and analysis of prey. The retina of each of the anterior medial eyes has a compact, high resolution, three dimensional array of receptors that can scan, saccade, and engage in slow tracking. Computer controlled data collection and randomized stimulus presentation were used in an electrophysiological study of high order visual neurons.

Receptive field mapping revealed neurons with well defined, stable receptive fields. Some of these receptive fields had an angular extent greater than that of the static retinal array. The stability with respect to the cephalothorax and the angular subtense of these receptive fields suggest integration of retinal information and eye position. Most neurons had receptive fields of constant angular subtense as the distance to the tangent screen varied. I found some neurons with fields which remain relatively constant in absolute physical size (changing in angular subtense) as screen distance varied. Such size constant cells are useful in assessing prey suitability. These characteristics require integration of depth information. These cells are driven monocularly, ruling out disparity cues. Jumping spiders do not use muscular accommodation. Instead, the retina has a layered, tiered structure. Depth of focus cues are probably the source of this information, as hypothesized previously (Land, M.F., J. Exp. Biol. 51: 443, 1969).

This work was supported by the Lawrence A. Hanson Fund at Caltech.

78.6 STARTLE RESPONSES OF BURROWED LAMPREY LARVAE. Scott Currie, Dept. Human Physiology, U.C. Davis, Davis, Ca. 95616.

When initially at rest in a pan of water, intact larval lampreys exhibit a robust, vibration-evoked startle response in which both sides of the body contract simultaneously (Currie 1984, Neurosci. Abstr. 10). EMG's recorded from mid-body reveal a response latency and duration of 15-30 ms and 10-20 ms respectively. The movement animals make during the response varies with their resting posture. Areas of inward curvature contract more forcefully than areas of outward curvature. Simultaneous spikes in both Mauthner axons are both appropriate and sufficient for this response (Currie 1984, Biol. Bull. 167).

Larval lampreys normally live burrowed in the sand or silt of fresh water streams. Therefore, it seemed prudent to study the startle response in the context of the animal's normal, burrowed posture. To observe burrowed animals, a thin-section aquarium (similar to an ant farm) was constructed of clear plexiglass. Fine glass beads (0.18 mm) were used as a burrowing substrate. Burrowed animals are visable in silhouette when shining light through the aquarium from behind. Their usual position is to have their mouthparts near or just above the surface of the "sand" while their bodies slant deeper into the burrow at a shallow angle. They are almost always dorsal side-up and curved along their backs so that their heads approach the surface nearly vertical. There are usually small lateral body bends visable in the trunk and tail.

A light hammer tap on top of the aquarium served as the stimulus and was recorded with a calibrated phono-stylus in contact with the aquarium wall. Tapping evokes a jerked withdrawal of the larva's head, 3-6 mm deeper into the burrow. To record head movement during this response, a light beam was shone through the aquarium toward a photoresistor on the other side. The light and photoresistor were positioned so that the animal's head was in between, and partially obstructed the light. Head withdrawal thus translated to increased light intensity and was recorded as a change in voltage across the photoresistor.

One of the larvae studied had a fine, monopolar electrode threaded into its spinal canal, making it possible to record both the extracellular Mauthner spike and trunk EMG. The aquarium water was replaced with Ringers and recordings made relative to an indifferent bath electrode. The animal was allowed to burrow and simultaneous recordings were made of trunk EMG and head withdrawal. Taps which elicited a time-locked EMG spike were always followed by withdrawal. Withdrawal never occurred in the absence of the EMG response. The latency from the start of the EMG response to the start of withdrawal was 19.0 ms ± 1.6 (mean ± s.d., N = 11). The stimulus-EMG latency was 18 ms.

78.7 HABITUATION TO ELECTROSENSORY STIMULI IN THE WEAKLY ELECTRIC FISH HYPOPOMUS OCCIDENTALIS. R. Zelick and W. Heiligenberg. Neurobiology Unit, Scripps Inst. of Oceanography, University of California, San Diego La Jolla, CA 92093.

During periods of inactivity, H. occidentalis produce 1-msec electric organ discharges (EODs) at stable rates of 20 to 40 Hz. An electronically generated electric pulse train of a slightly different frequency than that of the fish mimicks a neighboring conspecific. To such a stimulus the fish responds with a transient increase in EOD rate as each instance of pulse coincidence approaches. This jamming avoidance response (JAR) is asymmetric in that foreign pulse rates lower than the EOD rate typically produce larger responses than do higher rate foreign pulse trains. A short, connecting two parts of the body surface previously at unequal potential, also elicits a momentary acceleration in EOD rate. The first in a series of experimental stimuli (shorts or foreign pulses) typically produced the largest acceleration (a novelty response). Subsequent accelerations revealed a gradual habituation. In most cases the response to shorts habituated more quickly than that to foreign pulses. Identical behaviors were observed in curarized preparations in which an artificial signal (S1) is accepted by the fish as a substitute for its silenced EOD. (In this case the response is detected as the spinal nerve command signal to the tail electric organ.) Step amplitude modulation (1-5%) of the S1 yielded responses which, although initially of the same magnitude as those to a short or foreign pulses, showed little or no habituation. Recordings were made from axons of the anterior lateral line nerve while presenting S1 modulations and shorts (shorts directed to a small region of the body surface including the receptive field for the axon being tested). In no case was there evidence of habituation of the receptor response, even when such was obvious from the simultaneous measurement of the behavioral response. This implies a central mechanism for the habituation to such electrosensory input. Surprisingly, when similar experiments were performed on fish in which the telencephalon and optic tectum were removed, the asymmetric JAR, habituation to foreign pulses and to shorts all remained intact. Thus only the electrosensory hindbrain and midbrain structures play a role in these behaviors.

78.8 THE MULTIMODAL REPRESENTATION OF SPATIAL INFORMATION IN THE OPTIC TECTUM OF THE ELECTRIC FISH EIGENMANNIA. W.Heiligenberg and G.Rose. Neurobiology Unit, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093

Intracellular labelling of physiologically identified neurons has revealed classes of tectal neurons that are characterized by their laminar location, general morphology and functional properties Most types of neurons respond to moving objects and are sensitive to either visual, electrical, mechanical or auditory cues, or particular combinations of these modalities. The sensory modality of these neurons is closely correlated with the laminar location of their dendrites.

Electroreceptive neurons may respond to electric signals interfering with the animal's own electric organ discharges and may even discriminate the sign of the frequency difference, Df, between the interfering signals. This particular property suggests that these neurons participate in the Jamming Avoidance Response, a Df-sign-specific frequency shift of the medullary pacemaker. These same neurons may also respond to the electric images of moving objects, and their ability of detecting moving objects may be hampered by signal interference.

Large neurons in the deeper layers of the tectum are often multimodal, respond to moving objects with directional specificity and project to premotor areas in the reticular formation. The Df-sign-specific electrosensory neurons are of similar morphology although they send additional collaterals to a diencephalic region apparently not contacted by neurons that only respond to moving objects. This suggests that the Jamming Avoidance Response shares neuronal mechanisms with regular physical responses to moving objects and that it probably represents an evolutionary modification of more general motor responses.

78.9 MODULATION AND CONTROL OF THE PACEMAKER NUCLEUS OF WEAKLY ELECTRIC FISH. J. Dye and W. Heiligenberg. Dept. of Neurosciences and Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093.

The mesencephalic pacemaker nucleus of wave-type, weakly electric fish is the most precisely regular neural oscillator known. This nucleus, which commands the electric organ discharge of the animal, is quite simple as regards intrinsic cell types and sources of afferent input. The high degree of precision in its firing offers opportunities to monitor, reproducibly and quantitatively, subtle physiological effects through alterations in pacemaker frequency. Most interesting from the behavioral standpoint are those changes in frequency employed by the organism to effect various functions subserving electrolocation and intraspecific communication. Heretofore, attempts to identify the proximal mechanism by which the pacemaker frequency is modulated in such behaviors have yielded only negative results.

We report here studies on isolated pacemakers in a brain slice preparation which show a strong effect of the excitatory amino acid transmitters, aspartate and glutamate, on pacemaker firing frequency. Application in micromolar concentrations causes immediate acceleration of the rhythm. This short-term effect is transient due to rapid desensitization. Such exposures, however, prolong the activity of the nucleus in vitro from the usual period of 8 to 12 hours to several days. Millimolar concentrations of these amino acids result in the normally tightly-coupled and synchronously-active cells of the nucleus to fire incoherently for these extended periods.

The pacemaker frequency of these fish has long been known to be extremely temperature-sensitive. In order to accurately separate the effects due to a putative transmitter from unavoidable temperature variations accompanying rapid solution changes, we undertook to characterize the temperature dependence of pacemaker activity. We have found the frequency to change linearly across the physiological temperature range, and the regression coefficient to itself increase monotonically with the fish's individual frequency. Our hope is that an understanding of the temperature dependence may indicate the source of individual variation in baseline frequency and, hence, the mechanism of hormonal control of the pacemaker frequency.

78.10 PYRAMIDAL CELLS IN THE MULTIPLE ELECTROSENSORY MAPS OF THE ELECTROSENSORY LATERAL LINE LOBE (ELLL) HAVE A CENTER-SURROUND RECEPTIVE FIELD. C.A. Shumway, Scripps Inst. of Oceanography UCSD, La Jolla, CA 92093

The weakly electric fish Eigenmannia virescens has 3 tuberous electrosensory maps in the first-order nucleus, the electrosensory lateral line lobe (ELLL). Studies are actively underway to determine the anatomical and physiological differences among the maps in hopes of understanding their functional significance. I recently discovered that the maps differ anatomically by the presence or absence of a local axonal collateral on the amplitude-coding pyramidal cells in the ELLL. I am currently examining the receptive field properties of single pyramidal cells in the different maps. Whether or not the pyramidal cells have a center-surround receptive field has been in dispute for some years (see Maler et al.,J. of Comp. Neurol. 195:87-139,1981 and Bastian,J. of Comp. Physiol. 144:481-494,1981). The cells are first characterized as E or I units (E units are excited by a rise in amplitude, I units are excited by a fall in amplitude) by whole body stimulation with a positive amplitude modulated (AM) signal. To localize the receptive field, the whole body stimulation is turned off, and a small local AM signal is applied with an electrode positioned directly on the skin. The pyramidal cell is subsequently filled with Lucifer Yellow to determine its map origin.

Using electrodes positioned directly on the skin, these results convincingly demonstrate that pyramidal cells have a center-surround receptive field which should enhance the spatial contrast of an electric image. E units were excited by the AM in the center of their receptive field and inhibited in the periphery. An electrode placed in the center of the receptive field with an additional electrode in the periphery either inhibited or reduced the excitation of the E unit. I units were inhibited by the AM in the center of their receptive field and excited by the AM in the periphery. These physiological results correlate with the predictions made by Maler et al. on the basis of Golgi light and electron microscopic studies. Maler and coworkers found that E units receive direct excitatory input from the primary P-type afferents in the center of their receptive field and inhibitory input via granule cells in the periphery. An I unit receives inhibitory input in its receptive field center from granule cells and excitatory gap junction input in the periphery of its receptive fields from granule cells.

It is possible that the pyramidal cells in the maps differ in receptive field size,in response to objects with a given size, shape, or velocity, or in direction selectivity. One I unit from the C-M map was direction selective, responding more to movement of the electrode ventrodorsally than dorsoventrally.

78.11 **ELECTRORECEPTOR TUNING PREDICTS TEMPORAL ENCODING OF SPECIES SPECIFIC SIGNALS IN ELECTRIC FISH.**

S.I. Arnesen* and C. D. Hopkins. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Mormyrid electric fish communicate with pulse-like electric organ discharges (EODs) which are species-specific and often sexually different. EODs are coded as a unique time-domain pattern of spikes by peripheral electroreceptors (Knollenorgans) in mormyrids. Single units with differing frequency tuning characteristics produce different temporal codes. Our goal was to model the effect of frequency tuning on the pattern of spikes in the time domain.

Receptor potentials from single Knollenorgans were recorded non-invasively from the mormyrid, *Brienomyrus brachyistius*, while artificial stimuli were delivered to the unit via a bridge circuit. Frequency tuning curves (FTCs) and compound post-stimulus time histograms (PSTHs) were collected from each cell in response to sine waves, EODs, delta functions, step functions, and other stimuli.

Since Knollenorgans behave as linear bandpass filters for low amplitude stimuli, we used linear filter theory to describe the transfer characteristics of the electroreceptor. Tuning curves were matched by eye to the gain curves of known electronic bandpass filters. The matching was based on four parameters: the low and high frequency cutoff slopes, the damping coefficient, and the best frequency. Once the transfer function had been determined, we then could predict the response to any arbitrary test stimulus, by performing the convolution between the original stimulus, and the impulse response of the filter. The results are compared to the compound PSTH (fig. 1). [Since the receptors rectify electrical stimuli, compound PSTHs are assembled from PSTHs to a stimulus presented in one polarity, and then its inverse.]

For low amplitude stimuli, the compound histogram matches the predicted filtered stimlus. Knollenorgans respond to positive-going transitions in the stimulus waveform only, and are inhibited by negative-going transitions. The waveform of the compound PSTH and the relative amplitude of the peaks matches the predicted filter response. For high amplitude stimuli, non-linearities distort the PSTH.

Brienomyrus brachyistius has two classes of Knollenorgans tuned respectively to 800 Hz, and to 1.0-2.0 kHz. Each class fires spikes with a characteristic PSTH. Our modeled responses can account for the differences observed for the two classes of units.

A fish may require two types of Knollenorgans in order to encode its own species' EOD in a unique way.

Supported by NIMH grant * MH37972

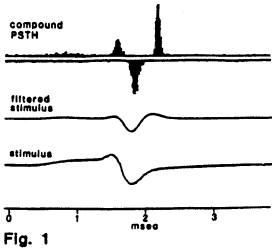


Fig. 1

78.12 BRAINSTEM CENTERS GENERATING MOTOR BEHAVIOR FOR ELECTRO-LOCATION IN THE ELECTRIC FISH Eigenmannia. K.Behrend Zool. Inst. Universität Mainz 65 Mainz,FRG

The electric fish Eigenmannia investigates objects in it's "electrosensory world" by a motor behavior termed "probing"(1).The fish bends it's tapering caudal end towards the object of interest thereby enhancing its electrosensory resolution.Since cerebellar activity is involved in this motor act(1) to further clarify it's role in motor co-ordination the sites where cerebellar output acts on had to be determined.Fish were anesthetized by perfusion of aerated MS 222 solution(1:15ooo) through the mouth,placed in a head holder and the brainstem was stimulated (5o Hz train,6o μsec square pulses for 55oms) in 2oo μ steps along its longitudinal extent.The trunk musculature which in this fish does not serve locomotion could be activated along a strip comprising the medial reticular nucleus.Two sites are of special interest: at the level of entrance of the VIII nerve stimulation elicits synchronous forward flapping of the pectoral fins followed after about 1oo ms by contralateral body bending.This corresponds to "backwards probing" as analyzed in free moving fish(1).The second site is in the midbrain tegmentum at its rostral border lateral to the nucleus of the f.l.m..Here,the pectorals move alternating and the body bends ipsilaterally which equals "forward probing"(1).The two sites comprise distinct groups of large(2o-5oμ)neurons projecting directly down the spinal chord,which has been shown by retrograde staining with HRP deposited at various spinal levels.The midbrain site seems to be a good candidate for the still much debated red nucleus in teleosts.

(1)Behrend,K. Neurosci.,13:171,1984

78.13 **ACOUSTIC SONG IN AN ELECTRIC FISH**

J.D.Crawford*, M.M.Hagedorn* and C.D.Hopkins (SPON:H. Howland). Section of Neurobiology and Behavior. Cornell University, Ithaca, New York 14853.
(Order of authors determined by coin toss)

Although African electric fish (*Mormyridae*) are known to have a highly- specialized auditory system based on a tiny inner-ear air bladder which contacts the saccular otolith, the function of this auditory system has been a mystery. We now describe a rich repertoire of underwater acoustic signals which accompany courtship and agression in one electric fish, ***Pollimyrus isidori***.

1. Two males and one female *Pollimyrus* were induced to spawn in a community aquarium by gradually decreasing the water conductivity over a period of several months. For weeks prior to each spawning, the males went through a phase of nest building and territorial defense while producing frequent underwater sounds [**"grunts"**, **"moans"** and **"growls"** (sound spectrogram in Fig.)]. Calling accompanied *hovering* and active *patroling* by males. It was most frequent at night, especially at dusk, and at dawn. Calling rate increased more than three fold when a gravid female entered a male's territory, but was unaffected by the presence of juvenile (non-breeding) females. Males and females were silent during time of actual spawning.

2. During agonistic encounters between males, between breeding and non-breeding females, or between male and female, we also recorded **"hoots"** and **"pops"**(not illustrated).

3. **Grunts** are a highly stereotyped bursts of about 15 clicks in 300 msec(c.v.-3%). Our two males produced grunts differing consistently by 10 Hz. **Moans** and **growls** are more variable; the two males overlap in characteristics of these calls. Males call from preferred sites which may signal future spawning spots to females.

4. *Pollimyrus* continues to discharge its electric organ during bouts of acoustic calling but is usually *electrically silent* during the the "grunt-moan" sequence.

5. The sonic organ has not yet been identified.

The diversity of acoustic signals suggests that acoustic communication is an important function for audition in mormyrids. Although mormyrids also communicate with electric signals, sound may be more useful for attracting mates from a longer distance.

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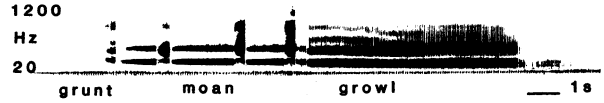


78.14 HAIR CELLS IN THE LATERAL LINE ARE TROPHICALLY DEPENDENT ON INNERVATION BOTH FOR MAINTENANCE AND FOR REGENERATION. P. C. Borden* and J. T. Corwin. Dept. of Zoology and Bekesy Lab., Univ. of Hawaii, Honolulu, HI 96822 (SPON: H. Gillary).

Trophic interactions between nerves and hair cells in lateral line neuromast organs were investigated in the aquatic salamander Ambystoma mexicanum. In one series of experiments unilateral transection and removal of a 2 mm segment of the lateral line nerve in the abdominal region of anesthetized salamanders resulted in dramatic reductions in the number of lateral line neuromasts on the ipsilateral side of the tail. The difference in the number of neuromasts between denervated and control sides of the tail was statistically significant ($P<0.05$, two-tailed) in specimens that survived for 110 to 140 days postoperatively.

In other experiments unilateral abdominal transection of the lateral line nerve was combined with amputation of the tip of the tail in anesthetized salamanders. These experiments provided evidence suggesting that the regenerative replacement of hair cells and neuromasts in this species is dependent on the local presence of a lateral line nerve. When the regenerating lateral line nerve grew into deep tissues so that it was not near the surface of the regenerated tail no neuromasts regenerated and persisted. In several specimens in both experimental series, foreign nerves invaded previously denervated regions, in these specimens neuromasts did not regenerate or persist in the denervated regions but they did appear to regenerate and persist in the regions that were innervated by the foreign nerve.

Our findings are essentially opposed to conclusions reported by Jorgensen and Flock (J. Neurocytol. 1976, 5:33) based on similar manipulations in the same species. However, we made comparisons between a control and an experimental side in each animal, recorded quantitative results, and noted the positions of neuromasts before and after the manipulations. We also operated on salamanders that were older and larger (47-68 days old, 38-52 mm) than those used by Jorgensen and Flock (14 days old, 24-27 mm) and we allowed longer postoperative survival times. Jorgensen and Flock reported on noninnervated neuromasts that they identified as newly regenerated neuromasts in salamanders where bilateral nerve transections and amputation of the tail were combined. The results of our study indicate that the lateral line neuromasts are strongly dependent on intact innervation. If the conclusions reported by Jorgensen and Flock are also correct, then this suggests that the postoperative survival period of sixty days used in their study was too short to allow an effect to develop or that there may be a critical period in the development of the lateral line system when hair cells and neuromasts are not yet dependent on a neurotrophic interaction that later becomes required.

(Supported by grants from NINCDS, the Deafness Research Foundation, and the Grass Foundation to J.T.C.)

78.15 INDIVIDUAL VARIATION IN VOCALIZATIONS AND AUDITORY SENSITIVITY OF THE GREEN TREEFROG, HYLA CINEREA. M. Penna*, R.R. Capranica and J. Somers*(Sponsor: P.M. Narins). Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Spectral characteristics of anuran mating calls vary individually according to the male's body size. Large males produce lower-pitched calls than smaller males. Variation in tuning of auditory sensitivity related to sex and age is also correlated with individual size differences. Female green treefrogs rely on spectral features for mating call recognition and subsequent orientation towards males. This species therefore provides an opportune model for estimating the importance of individual variations in vocal and auditory tuning for sound communication during the breeding season.

The experimental group consisted of 32 males and 21 females which were induced to call after subcutaneous implants of testosterone pellets and injections of arginine-vasotocin. Vocal responses of the animals to a ten-minute presentation of conspecific calls were tape recorded and analyzed with a Mini-Ubiquitous Spectrum Analyzer (Nicolet 444). Multiunit auditory responses to pure tones were recorded in the torus semicircularis of the midbrain with gross tungsten electrodes (75 μm width) in these same frogs all at the same temperature (21± 1°C).

The calls of males have a low-frequency peak centered at 1030 Hz (range 817-1300 Hz) and a high-frequency peak at 2742 Hz (range 2242-3211 Hz). A mid-frequency peak at 1897 Hz (range 1617-2180 Hz) is also present in the calls of some individuals. The audiograms for both sexes have three dips of improved sensitivity which in males are centered at 550, 990 and 2690 Hz. In females the region of optimal high-frequency sensitivity is at 2530 Hz. A significant negative regression with body size exists for the low frequency peak of the males' calls ($F=9.96$, $p<0.01$) and for the region of high-frequency sensitivity in their audiograms ($F=12.34$, $p<0.05$).

The lack of correlation between the low-frequency region of auditory sensitivity and size is in agreement with behavioral data which have shown that body size in H. cinerea females is not correlated with a preference for lower-frequency components in the cells of males as tested in discrimination experiments (Gerhardt, H.C., Am. Zool. 22:581-595, 1982). The significance of size-dependent variation in the region of high-frequency sensitivity is unclear and deserves further behavioral study.

Supported by U.S. Public Health Service International Research Fellowship 1 F05 TW03411-01 to M.P. and NIH grant NS-09244 to R.R.C.

78.16 DETECTION OF COMPLEX SOUNDS BY THE GREEN TREEFROG. A. Megela-Simmons and C.F. Moss. Dept. Psych., Brown Univ., Providence, RI 02912

Green treefrogs (Hyla cinerea) can detect harmonically-structured complex sounds that mimick essential features of their conspecific mating calls with greater sensitivity than non-harmonic complex sounds or pure tones. Using the technique of reflex modification, we measured detection thresholds for pure tones and complex acoustic signals against a background of broadband noise. The frog detects pure tones at communication frequencies of 900 Hz and of 3000 Hz with power signal-to-noise ratios of about 20 dB, detects non-harmonic complexes with frequencies near 900 and 3000 Hz with power signal-to-noise ratios of about 18 dB, and detects harmonically-structured complex sounds near 900 and 3000 Hz with signal-to-noise ratios as low as 11 dB. The superior ability to resist noise masking of harmonic complexes may be due to perception of coherence, or phase-locking, of the harmonics, possibly through detection of the first-harmonic periodicity. Coherence detection alone provides the frog with about 7 dB of noise rejection for complex sounds beyond what occurs as a consequence of conventional critical-band rejection of noise. Because reception of the 900 Hz and 3000 Hz components occurs in anatomically separate sense organs in the treefrog's inner ear, the coherent comparison of these components must take place centrally. The very strong frequency selectivity (narrow critical ratio-bands) implied by low signal-to-noise ratios for detection of harmonic complexes may be achieved by temporal processing in the frog's central auditory system.

78.17 ALLIGATOR BAEPS: EFFECTS OF TEMPERATURE AND HYPOXIA. G.M. Strain and T.A. Tucker*. Louisiana State Univ., Sch. of Vet. Med., Baton Rouge, LA 70803.

Brainstem auditory evoked potentials (BAEPs) were recorded from 10 young alligators (Alligator mississippiensis), and the effects of hypothermia, hyperthermia and hypoxia on the waveforms was determined. Average weight and length were 406 gm and 54 cm, corresponding to an age range of 1-3 years. Recordings were made with needle electrodes placed subdermally at vertex and just below the left external ear flap, with ground on the tail. Click stimuli (21/S, 100 μsec) were presented through an insert earphone 1 cm from the ear flap. Body temperature was controlled by submersion in a water bath with a temperature-regulating circulator. Body temperature was monitored with a cloacal thermistor. The waveform shape was similar to the human BAEP, although extra waves were occasionally seen. The responses were highly repeatable, and varied in a predictable manner as a function of stimulus frequency, polarity, intensity, and body temperature. Rarefaction clicks produced waveform latencies longer than those from condensation clicks. BAEPs were present over the entire temperature range studied (0-36°C). In contrast, mammalian BAEPs disappear over the temperature range of 20-27°C, and seizures occur at 20-21°C. At temperatures below 20°C, the alligator BAEP peak amplitudes decreased with decreased temperature, but latencies did not greatly decrease. At temperatures above 20°C the peak amplitudes increased, and the latencies decreased with temperature. Transient brain hypoxia, achieved by inverting the alligator, produced a progressive decrease in BAEP waves to an isoelectric amplitude without greatly altered latencies. The reverse sequence of changes was seen during recovery. Metabolic differences between mammals and the alligator likely account for the alligator's resistance to hypothermia, hyperthermia and hypoxia.

Supported by grant LA SVM 384.

78.18 THEORETICAL ANALYSIS OF VELOCITY DEPENDENCE IN THE RESPONSE OF TECTAL CELL T5(2).[1] F. Cervantes-Pérez* and M. A. Arbib (SPON: A. Barto). Centro de Inv. en Fis. Celular, UNAM, México 04510 and Center for Systems Neuroscience, Univ. of Mass., Amherst, MA 01003

Electrophysiological studies have identified different types of cells within the anuran's optic tectum. Some of the properties exhibited by specific tectal cell responses are still a matter of debate. In toad (Ewert, 1976) and in frog (Schuerg-Pfeiffer & Ewert, 1981), Ewert and coworkers identified a tectal cell they classified as T5(2) that responded to different moving configurational visual stimuli with firing levels that resembled the overt behavior of the animal. These authors also found that a T5(2) cell preferred "worm-like" to "square" or "antiworm-like" stimuli independently of the stimulus direction of motion (Ewert et al., 1979) and velocity function (Ewert, 1980, 1984).

Roth and collaborators have also recorded cells of this type in toads (Roth & Jordan, 1982) and in salamanders (Himstedt & Roth, 1980). However, they found that a subgroup of this type of cell changed their preference as the stimulus velocity was varied. Roth and Jordan reported that 16 out of 41 cells, which behaved as T5(2), preferred worm-like to square stimuli at low velocities while at high velocities squares were more effective.

In Cervantes-Perez, Lara and Arbib (1985) we developed a family of neural models of the anuran's optic tectum and its interactions with the pretectum. Following Ewert's original hypothesis, the aim was to analyze how an inhibitory effect from a pretectal cell he classified as TH3 to tectum enables T5(2) cell to discriminate between prey and non-prey stimuli. Ewert (1980) and Ingle (1981) showed that such inhibition is modulated by the motivational state of the animal. Additionally, Himstedt (1982) suggested that the "inversion phenomenon" reported by Roth's group could depend on the animal's experience with certain type of prey. Based on these observations, we hypothesize that the mechanisms responsible for the difference between the results obtained by the Ewert and Roth laboratories might be related to parameters that produce changes in the level of pretectal inhibition upon tectum.

Computer experiments simulating variations in TH3 neuron parameters were conducted to study the ways in which T5(2) responses might be velocity dependent. Results from these simulations suggest that if pretectal inhibition over tectum is delayed or reduced then retinal axons impinging upon tectum build up a strong effect that causes a switch in T5(2) preference between worm-like and square stimuli as stimulus velocity is increased. We want to stress that the interplay of theoretical and empirical studies is necessary and will clarify the real nature of these processes.

[1]Supported in part by CONACYT under contract PCCBANA-021005, and by N.I.H. under grant NS14971-06.

79.1 BENZODIAZEPINE RECEPTOR INVOLVEMENT IN THE POSTICTAL REFRACTORY PERIOD OF THE SPONTANEOUS SEIZURE PRONE HAMSTER. J. E. Fisher, Jr.* and W. B. Iturrian. Pharmacology Dept., Univ. of Georgia, Athens, Ga. 30602 (SPON. J. M. Bowen).

Several lines of evidence indicate that the postictal period following electroconvulsive seizure is characterized by a neural hypometabolic state and that maximal electroshock (MES) has anticonvulsant properties. Animal and human research has shown that MES results in an elevation in threshold for pharmacological as well as electrical induction of seizures. Post et al. (1984) showed that electroconvulsive seizures inhibit amygdala kindling. While the mechanism of this anticonvulsant effect of MES is unknown, post seizure changes in ligand binding properties in a variety of seizure models have reinforced the involvement of the BDZ receptor in the regulation of seizure activity. A transient, time dependent increase in number of Diazepam binding sites has been found after experimentally induced (MES, Metrazol, amygdala kindling) seizures. We demonstrated an inherent difference in the nature of BDZ receptors during spontaneous seizures in genetically epileptic hamsters (sz, BIO 89.93).

We used paired MES to measure the time course of hyperexcitability and depression during the postictal period. The hyperexcitability phase in which a second flexor-extensor seizure occurs was extended to over 80 sec. in sz hamsters in contrast to only 10 sec. in controls. The optimal depression inter-test interval for control animals occurs at 32 sec. with 31% (N=52) having a second seizure. Insertion of the sz trait onto the LVK background increased the incidence to 83% (N=78), suggesting a hereditary deficit in the inhibitory system producing the depression phase. Breeding experiments demonstrate a linkage between the sz trait and the prolonged hyperexcitability phase. CGS 9896 (20 mg/kg), a partial agonist at the BDZ receptor, reduced the incidence of second seizures by 50% in sz hamsters without altering the initial seizure. The prolongation of the hyperexcitability phase does not develop until the sz hamster outgrows its susceptibility to spontaneous seizure, which occurs at about 60 days of age. MES increases the number of available BDZ binding sites and we find that it reverses sz seizures with a temporal course resembling the receptor effect. CGS 8216, a BDZ receptor antagonist, blocks this protective effect of MES, suggesting that the sz trait may be the result of a BDZ receptor or endogenous ligand alteration.

79.2 ANTICONVULSANT ACTIVITY OF A BENZODIAZEPINE INJECTED INTO SUBSTANTIA NIGRA PARS RETICULATA. H.C. Rosenberg, E.I. Tietz and T.H. Chiu. Medical College of Ohio, Toledo, OH 43699.

Several studies have suggested that the pars reticulata of the substantia nigra (SNpr) is part of a system that regulates seizure spread. Activating nigral GABA systems, and thereby suppressing SNpr neuronal activity, appears to be an important mechanism for regulating seizure susceptibility. Benzodiazepines potentiate GABA activity, and can suppress the discharge of SNpr neurons. Injection of clonazepam into SNpr suppressed kindled seizures (King and McNamara, Soc. Neurosic. Abst. 10:343), and rats made tolerant to benzodiazepines show down-regulation of benzodiazepine receptors which is especially prominent in SNpr (Tietz et al., Soc. Neurosci. Abst. 10:644). This suggests that SNpr may be an important site for the anticonvulsant action of benzodiazepines, and that tolerance to benzodiazepines may occur within SNpr. Experiments were begun to evaluate acute and chronic benzodiazepine actions in SNpr.

Male, Sprague-Dawley rats (250-300 gm) were deeply anesthetized with pentobarbital. Using stereotaxic technique, bilateral stainless steel guide cannulas with close-fitting obturators were implanted, aimed at the SNpr. A week was allowed for recovery from the surgery before testing. The water soluble benzodiazepine, midazolam, was infused bilaterally over 1 min, 5 µg per side in 1 µl. The injectors were steel hypodermic tubing that extended 1 mm past the end of the guide cannulas. Injectors were left in place another minute, then withdrawn slowly and replaced with obturators. 5 min after midazolam, pentylenetetrazol (PTZ), 100 mg/kg was given IP. Rats were observed for another 12 min and any seizure activity recorded. Appearance of a tonic-clonic, or severe clonic seizure with loss of posture was considered a negative midazolam response, and 40 mg/kg Na pentobarbital was immediately given to prevent death. If less intense, or no seizures were seen for 12 min, animals were considered protected from PTZ, and were given Na pentobarbital to preclude later appearance of lethal seizures. After 5-7 days, rats were anesthetized, and brain tissue was taken for histological verification of injection sites.

In previously untreated rats, 100 mg/kg PTZ produced tonic-clonic seizures within 3 min in virtually all cases. After intranigral midazolam injection, all rats had some response, ranging from mild jerks to tonic-clonic seizures. Results are shown grouped according to sites of midazolam injection:

Midazolam Injection Sites	#Protected/# Tested
Both sides in SNpr	5/5
One or both sides < .2 mm out of SNpr	4/5
One or both sides > .2 mm out of SNpr	0/5

These data show that injection of a benzodiazepine into SNpr produced a clear anticonvulsant action. SNpr may prove useful for studying mechanisms of tolerance to benzodiazepines.

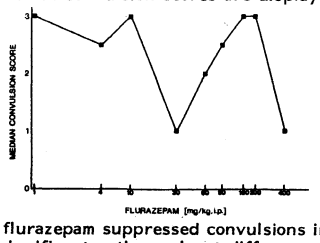
Supported by DHHS grants RO1-DA02194, RO1-NS16595, F32-DA05265.

79.3 ANTICONVULSANT AND CONVULSANT PROPERTIES OF FLURAZEPAM. M.W.Kalichman. V.A. Med. Ctr. & Univ. of Calif., San Diego, Dept. of Anesthesiology, V-151, La Jolla, CA 92093.

The benzodiazepine drug flurazepam is one of the most widely-prescribed sleeping pills. Although benzodiazepines are typically effective anticonvulsants, several investigators (Randall & Kappell, The Benzodiazepines, Garattini et al., eds., Raven Press, New York, 1973, pp. 27-51; Rosenberg, Pharmacol. Biochem. Behav. 13: 415, 1980; Barr & Lithgow, Nature 302: 431, 1983) have provided evidence that flurazepam may be convulsant in rats, cats, and mice.

The significance of flurazepam's convulsant property may be related to the demonstration by MacDonald & Barker (Brain Res. 246: 257, 1982) that low concentrations (500 picomolar) of flurazepam could effectively antagonize the paroxysmal depolarizing events produced by picrotoxin in cultured mouse spinal neurons, but higher doses (10 nanomolar) produced minimal antagonism of the picrotoxin-induced activity. In light of the evidence of a convulsant property of flurazepam as well as a dose-dependence for antagonizing an effect of the convulsant drug picrotoxin, 30 male Sprague-Dawley rats were tested two to three times each with different doses of flurazepam hydrochloride against convulsions produced by picrotoxin (4 mg/kg, i.p.).

Median picrotoxin convulsion scores are displayed in figure 1.



No dose of flurazepam suppressed convulsions in all subjects tested, but a significant anticonvulsant difference was present for a dose of 30 mg/kg when compared to doses of greater than 150 mg/kg ($P<0.05$). At a dose of 400 mg/kg, all subjects died (with or without convulsions). These observations are consistent with the reports of direct convulsant properties of flurazepam in other animal models and are a clear behavioral demonstration of a dose-dependent anticonvulsant property similar to that described for cultured mouse spinal neurons. At this point it is not clear whether flurazepam epileptogenicity may simply be a pharmacokinetic interaction; however, the study of MacDonald & Barker demonstrates the possibility that the mechanism may be found at flurazepam's site of action in the central nervous system.

79.4 INTRANIGRAL MUSCIMOL ATTENUATES ELECTROGRAPHIC SEIZURES INDUCED BY INTRAVENOUS BICUCULLINE IN RATS. D.S. Garant* and K. Gale (SPON: S.J. Potolicchio). Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Previous work in our laboratory demonstrated that muscimol infused bilaterally into the substantia nigra (SN) reduces the severity of behavioral seizures induced by chemoconvulsants (Science 218:1237, 1982). We now report that bilateral infusion of muscimol into the SN attenuates electroencephalographic epileptic activity induced by i.v. bicuculline.

Bilateral guide cannulae directed to the SN were implanted along with epidural and depth electrodes in 350 g male rats. Records were made using a Grass Model 6 electroencephalograph (EEG). After at least a 24 hr recovery from surgery, rats were tested on alternate days with either i.v. bicuculline 30-60 min following intranigral muscimol (25-50 ng), or with the identical dose of bicuculline alone as a control, with order of treatment randomized. EEG recordings and drug injections were performed without restraint or anesthesia. Doses of bicuculline (0.15-0.23 mg/kg) were selected which were just subthreshold for inducing clonic activity and which produced marked bilateral epileptic activity in the EEG. Following intranigral muscimol, the duration of electrographic seizure activity was reduced by more than 75% in the majority of animals. The animals had control seizures with recurrent epileptic EEG activity lasting 3 min or more; after muscimol, they usually experienced only a single brief episode averaging 15 sec.

These results indicate that GABAergic inhibition of nigral output neurons can attenuate not only motor seizures, but electrographic seizure activity as well. Thus, SN efferents must normally facilitate seizures, either by maintaining or promoting propagation of epileptic activity. This further supports our findings that inhibition of SN outflow produces a shift in the dose-response for chemoconvulsants, rather than a selective blockade of any specific motor component of the seizures (Brain Research 273:156, 1983).

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79.5 ANXIOLYTIC AND ANXIOGENIC DRUG EFFECTS IN A NEW ANIMAL TEST OF ANXIETY. Sharon Pellow and Sandra E. File. MRC Neuropharmacology Research Group, The School of Pharmacy University of London, London, U.K.

We have recently carried out an extensive behavioural physiological and pharmacological validation of a novel test for the identification of anxiolytic and anxiogenic drug effects in the rat (Pellow, Chopin, File & Briley, J. Neurosci. Meths. submitted 1985). The apparatus consisted of a +-maze elevated to a height of 50 cm, with 2 open arms opposite to each other, and 2 closed arms with an open roof. Rats were placed in the centre of the maze and their behaviour noted for 5 min. The percentage of the total time that rats spent on the open arms, and the percentage of open arm entries, provided a measure of fear-induced inhibition of exploratory activity. The total number of arm entries provided a measure of overall activity. Clinically effective anxiolytics (benzodiazepines) significantly elevated, and drugs that are anxiogenic in man (yohimbine, caffeine and pentylenetetrazole) significantly attenuated, the percentage of time spent on the open arms. Antidepressants and neuroleptics non-specifically reduced overall activity.

In the present investigation we examined the effects of several novel putative anxiolytic and anxiogenic agents that are believed to produce their effects by interactions with the GABA-benzodiazepine receptor complex. The following compounds had anxiogenic activity - FG 7142 (1-5 mg/kg); CGS 8216 (3-10 mg/kg); Ro 5-4864 (1-5 mg/kg). The benzodiazepine receptor antagonists Ro 15-1788 (10-20 mg/kg) and ZK 93426 (5-10 mg/kg) that can have anxiogenic activity in certain tests, were ineffective. The following compounds had anxiolytic activity: CGS 9896 (10-20 mg/kg); tracazolate (5 mg/kg); CL 218,872 (10-20 mg/kg). Ineffective compounds included the 3,4-benzodiazepine tofisopam (25 mg/kg), PK 8165 (10-25 mg/kg) and buspirone (0.5-20 mg/kg), consistent with their lack of efficacy in other animal tests of anxiety.

Thus, it appears that the present procedure provides a valid and reliable test for the detection in the rat of anxiolytic and anxiogenic drug effects. The test is simple and rapid, and its advantages over other tests include the lack of necessity for food or water deprivation, or for the use of electric shock.

79.6 CAN ANIMAL TESTS OF ANXIETY DETECT ANTI-PANIC COMPOUNDS? Sandra E. File, Sharon Pellow and Philippe Chopin* MRC Neuropharmacology Research Group, The School of Pharmacy University of London U.K. &*Centre de Recherches Pierre Fabre, 81100 Castres, France.

Several lines of clinical evidence suggest that the neural mechanisms underlying generalised anxiety states and panic disorders are distinct. For example, the benzodiazepines (BDZs), that are clinically effective in generalised anxiety, are generally ineffective in the prevention of panic attacks; whereas for tricyclic antidepressants (ADs) the reverse is true. The discovery of the triazolobenzodiazepines has for the first time provided a group of compounds that are effective in both types of disorder. It has also been suggested (though the evidence is controversial) that the α_2-adrenoceptor agonist clonidine may be effective in both disorders.

The purpose of the present investigations was to compare the effects of these different classes of compound in two extensively validated animal tests of anxiety: the social interaction (SI) test (File, J. Neurosci. Meths. 2:219-238, 1980) and the elevated plus-maze (Pellow, Chopin, File & Briley, J. Neurosci. Meths. submitted; see also Pellow & File, this meeting). Classical 1,4-BDZs have an anxiolytic profile in both tests, although chronic pretreatment is necessary in the social interaction test. After acute treatment, the triazolobenzodiazepines alprazolam, adinazolam and U-43,465 had strongly anxiolytic effects in the +-maze, but only U-43,465 was effective in the social interaction test. Several antidepressants, after both acute and chronic treatment, have been found to be inactive in the social interaction test. These include imipramine, clomipramine and 5-HT uptake inhibitors. In the +-maze, neither imipramine nor mianserin (after acute treatment) had an anxiolytic-like effect; in fact, mianserin was weakly anxiogenic. Results after chronic treatment will be presented. Clonidine did not have anxiolytic activity in either test; its effects also were mildly anxiogenic, but sedation was a confounding factor.

In conclusion, it is apparent that current animal tests that have been extensively validated to indicate the specificity of their measurement of anxiety-related behaviours, are sensitive to the effects of compounds that are effective in the treatment of generalised anxiety states, but not to those effective in the prevention of panic attacks.

79.7 TRIAZOLOPYRIDAZINES WITH ANTAGONISTIC PROPERTIES OF BENZODIAZEPINE ACTIONS IN RODENTS. J.P. Chambon (1), S. Tebib (2), J.J. Bourguignon (2), A. Perio (1), M. Heaulme (1), K. Bizière (1) and C.G. Wermuth (2). (1)Sanofi Recherche, rue du Prof. J. Blayac, 34082 Montpellier Cedex, France ; (2)Laboratoire de Pharmacochimie Moléculaire, UA 501, Centre de Neurochimie du CNRS, Université Louis Pasteur, 67084 Strasbourg Cedex, France.

The benzodiazepine-like activity of the triazolopyridazine CL 218872 and its specific action at the BZD-1 central receptor site has been extensively established since the original work of Lippa et al. (Pharmacol. Biochem. Behav. 11 : 96-106, 1979).

In order to investigate the structural requirements which are needed in this chemical series for the interaction with the BZD central receptor sites, we synthesized triazolopyridazines substituted in the 5-position of the pyridazine ring. These compounds exhibited lower affinity for the BZD central receptor sites than CL 218872 with IC50 values ranging from 1 to 10 µM. When administered orally to mice these derivatives did not elicit the classical sedative pattern of activity of BZD. These derivatives did not induce myorelaxant effects in mice (traction test) but administered orally (12.5 to 100 mg/kg) 30 min before diazepam 3.5 mg/kg i.p., they dose-dependently suppress the BZD-induced myorelaxation in this test. These compounds were found inactive in antagonizing pentylenetetrazole-elicited seizures in mice but prevented the anticonvulsant effect of diazepam against pentylenetetrazole-induced clonic seizures. Finally, in the conflict test in rats performed according to the Geller and Seifter procedure these compounds (0.7 to 25 mg/kg i.p.) antagonized the desinhibitory effect of diazepam 4 mg/kg i.p..

In conclusion, the data strongly suggest that triazolopyridazines substituted in the 5-position of the pyridazine ring have potent antagonistic properties of BZD actions in rodents.

79.8 CENTRAL CARDIOVASCULAR EFFECTS OF BACLOFEN AND ITS INTERACTION WITH BARORECEPTOR REFLEXES IN SPONTANEOUSLY HYPERTENSIVE RATS. R. Singh* and M.K. Ticku (SPON: A. Modak). Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

Our previous studies have demonstrated that specific [^{3}H]-baclofen binding to $GABA_B$ receptors in brain is significantly different between spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats (Neurosci. Abstracts 10:387, 1984). Here we report the effects of intracerebroventricular (icv) administration of baclofen on mean arterial pressure (MAP) and heart rate (HR) in conscious SHR and WKY. In 12 week old SHR (n = 8) and WKY (n = 8), steel cannula (21-gauge) were implanted into the lateral cerebral ventricle. One week after cannulation, catheters were placed in femoral artery and vein for recording of MAP and intravenous (iv) injection, respectively. MAP and HR were monitored on a Beckman R-611 polygraph with an Ailtech pressure transducer (MS-20). ICV injections were made with 30-gauge injector connected to 10 µl syringe with PE20. Administration of (±)baclofen (0.5 - 1.5 µg/kg) icv elicited a dose-dependent increase in MAP and HR in both SHR and WKY. The peak increases after 60 min of icv baclofen (1.0 µg/kg) was 13 ± 3 mmHg in SHR vs 27 ± 5 mmHg in WKY. Changes in HR were variable and not different between the two strains. Baclofen (1.0 µg/kg) icv significantly suppressed the reflex bradycardia to phenylephrine (3.0 µg/kg, i.v.) in conscious SHR (-49 ± 3 vs -24 ± 2 beats per minute [bpm], n = 7) but not in WKY (-33 ± 5 vs -33 ± 4 bpm, n = 7). Similarly, reflex increases in HR to nitroprusside (10 µg/kg, i.v.) was significantly lowered after baclofen in SHR (99 ± 8 vs 58 ± 8 bpm) than in WKY (87 ± 10 vs 89 ± 8 bpm). However, treatment with sympathetic ganglionic blocker hexamethonium (HX; 25 mg/kg, iv) produced an equivalent fall in MAP between SHR (n = 6) and WKY (n = 6) rats injected icv with either baclofen or 0.9% saline alone. These findings indicate that baclofen effect on baroreceptor reflexes in SHR may not be completely dependent on the total functional contribution of the sympathetic nervous system.

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79.9 EFFECTS OF METHYL ß-CARBOLINE-3-CARBOXYLATE, RO 15-1788 AND CGS 8216 ON THE MUSCLE TONE IN GENETICALLY SPASTIC RATS. M. Schwarz *, C. Ikonomidou *, L. Turski *, T. Klockgether * and K.-H. Sontag * (SPON:U. Kuhnt), Max-Planck-Institute for Exp. Medicine, Hermann-Rein-Str. 3, D-3400 Göttingen, F.R.G.

Benzodiazepines (BDZs) are supposed to exert their anticonvulsant, anxiolytic and muscle relaxant action by interacting with specific BDZ receptors. Substances which antagonize the action of BDZs have been classified as either 'neutral antagonists' due to their lack of intrinsic activity or as 'inverse agonists' due to their ability to exert actions opposite to those of benzodiazepines. In the present study the effect on the muscle tone of ß-carboline-3-carboxylic acid methylester (ß-CCM), an inverse benzodiazepine (BDZ) agonist, and that of Ro 15-1788 and CGS 8216, both putative neutral BDZ antagonists were studied in genetically spastic rats. These animals exhibit pathologically increased muscle tone, which can be recorded and quantified in the electromyogram (EMG) of the gastrocnemius (GS) muscle. ß-CCM, 2.5 and 3.0 mg/kg i.p., augmented the tonic activity in the EMG of GS muscle in spastic rats, while did not modify the muscle tone at doses of 1.0 and 2.0 mg/kg. Diazepam, 0.4 mg/kg i.p. and Ro 15-1788, 5 mg/kg i.p., but not CGS 8216, 5 mg/kg i.p., antagonised the effect of the ß-carboline on the muscle tone. Ro 15-1788, at doses of 0.1-5 mg/kg i.p., did not affect the muscle tone in genetically spastic rats, whilst at doses of 25-200 mg/kg the imidazodiazepine dose-dependently increased the tonic activity in the EMG. The action of Ro 15-1788, 50 mg/kg i.p., was reversed by diazepam, 0.4 mg/kg i.p., and ß-CCM, 2 mg/kg i.p., while CGS 8216, 5 mg/kg i.p., facilitated the effect of Ro 15-1788 on the muscle tone. CGS 8216 at doses of 5 and 25 mg/kg i.p. was devoid of any effect on the muscle tone, whilst at doses of 50 and 200 mg/kg the pyrazoloquinoline increased the tonic activity recorded in the EMG from the GS muscle of spastic rats. The effect of CGS 8216, 50 mg/kg i.p., on the muscle tone was blocked by diazepam, 0.4 mg/kg i.p., while Ro 15-1788, 5 mg/kg i.p., and ß-CCM, 2 mg/kg i.p., augmented the action of CGS 8216. Diazepam, 0.4 mg/kg and ß-CCM, 2.0 mg/kg, both did not influence the activity in the EMG of genetically spastic rats by themselves. This study demonstrates that increase in the muscle tone may be achieved via inverse agonist action at BDZ receptors. The results indicate that BDZ receptors are involved in the effect ß-CCM and Ro 15-1788 displayed on the muscle tone and suggest that at a critical dose range Ro 15-1788 possesses inverse agonist activity at BDZ receptors in vivo. The failure of both ß-CCM and Ro 15-1788 to prevent the augmenting effect of CGS 8216 on the muscle tone might indicate a different mechanism of interaction of the pyrazoloquinoline at the BDZ receptor.

79.10 CAFFEINE ANTAGONIZES BENZODIAZEPINE EFFECTS ON SACCADIC EYE VELOCITIES, SEDATION, AND VIGILANCE. V. Matsuo, D.W. Hommer, O. Walkowitz*, G.A. Chrousos* and H. Weingartner*. NEI, Clinical Branch, and NIMH, NSB, NIH, Bethesda, MD 20205.

Previous studies have established that intravenous benzodiazepine (BZ) reduces the peak velocity of horizontal saccades and impairs cognitive functions in a dose dependent manner. The purpose of this series of experiments was to determine whether pretreatment with caffeine, a methylxanthine with recognized diazepam antagonist properties, had any effect on BZ effects on eye velocity and cognitive processes.

Five healthy normal volunteers were used. They gave informed consent. Caffeine or placebo pretreatments were administered double blind on three occasions at least one week apart. Each subject received 3 mg/kg caffeine, 10 mg/kg caffeine or quinine placebo orally 50-60 min before BZ. Diazepam was given in serial doses of 25, 25, 50 and 100 µg/kg i.v., at 15 min intervals. Subjects performed a saccadic tracking task within 2-5 minutes of receiving each dose. They viewed a horizontal array of 5 LED's placed centrally, and 7.5° and 15° to the right and left. The LED's were activated in a quasi-random order and the subject was instructed to move his eyes to the target as quickly and accurately as possible. Eye position was monitored using IR reflection, and differentiated to give eye velocity. The effects of diazepam were also evaluated on self-rated sedation, on vigilance, recent episodic memory, and semantic memory.

The slowing of saccadic velocities following placebo pretreatment was consistent with earlier results. After the highest dose of BZ the velocity of 15-30° saccades was reduced by as much as 200-300°/s. Smaller saccades were slowed to a lesser degree. In contrast, after 10 mg/kg caffeine, 15-30°saccades were slowed by approximately 50°/s. Other BZ-induced changes in saccadic following were also blocked, viz., instability of fixation and increased variability in the amplitude-velocity curve. Pretreatment with 3 mg/kg caffeine did not appear to have a consistent effect on saccadic velocities.

Following placebo pretreatment, diazepam increased sedation and reduced vigilance and recent memory. Retrieval of information from long-term memory was unaffected. Pretreatment with 3 mg/kg of caffeine had no consistent effect on any of these measures. Pretreatment with 10 mg/kg of caffeine reduced the self-rated sedation produced by diazepam and blocked the vigilance deficit produced by diazepam ($p=0.05$). Caffeine pretreatment had no effect on diazepam induced impairment of recent memory.

We conclude that 10 mg/kg of caffeine pharmacologically antagonizes several of the effects of BZ. This antagonism appears to be selective for less complex cognitive and motor tasks.

79.11 GABA UPTAKE INHIBITORS PRODUCE A GREATER ANTINOCICEPTIVE RESPONSE THAN OTHER TYPES OF GABAERGIC DRUGS. S.H. ZORN AND S.J. ENNA. Depts. Pharmacology and of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77025.

GABAergic drugs produce antinociception in laboratory animals and man. It has been reported that the ethyl ester of nipecotic acid (NAEE), a GABA uptake inhibitor, is a more efficacious antinociceptive agent than direct-acting GABA receptor agonists or GABA transaminase inhibitors. However, it is difficult to determine whether this greater activity was due solely to an ability to enhance GABAergic transmission or whether the cholinomimetic properties of NAEE were primarily responsible for the observed response. This issue was addressed in the present study by comparing the antinociceptive responses to some new GABA uptake inhibitors, SKF-89976A and SKF-100330A with NAEE, direct-acting $GABA_A$ and $GABA_B$ receptor agonists (THIP and baclofen, respectively) and the GABA transaminase inhibitor γ-acetylenic GABA (GAG).

Unlike NAEE, the SKF compounds have no appreciable affinity for cholinergic muscarinic receptor binding sites in rat brain, suggesting that they are devoid of cholinomimetic activity. Uptake experiments with rat brain synaptosomes revealed that the SKF compounds are selective inhibitors of GABA transport, having no effect on the accumulation of aspartate, glutamate, ß-alanine or glycine. Using a mouse tail-immersion assay it was found that, as with uptake inhibition, the antinociceptive response to SKF-89976A was stereoselective, with the d-isomer possessing most of the activity. A comparison at maximally effective doses revealed that the SKF compounds were substantially more efficacious than the GABA transaminase inhibitor and the direct-acting receptor agonists, even when the latter drugs were given in combination with one another. The results suggest that GABA uptake inhibitors are more efficacious antinociceptive agents than other types of GABAergic compounds, suggesting that they may be facilitating GABAergic transmission in a system that is less affected by THIP, baclofen, or GAG. (Supported in part by grants from the National Science Foundation, the National Institute of Mental Health and Bristol-Myers, Inc.).

79.12 REVERSIBLE CONFORMATIONAL CHANGES ACCOUNT FOR CONVULSANT/BARBITURATE RECEPTOR ACTIVITIES ON THE GABA/BENZODIAZEPINE RECEPTOR/IONOPHORE COMPLEX. Ronnie G. King, Greg B. Stauber* and Richard W. Olsen. Department of Biochemistry, University of California, Riverside, and Department of Pharmacology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Convulsant/Barbiturate activities of the GABA/benzodiazepine (BZ) receptor complex, abolished by membrane exposure to or solubilization in Triton X-100, can be recovered by removal of Triton and exchanging into CHAPS detergent, suggesting that changes in conformational states account for the presence or absence of this activity. Receptor sites for BZ, barbiturates, and a variety of convulsant drugs are intimately related to the GABA receptor-Cl^- channel complex which mediates the central inhibitory synaptic action of this neurotransmitter. The cage convulsant [^{35}S]t-butyl bicyclophosphorothionate (TBPS) labels the picrotoxin/convulsant receptor site on the GABA/BZ receptor-ionophore complex. [^{35}S]TBPS binding activity has been solubilized with the zwitterionic detergent 3-[(3-cholamidopropyl)-dimethylammonio] propanesulfonate (CHAPS) and co-purifies with the GABA/BZ receptor protein (King and Olsen, Biochem. Biophys. Res. Comm. 119, 530-536 (1984); Sigel and Barnard, J. Biol. Chem. 259, 7219-7223 (1984); Olsen et al., Neuropharmacology 23, 853-854 (1984). [^{35}S]TBPS binding activity and barbiturate enhancement of GABA and BZ binding were not detected in extracts solubilized and purified in Triton X-100 or deoxylcholate (DOC) and appeared to be irrversibly lost, while BZ binding was unaffected and GABA binding was improved. This could be due to one or more of four possible explanations: selective denaturation of part of the complex, loss of peptide subunits or other essential membrane molecules, different aggregation states of a protein complex of constant subunit composition, or different conformational states of a single protein complex of constant size and subunit composition.

Convulsant/barbiturate activities present in 1.0% CHAPS extracts were lost rapidly upon addition of 0.5% Triton X-100. However, the activity could be recovered upon removal of Triton by gel filtration column chromatography in 0.5% CHAPS, with no change in apparent molecular weight. Similarly, maximal yield of GABA and BZ binding activity is obtained in 0.5% DOC, but negligible TBPS/barbiturate activity is observed. [^{35}S]TBPS binding and barbiturate modulation of GABA/BZ binding are found after exchanging DOC for CHAPS on a Trisacryl column. Finally, a CHAPS extract, spiked with 0.5% Triton, showing no convulsant/barbiturate activity before or after purification on a BZ affinity column, recovered those activities upon exchange of Triton for CHAPS. These results suggest that conformational states (4th hypothesis above) account for convulsant/barbiturate activity, the active state being associated with low affinity GABA agonist binding.

79.13 INHERENT DIFFERENCES IN CONVULSANT SENSITIVITY TO RO 5-4864, PICROTOXININ AND PENTYLENETETRAZOLE AMONG INBRED STRAINS OF MICE. T.W. Seale, G.T. Bolger* and P. Skolnick. Lab. of BioOrganic Chemistry,NIADDKD, National Institutes of Health,Bethesda,MD 20205.

The neurophysiological significance of specific,high affinity binding sites in the brain for peripheral type benzodiazepines (PTB) has remained unknown. Until recently these binding sites were thought to be associated primarily with non-neuronal elements and no function could be assigned to them. The discovery that the PTB Ro 5-4864 possessed potent convulsant activity suggested that these binding sites are receptors with important biological functions in the CNS. However, because Ro 5-4864 and its analogs are potent competitors for displacement of ^{35}S t-butylbicyclophosphorothionate(TBPS) from the picrotoxinin binding site, there remained the possibility that the convulsant action of Ro 5-4864 resulted from its interaction with the picrotoxinin binding site, rather than being mediated by the PTB binding site. We have used genetic techniques to resolve the site of action of Ro 5-4864. Variants with altered dose dependent responses to Ro 5-4864 were identified by scoring the occurrence of clonic and tonic seizures in a 30 minute time period following i.p. administration of the convulsant (0.1ml/mouse in a vehicle composed of 70% saline,30% of a 1:1 mix of Emulphor and dimethylsulfoxide). Male mice 10 weeks old were used. Strain BALB/cBy was found to be hyporesponsive to both the clonic- and tonic- seizure inducing action of Ro 5-4864 compared to 8 other inbred strains of mice (e.g. in DBA/2 the EC_{50} for clonic seizure induction is 17mg/kg, in BALB/cBy it is 34 mg/kg). This difference in sensitivity to Ro 5-4864 is specific because no difference between DBA/2 and BALB/cBy exists for the dose dependent induction of seizures by strychnine or bicuculline. However,BALB/c By mice are markedly hyporesponsive to the convulsant action of picrtoxinin and pentylenetetrazole compared to DBA/2 mice. Entry of Ro 5-4864 into the brains of the two mouse strains did not differ significantly. Binding of 3H Ro 5-4864 to cerebrum membrane preparations showed a reduction of 15-20% in the number of Ro 5-4864 binding sites in BALB/cBy compared to DBA/2 but no change in their affinity (B_{max}=1005 fmol/mg, K_d=0.8 nM for DBA/2). The number of PTB binding sites in kidney of the two strains did not differ in this way. No difference in the number of TBPS binding sites or their affinity was found when the brains of these two strains were compared. We conclude that hyporesponsiveness to the convulsant action of Ro 5-4864 in BALB/cBy mice results from a genetically determined,brain-specific decrease in PTB sites and that these sites are functional receptors. A second qualitative change in the picrotoxinin binding site probably accounts for the alteration in response to picrotoxinin and pentylenetetrazole.

79.14 PICROTOXIN REVERSAL OF BARBITURATE-STIMULATED CHLORIDE EFFLUX FROM SYNAPTONEUROSOMES PARALLELS DIFFERENCES IN IN VIVO CONVULSANT SENSITIVITY BETWEEN TWO INBRED STRAINS OF MICE. Rochelle D. Schwartz Thomas W. Seale , Phil Skolnick* and Steven M. Paul*. Clinical Neuroscience Branch, NIMH and Lab. Bioorganic Chem. NIADDKD, NIH, Bethesda, MD 20205.

Differential seizure sensitivity to convulsants active at the GABA receptor complex has been recently identified in 2 inbred mouse strains (Seale et al., this vol.). The Balb C/By (BC) strain is hypo-responsive to convulsants such as picrotoxinin and pentylene tetrazole while the DBA/2 (DB) strain is hyper-responsive to these agents. In the present study, we have investigated the interaction of pentobarbital (PB) with picrotoxinin-induced convulsions in these 2 strains of mice. The ED_{50}s for picrotoxinin-induced clonic seizures in the BC and DB mice were 3.2 and 18 mg/kg, i.p., respectively. When varying concentrations of PB were given 10 min prior to the administration of picrotoxinin (5 mg/kg, ED_{100}), the IC_{50} for PB protection from picrotoxnin-induced tonic seizures was less in the BC strain (5.5 mg/kg) than in the DB strain (18 mg/kg). We have also investigated possible neurochemical correlates to the behavioral differences observed in these 2 mouse strains using ^{36}chloride (Cl^-) efflux and receptor binding assays. Recently we reported the use of a novel subcellular brain preparation ("synaptoneurosome") to study $^{36}Cl^-$ efflux associated with the GABA/picrotoxin/ barbiturate receptor complex (Schwartz et al., FEBS Lett., 175, 193-196, 1984; Schwartz et al., J. Neurosci., in press). In this study, the effects of PB and picrotoxin on $^{36}Cl^-$ efflux from mouse forebrain synaptoneurosomes were determined. PB (500 uM) stimulated $^{36}Cl^-$ efflux to the same degree in both strains of mice. Picrotoxin (1 mM) had no significant effect on basal $^{36}Cl^-$ efflux. However, a significant difference in the ability of picrotoxin to reverse the PB-induced $^{36}Cl^-$ efflux was observed in the BC and DB strains (38.3 ± 6.5 vs 74.8 ± 7.4% inhibition, resp.). In binding studies there was no difference between the 2 strains in either the EC_{50} for PB-enhancement of ^{3}H-muscimol binding, nor the picrotoxin reversal of PB-enhanced ^{3}H-muscimol binding (low or high affinity sites). However, the ability of PB to decrease ^{35}S-TBPS (which putatively binds to a picrotoxin-like site on the Cl^- ionophore) binding was greater in the BC strain than in the DB strain (IC_{50} = 74 uM and 130 uM, resp.) These behavioral and biochemical studies suggest that the altered sensitivity of the binding sites on the Cl^- ionophore associated with the GABA receptor complex and the subsequent functional alterations in Cl^- transport may account for the genetic differences in convulsant sensitivity in these 2 strains of mice.

79.15 CHLORIDE TRANSPORT STUDIES IN BRAIN SYNAPTONEUROSOMES: EFFECTS OF GABA AGONISTS AND ANTAGONISTS, PENTOBARBITAL, FLURAZEPAM AND THE ROLE OF PHOSPHOLIPASE A_2. Steven M. Paul*, Rochelle D. Schwartz, Phil Skolnick, and Jane Jackson*, (SPON: C.R. Mantione), Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205.

Recently, we have reported the use of a novel subcellular brain preparation ("synaptoneurosome") to study chloride (Cl^-) transport associated with the GABA/barbiturate/picrotoxin receptor complex (Schwartz et al., FEBS Lett. 175, 193-196, 1984; Schwartz et al., J. Neurosci., in press). In the present study we have investigated the effect of the GABA receptor agonist muscimol, GABA and the water soluble benzodiazepine, flurazepam on $^{36}Cl^-$ transport from rat cerebral cortical synaptoneurosomes. Muscimol and GABA caused a dose-dependent increase in $^{36}Cl^-$ efflux ($EC_{50} \simeq 2\mu M$ and 500 μM, resp.). These effects were reversed by bicuculline (500 μM) which also decreased $^{36}Cl^-$ efflux ($EC_{50} \simeq 150$ μM). However, in experiments where bicuculline had no effect on basal $^{36}Cl^-$ efflux, it significantly decreased muscimol-stimulated $^{36}Cl^-$ efflux. Flurazepam was inactive at low concentrations (< 100 μM) but stimulated $^{36}Cl^-$ efflux at concentrations greater than 300 μM (8-20%). This effect was not reversed by the benzodiazepine antagonist, Ro15-3505, indicating a probable action of flurazepam directly with the Cl^- channel. In separate studies $^{36}Cl^-$ uptake was also measured. Muscimol stimulated $^{36}Cl^-$ uptake in a multiphasic manner ($EC_{50} \simeq 10$ μM). the effect of muscimol was reversed by bicuculline (100 μM). The advantages and disadvantages of measuring $^{36}Cl^-$ uptake vs. $^{36}Cl^-$ efflux will be discussed. The effects of phospholipase A_2 (PLA_2) on $^{36}Cl^-$ efflux were also investigated. Pretreatment of synaptoneurosomes with PLA_2 (naja naja venom, 0.05 units/ml) decreased the stimulation of $^{36}Cl^-$ efflux by muscimol and pentobarbital (30-70%) and decreased the inhibition of $^{36}Cl^-$ efflux by bicuculline and picrotoxin (30-92%). The effects of PLA_2 were reversed by mepacrine (100 μM) and EGTA (2.5 mM). Pretreatment of synaptoneurosomes with mepacrine had the opposite effect; the stimulation of $^{36}Cl^-$ efflux by muscimol and pentobarbital and the inhibition of $^{36}Cl^-$ efflux by bicuculline and picrotoxin were enhanced by 116-120% amd 50-80%, respectively. These data indicate that the functional properties of the GABA/barbiturate/picrotoxin receptor complex may be regulated by alterations in phospholipids closely associated with the membrane-bound receptor complex.

79.16 FUNCTIONAL RESPONSES OF THE γ-AMINOBUTYRIC ACID RECEPTOR FROM BRAIN. K. Subbarao* and D.J. Cash. Neurochemistry Unit, Missouri Institute of Psychiatry and Department of Biochemistry, University of Missouri-Columbia, School of Medicine, St. Louis, MO 63139.

A combination of two new tools is being used to investigate the functional response of the γ-aminobutyric acid (GABA) receptor from rat brain. Harris has recently observed a GABA mediated uptake of radioactive chloride ion using a "microsac preparation" from whole mouse brain (Allan, A.M. et al., abstracts FASEB meeting, Anaheim, CA, 1985). Based on this observation we have developed a preparation from rat brain regions suitable for use with quench flow technique. Quench flow technique has been used to measure acetylcholine mediated cation transmembrane ion flux in vesicle preparations from electric fish, and on this basis a minimal kinetic scheme for the functional response of acetylcholine receptor was proposed (Cash, D.J. and Hess, G.P. (1980) Proc. Natl. Acad. Sci. USA 77, 842-846; Hess, G.P. et al., (1983) Ann. Rev. Biophys. Bioeng. 12, 443-473).

A homogenate of rat cerebral cortex was centrifuged at 300 g, 5 min. The supernatant was pelleted, resuspended in physiological saline and centrifuged on a 4-12% Ficoll-400 gradient at 100,000 g, 1 hr. A middle band containing the functionally active vesicles was separated from a lighter "myelin" fraction and a heavier "mitochondrial" fraction. The transmembrane ion flux was initiated on addition of GABA and terminated by addition of bicuculline or N-methyl bicuculline. The ^{36}Cl content of the vesicles was determined by a filter disc assay. This vesicle preparation had a specific internal volume (accessed via GABA mediated transmembrane chloride flux) of 5 x 10^{-2} μl/mg protein. Transmembrane equilibration of chloride was completed in two phases of ion flux at saturating concentrations of GABA ($\geq$100 μM). The first phase was complete in less than 100 ms and the second in about 4 s. Experiments in which the membrane vesicle preparation was preincubated before the addition of chloride tracer ion showed that this behavior involves a single population of membrane vesicles with GABA receptors which are desensitized in two processes, one with a halftime of $\leq$ 50 ms and the second with a halftime of about 1 s. With decreased GABA concentrations both phases are decreased in amplitude. The ligand dissociation constants corresponding to the measured responses are in the region of 30 μM and desensitization and ion flux have a different dependence on GABA concentration.

These results, complementary to measurements of ligand binding and electrophysiology, indicate that the GABA dissociation constant of the active state of the receptor is higher than previously reported and that the receptor is rapidly converted to desensitized states in the presence of GABA.

79.17 CONTRASTING EFFECTS OF PHENOBARBITAL AND PICROTOXIN IN THE MEDIAL AND LATERAL SEPTUM ON HIGH AFFINITY CHOLINE UPTAKE IN THE HIPPOCAMPUS. J.A. Richter and J.M. Gormley*. Depts. Pharmacology and Psychiatry, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

Systemic in vivo administration of phenobarbital and picrotoxin cause an inhibition and stimulation respectively of septal-hippocampal cholinergic neurons as measured by changes in high affinity choline uptake in hippocampal synaptosomes in vitro. In order to determine where within the brain these drugs act to cause these effects, we have administered these drugs to selected brain sites in the awake rat via permanently implanted cannulae.

Phenobarbital injected into the medial septum caused a decrease in choline uptake in the hippocampus. Similar effects were obtained with barbital and with muscimol. When tested in the lateral septum phenobarbital was much less effective. In contrast, picrotoxin had no effect when injected in the medial septum but it stimulated hippocampal choline uptake when it was injected in the lateral septum. Picrotoxin coadministered with phenobarbital in the medial septum inhibited the effect of phenobarbital alone.

These data are consistent with the results of Brunello and Cheney (JPET 219: 489, 1981) on the effects of muscimol and bicuculline in the medial septum on hippocampal ACh turnover. In addition we have demonstrated that phenobarbital and barbital have inhibitory effects in the medial septum on septal-hippocampal cholinergic neurons. Together the results suggest that GABAergic receptor complexes exist in the medial septum but they are not tonically active since the antagonists alone have no effect there.

Our data further suggest that there are other GABAergic receptor complexes in the lateral septum. These complexes are tonically active and influence the medial septal cholinergic neurons, since picrotoxin in the lateral septum stimulates hippocampal choline uptake. Costa et al. (Life Sci. 32: 165, 1983) have shown GAD-positive cell bodies in both the medial and lateral septum and have suggested that both have terminals in the medial septum. Our data are consistent with this possibility, and our data also indicate there are other GABAergic receptor complexes in the lateral septum which are tonically active and which influence the medial septal cholinergic neurons by an unknown pathway. (Supported by PHS grant DA 00796).

79.18 PIPECOLIC ACID: A NEW NEUROMODULATOR? E. Giacobini (SPON: D. Hoffman). Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708 USA

Pipecolic acid (PA), one of the three cyclic secondary amino acids or imino acids present in the brain, along with proline and hydroxyproline, represents the major metabolite of lysine in the CNS. Its presence in the rodent brain has been unambiguously demonstrated with TLC-MS (Schmidt-Glenewinkel et al., 1977) and its regional distribution described with a new HPLC-EC method (Kim and Giacobini, 1984). Pipecolic acid levels and transport in brain are particularly high during the perinatal period (Kim and Giacobini, 1984). A high affinity, Na^+-dependent, uptake of PA in brain synaptosomes and a Ca^{++}-dependent release from brain slices have been demonstrated in our laboratory. In addition, a saturable transport across the blood-brain-barrier and high affinity specific binding of ^{3}H-PA have been described (Nishio et al., 1981, 1982; Gutierrez and Giacobini, 1983). The specific binding of ^{3}H-PA shows a regional distribution. This evidence strongly suggests that PA containing neurons and terminals may exist in the brain. Evidence for a reciprocal modulatory effect of PA and GABA has been made recently available (Giacobini and Gutierrez, 1983). These results, taken together with the evidence for localization, distribution, metabolism and action of PA in brain neurons, strongly support the role of PA as an endogenous and physiological modulatory substance in the mammalian brain. (Supported by U.S. Public Health Service Grants NS 11430 and NN 14086 and by SIU Cent. Res. grant 2-40202 to E.G.)

GABA AND BENZODIAZEPINES: RECEPTOR CHARACTERIZATION AND LOCALIZATION I

80.1 ASSOCIATION WITH REGULATORY PROTEINS: AN ALTERNATIVE TO MULTIPLE BENZODIAZEPINE RECEPTORS, W. F. Herblin, Central Research and Development Department, E. I. du Pont de Nemours & Company, Experimental Station, Wilmington, Delaware 19898

A mathematical model is presented which describes the association of a ligand-receptor complex with a regulatory subunit. This is meant to simulate the situation in which a receptor is coupled to a regulatory protein or subunit which serves as a tranducer between the ligand binding event and the subsequent second messenger changes, as has been found in the case of theβ -adrenergic receptor.

We have used experimental data obtained with the benzodiazepine receptor to evaluate the parameters of this model and to compare its performance to that of the alternative multiple-site hypothesis. Results obtained from experiments using equilibrium binding, competition by the same or different ligands, or time-dependent kinetics can be individually accommodated by either model, but the association model more easily encompasses their combination.

The major advantage of the association model is that it is based on a biochemical process that has been experimentally demonstrated to exist. Therefore, many of the assumptions that were required in the development of the model as well as many of the derived predictions from the model are subject to direct test and experimental verification.

80.2 PHOTOAFFINITY LABELLING OF THE BENZODIAZEPINE RECEPTOR OF THE GABA RECEPTOR COMPLEX IN LOCUST CNS. G.G.Lunt*, D.MacAllan*, and T.Robinson* (SPON: R.W.Olsen). Dept. of Biochemistry, University of Bath, Bath, England, BA2 7AY.

We have previously shown that membranes from the supraoesophageal ganglion of the locust (Schistocerca gregaria) have GABA receptors that interact with benzodiazepine binding sites in a manner similar to that seen in mammalian brain (Robinson et al., Neurochem. Int. in press 1985; Biochem. Soc. Trans. in press, 1985). We have now extended these studies and have used [^{3}H]flunitrazepam to specifically photoaffinity label the locust receptor complex.

Membranes were prepared as described previously (Robinson et al., Neurochem. Int. in press, 1985) and resuspended in 10mM Tris HCl, 4mM $CaCl_2$, 4mM $MgCl_2$, 0.15M choline chloride, pH 7.4 and preincubated in the presence or absence of 10^{-4}M diazepam at 0°C for 30 min. Following the addition of [^{3}H]flunitrazepam (74 Ci/mmol) at a final concentration of 20nM the membrane suspension was incubated at 0°C for 90 min and then irradiated for 3 min at 350nm.

Membranes were extensively washed and the membrane proteins separated by SDS-PAGE. Gels were either sliced at 2mm intervals and counted or subjected to autoradiography. Specific labelling was observed in a band at 50K. This specific labelling was enhanced when membranes were incubated in the presence of 10^{-8}M GABA. In other studies we have found that the equilibrium binding of [^{3}H] flunitrazepam to the membranes is enhanced maximally by 10^{-8}M GABA.

Our results show that locust GABA receptor complex has a benzodiazepine binding component of a similar size to that seen in mammalian brain. The pharmacology of the locust benzodiazepine site is however different from that seen in mammalian brain (Robinson et al., Biochem. Soc. Trans. in press, 1985).

We are grateful to the SERC (T.R.) and the AFRC (D.M.) for support.

80.3 REEVALUATION OF SATURATION BINDING TO BENZODIAZEPINE RECEPTORS. E.I. Tietz, M. Ito*, T.H. Chiu and H.C. Rosenberg, Medical College of Ohio, Toledo, OH 43699.

Overwhelming evidence favors benzodiazepine receptor heterogeneity despite a few incongruent pieces of evidence. The most widely noted, is the linearity of Scatchard plots reported by our laboratory and most others. Recently, we noticed a consistent curvilinearity in plots generated from [^{3}H]flunitrazepam ([^{3}H]FNP) assays in which we used higher than usual concentrations of [^{3}H]FNP to compete for residual drug following chronic treatment. Experiments in untreated rats were done to verify this observation.

Centrifugation assays provide the most accurate estimation of bound and free ligand concentrations. The cerebral cortex (CTX), hippocampus (HIP), or cerebellum (CEB) was dissected from male rats (200-250 gm, .7 - 1 gm tissue). The P_2 pellet was triple washed in Tris-HCl (pH 7.4 at 0°C). Protein (.6 - .75 mg/ml) was incubated in duplicate with 16 concentrations of [^{3}H]FNP (.25 - 32 nM) or [^{3}H]Ro15-1788 ([^{3}H]RO) (.125 - 24 nM) for 1 hr at 0°C. Nonspecific binding was in the presence of 10^{-6} M clonazepam. The reaction was terminated by centrifugation (20,000g X 20 min at 4°C). The supernatant was removed by suction and the bound pellet was gently washed 2X with ice-cold Tris buffer, was solubilized (Protosol) with mixing, and counted. Each experiment was repeated 2 or 3 times.

Results were analyzed with the LIGAND program. [^{3}H]FNP binding to CTX and HIP revealed two high affinity binding sites of different proportions. Binding to CEB tissue was less conclusive but suggested one site. [^{3}H]RO labelled only one site in the three areas and the number of sites was less than that labelled by FNP.

Nonspecific binding was linear across all concentrations for both ligands. These findings do not reflect binding to "peripheral type" sites since [^{3}H]FNP binding in the presence of Ro5-4864 was still curvilinear.

		Kd1 (nM)	Kd2 (nM)	Bmax1	%	Bmax2	%	BmaxT
[^{3}H]FNP	CTX	.24±.08	3.86±.12	.64±.19	(20)	2.44±.10	(80)	3.08±.28
	HIP	.55±.01	10.2±.78	1.54±.04	(49)	1.62±.06	(51)	3.16±.09
	CEB	1.48±.46		2.43±.22	(100)			
[^{3}H]RO	CTX	.77±.02		2.65±.04	(100)	pmol/mg protein		
	HIP	.70±.01		2.16±.20	(100)			
	CEB	.81±.04		2.66±.00	(100)			

The curvilinear Scatchard plots are compatible with either two binding sites or negative cooperativity. 2 nM [^{3}H]FNP was dissociated by 100-fold dilution, with or without addition of excess cold FNP. With the addition of FNP, dissociation after 60 min was only increased 3-4%. Thus negative cooperativity is unlikely.

The results suggest that the benzodiazepines bind to two classes of high affinity sites in CTX and HIP. The proportion of sites is similar to that reported using other methods.

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80.4 ENHANCEMENT OF ^{3}H-GABA BINDING BY COMPOUNDS THAT BIND TO THE BENZODIAZEPINE RECEPTOR BUT DIFFER IN EFFICACY. B. A. Meiners* and A. I. Salama (SPON: R. D. Krell). Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, DE 19897.

Compounds known to interact at the benzodiazepine binding site have been shown to either mimic the actions of benzodiazepines (diazepam, CL218872), antagonize the actions of benzodiazepines (RO15-1788, CGS8216) or share in some but not all the actions of the benzodiazepines (CGS9896, CGS9895). This categorization has largely been obtained through behavioral studies. The effect of these compounds on the binding of ^{3}H-GABA to rat brain membranes was measured to determine whether it could be possible to distinguish between these classes. The assay was performed at 37°C in the presence of EGTA and the bound ^{3}H-GABA separated from the free by centrifugation. Diazepam and CL218872 caused a 20% to 45% increase in the binding. In contrast, RO15-1788 and CGS8216 caused a marginal enhancement of GABA binding (8% to 10%). In addition, these agents were capable of antagonizing the diazepam enhancement of GABA binding. On the other hand, CGS9896 (partial agonist) caused an intermediate level of enhancement (15% to 20%). The effect of CGS9896 was statistically distinguishable from that of both a full agonist and a full antagonist. These data suggest that enhancement of ^{3}H-GABA binding by compounds that interact with the benzodiazepine receptor may reflect the efficacy (agonist, antagonist, mixed type) of the compounds. CGS9895 caused an increase in GABA binding that was equal to that of diazepam but unlike diazepam, the effect was not reversible by either RO15-1788 or by beta-carboline ethylester suggesting that this enhancement of GABA binding was not mediated at the benzodiazepine binding site.

80.5 **KINETICS OF [^{3}H]FLUNITRAZEPAM BINDING TO BRAIN CELL CULTURES IN SITU** G. D. Schiller* and D. H. Farb (SPON: W. Riss). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Bklyn., NY 11203.

The enhancement of [^{3}H]flunitrazepam (FNZM) binding by GABA decreases during chronic exposure to flurazepam (Farb et al., Ann. N.Y. Acad. Sci. 1984, 435, 1). As a first step to examine the interactions between the benzodiazepine (BZD)- and GABA-binding sites in situ, we have investigated the kinetics of [^{3}H]FNZM binding to living primary brain cell cultures, (derived from 7d old embryonic chick, maintained in culture for 6 to 7d). Association of [^{3}H]FNZM (1,2.5,5,10nM) was determined from 0.5 to 90min at 0°C. Nonspecific binding was defined as [^{3}H]FNZM bound in the presence of 0.1mM FNZM. The kinetics for association were biphasic with fast and slow exponential components. The k_{obs} increased for both components as ligand concentration was increased. A plot of k_{obs} for the fast and slow components versus ligand concentration yielded 2 straight lines ($r^2 > 0.9$). The pseudo-first-order association rate constants (k_1) were 0.286×10^9 and 0.042×10^9 $M^{-1}min^{-1}$; the dissociation rate constants (k_{-1}) were 0.64 and $0.042min^{-1}$ for the fast and slow components respectively. The resulting affinities were 2.2 and 4.2nM in proportions of 36±5% and 64±5% for the fast and slow components, respectively. Analysis of equilibrium binding to intact cells (0°C) yielded a single high affinity binding site of 4nM (n=5 experiments). Biphasic dissociation was confirmed independently (2 experiments): Cells were equilibrated with 5nM [^{3}H]FNZM at 0°C and dissociation was initiated either by dilution (k_{-1}, 0.52 and $0.035min^{-1}$) or by addition of 0.1mM (final conc.) FNZM (k_{-1}, 0.78 and $0.055min^{-1}$). This shows that high-affinity BZD binding to neurons in situ does not follow simple one-site Michaelis Menten kinetics. We cannot yet say whether the BZD-binding sites exist as interconvertible conformers or as distinct receptors. It remains to be determined whether or not both of the sites reported here are coupled to a GABA-receptor and if binding of [^{3}H]FNZM to both of these sites is subject to enhancement by GABA. It will be important to examine the potential role of these two sites in the functional regulation by BZDs of GABA-receptor action.

80.6 **CHLORDIAZEPOXIDE STIMULATES DESENSITIZATION OF THE GABA RESPONSE** D. Mierlak* and D. H. Farb. Dept.of Anatomy & Cell Biology, SUNY Downstate Med. Ctr. Brooklyn, NY 11203.

GABA receptor (GABA-R) modulation by benzodiazepines (BZDs) has been previously described. Electrophysiological studies have established that (1) GABA induces an increase in chloride conductance, (2) BZDs potentiate GABA-mediated conductance increases and (3) GABA responses desensitize during prolonged application of GABA. Studies demonstrating GABA enhancement of BZD binding utilize long incubation times with respect to the time course of GABA-R desensitization, presumably reflecting enhanced BZD binding to the desensitized GABA-R. This predicts that BZDs have a greater affinity for the desensitized GABA-R and should promote GABA-R desensitization. We have begun to investigate these paradoxical observations by determining the apparent rate constants of desensitization of the GABA response in the presence and absence of a saturating concentration (300uM) of chlordiazepoxide (CDPX).

Intracellular recordings were made from spinal cord cultures under standard current-clamp conditions with 3M KCl electrodes. Drug solutions were delivered by pressure ejection from multi-barrel pipets. Application of GABA in the absence or presence of CDPX caused a conductance increase (gGABA), which faded with time to a steady-state (gSS). CDPX alone caused small or no changes in potential or conductance. gGABA values were corrected by subtracting gSS and plotted semi-logarithmically against time. The best straight line was used to determine the apparent rate constant of desensitization for GABA alone (kapp) or in the presence of CDPX (kapp'). Extent of desensitization (%D) was determined as follows:[(peak gGABA - gSS) / peak gGABA] X 100%.

In three neurons, kapp for 10uM GABA was 0.041 ± 0.006 s^{-1}. In the presence of 300uM CDPX, this increased to kapp' = 0.146 ± 0.032 s^{-1}, representing a 3.5 ± 0.5 fold stimulation of kapp. In addition, the presence of CDPX increased %D by 18% ± 5%. In two initial experiments where multiple GABA concentrations were applied to the same neuron, kapp and %D increased with increasing peak gGABA. The range of GABA concentrations used produced gGABA values above and below the CDPX potentiated 10uM GABA response. In both cells, kapp' and %D determined for the CDPX potentiated 10uM GABA response was greater than the predicted kapp and %D for an equivalent gGABA produced by GABA alone: kapp' was enhanced 2.4 & 2.0 fold; %D was enhanced 18% & 8%. This result suggests that the stimulation of kapp and %D seen in the presence of CDPX cannot be accounted for solely by the increase in gGABA produced in the presence of CDPX. The stimulation of GABA desensitization by CDPX is consistent with a model in which CDPX has a greater affinity for a GABA-bound desensitized GABA-R while displaying low affinity for the unoccupied desensitized GABA-R.

80.7 EFFECT OF BENZODIAZEPINE BINDING SITE LIGANDS ON t-BUTYLBICYCLO-PHOSPHOROTHIONATE([^{35}S]TBPS) BINDING TO RAT BRAIN. A. Concas[1]*, D. Gehlert[2]*, J.K. Wamsley[2] and H.I. Yamamura[1](SPON: S.P. Duckles). Dept. of Pharmacology, Univ. of Arizona,AZ 85724[1] and Dept. of Psychiatry, Univ. of Utah, UT 84132[2].

[^{35}S]t-Butylbicyclophosphorothionate binds with high affinity to brain specific sites within the γ-aminobutyric acid (GABA) receptor-ionophore complex. TBPS binding in rat membranes is inhited potently by GABA and a number of GABAmimetics, and is also modulated by many sedatives-hypnotics and anxiolytics. Studies of benzodiazepine (BZ) interactions with[^{35}S]TBPS binding have in general yielded inconsistent results showing inhibition, no effect and stimulation of[^{35}S]TBPS binding by BZ. We now report tissue mounted slide binding and autoradiographic data that low concentrations of BZ allosterically modulate TBPS binding sites.

Slide mounted 20 μ sections of rat brain were labeled by immersion for 90 min in Na/K phosphate buffer (pH 7.4) containing 0.2 M NaCl and 2 nM[^{35}S]TBPS (initial spec. act. 109.8 Ci/mmol, NEN) in the presence or absence of 1 μM of unlabeled TBPS, followed by 2 rinses of 5 min each in the same buffer and a dip in distilled water. The sections were dried, stored overnight and apposed to a sheet of LKB Ultrofilm and the autoradiograms generated were analyzed by DADS Model 560 computer interfaced with an MPV compact microphotometry system attached to a Leitz Orthoplan microscope. For tissue mounted biochemical experiments, tissue sections were wiped off the slides using Whatman GF/B filters after the incubation and the final rinse, and placed in scintillation fluid and counted 24 h later. To prevent the inhibitory effect of GABA on [^{35}S]TBPS binding, tissue sections were preincubated for 30 min at 25°C in buffer alone. This procedure stimulated[^{35}S]TBPS binding to tissue sections, presumably by depleting the endogenous GABA from the tissue.

[^{35}S]TBPS slide mounted tissue binding was inhibited by 1 μM clonazepam when the tissue sections were not preincubated most likely GABA was not present in the tissue. In preincubated sections clonazepam did not alter [^{35}S]TBPS binding. Moreover, when 5 μM GABA was added in slide mounted tissue sections previously preincubated, the effect of clonazepam was similar to that seen in the sections without preincubation. Interestingly, the BZ inverse agonist, methyl β-carboline-3-carboxilate (MCC) enhanced [^{35}S]TBPS binding under non-preincubated conditions as would be predicted from its pharmacological profile.

These results suggest that the presence of GABA is an absolute requirement for the modulation by BZ receptor ligands of [^{35}S]TBPS binding and that the low affinity GABA receptor is involved in BZ anticonvulsant and anxiolytic actions.

Supported by NIMH Grants.

80.8 PERIPHERAL BENZODIAZEPINE BINDING SITES IN HUMAN BRAIN. J. Bénavidès*, N. Vaucher*, M. Daniel*, C. Malgouris*, A. Doble*, A. Özan, C. Guérémy* and G. Le Fur. PHARMUKA Lab., Groupe RHONE POULENC SANTE, 92231 Gennevilliers, France.

The pharmacological properties of peripheral-type benzodiazepine binding sites (PBBS) and their subcellular and anatomical distributions, have been examined in human brain using the radioligand [^{3}H]PK 11195.

In cortex, this ligand binds reversibly to a homogeneous population of high-affinity binding sites with an affinity of 3.6 ± 1.18 nM and a capacity of 400.7 ± 43.6 pmol/g protein. The affinities of a variety of compounds for this site are consistent with the pharmacological properties of the PBBS characterized elsewhere, although it should be noted that, as in the cow, cat and dog, the affinity of the benzodiazepine RO5-4864 for this site is 200-fold lower than it is in rat brain. The association and dissociation of [^{3}H]PK 11195 to and from its binding sites are monoexponential with kinetic constants of k_1 = 3.5 ± 0.5 x 10^{9} sec^{-1} M^{-1}, k_{-1} = 3.8 ± 1.3 x 10^{-4} sec^{-1} and k_{-1}/k_1 = 1.10 x 10^{-9} M. The binding was not temperature-dependent.

The subcellular distribution of this binding site in cortex parallels that of the binding of [^{3}H]flunitrazepam and of particulate lactate dehydrogenase. This indicates that these sites are likely to be localized to synaptic structures in human brain. This conclusion is supported further by autoradiographic studies of the distribution of these sites, which is very heterogeneous and localized predominantly to grey matter.

The binding of [^{3}H]PK 11195 to slide-mounted tissue sections has identical characteristics to that to tissue homogenates. In spinal cord, binding is found mainly in the outer laminae of the dorsal horn ; in medulla, there is an intense labelling in the inferior olive. Several pontine nuclei are also labelled strongly, as are the cerebellar cortex and deep cerebellar nuclei. Labelling is also found in the hippocampus, caudate nucleus and cerebral cortex.

This distribution is completely different from that of the brain-type benzodiazepine receptor, and resembles that described previously in cat, but not rat, brain.

These studies suggest a role, as yet unknown, for the PBBS in neuronal function in man, and encourage further studies to elucidate this role. Furthermore, the cat may prove a more relevant subject that the rat for such studies.

80.9 BENZODIAZEPINE RECEPTOR CHANGES IN DISCRETE AREAS OF RAT BRAIN FOLLOWING ADRENALECTOMY. N.E. Goeders, M.J. Kuhar and E.B. De Souza. Laboratory of Neuroscience, NIDA Addiction Research Center and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

A variety of clinical and experimental data suggest that glucocorticoids can enhance anxiety while benzodiazepines are effective anxiolytic agents. However, a more direct relationship may exist between glucocorticoids and benzodiazepines. For example, benzodiazepine administration inhibits stress-induced increases in plasma glucocorticoids and glucocorticoid administration can alter the anxiolytic effects of benzodiazepines in conflict procedures. This investigation was designed to determine the effects of bilateral adrenalectomy on benzodiazepine receptors in discrete regions of the rat brain.

Saturation studies were carried out with [^{3}H]-flunitrazepam to characterize changes in benzodiazepine receptors (ie., K_d and B_{max} values) resulting from the treatment conditions. The concentration (B_{max}; mean fmol/mg protein ± SEM) of benzodiazepine receptors was significantly increased in the hypothalamus (1046 ± 49 vs. 1699 ± 180, $p < 0.01$, sham-operated vs. adrenalectomized) and the striatum (559 ± 89 vs. 844 ± 36, $p < 0.01$) at two weeks after adrenalectomy. Adrenalectomy resulted in variable effects on the concentration of benzodiazepine receptors in the hippocampus although a slight increase was observed. In contrast, the adrenalectomy did not significantly affect the concentration of benzodiazepine receptors in the cerebral cortex, olfactory bulb or cerebellum. No significant differences in K_d values were seen in any brain region examined. The adrenalectomy-induced increase in benzodiazepine receptors in the hypothalamus and striatum was completely reversed by glucocorticoid replacement with dexamethasone.

In summary, the results of this investigation demonstrate that endogenous glucocorticoids are capable of selectively modulating benzodiazepine receptors in discrete regions of the rat brain that may be involved in glucocorticoid negative feedback. These data further suggest a mechanism whereby glucocorticoids can be anxiogenic. (Supported by USPHS Grants DA 00266, MH 00053, NS 15080 and MH 09111 and the McKnight Foundation).

80.10 **EVIDENCE FOR CELL SURFACE AND SEQUESTERED BENZODIAZEPINE RECEPTORS IN CHICK BRAIN CULTURES** C. Czajkowski* and D. H. Farb (SPON: J. Jakway). Department of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203.

Recent experiments from our laboratory have shown that it is possible to measure the turnover of the benzodiazepine receptor (BZD-R) in monolayer brain cell culture, using flunitrazepam (FNZM) as a specific irreversible label (Science 226:857,1984). To explore the mechanisms involved in receptor degradation the subcellular distribution of BZD-Rs in living cells was examined. Intact and saponin-treated brain cells were treated with trypsin and specific reversible and irreversible binding of (^{3}H)FNZM measured. After cells were treated with trypsin (0.5mg/ml, 37°C, 90'), soybean trypsin inhibitor (0.5 mg/ml) was added, and cultures were scraped, homogenized, centrifuged (30,000xg,20'), resuspended in PBSS and specific reversible and irreversible binding of 5nM (^{3}H)FNZM measured by filtration. Following trypsin treatment, specific reversible binding was reduced to 43% ± 2% (n=11) and irreversible binding to 41% ± 8% (n=4) of control. No further reduction in binding was observed when trypsin treatment was extended to 3 hours. When cultures were permeabilized with 0.5% saponin prior to trypsin treatment, 7% ± 1% (n=7) of the reversible binding and 11% ± 5% (n=3) of the irreversible binding remained. Saponin treatment alone did not affect binding. Binding affinity for FNZM (K_d = 5.8nM by competition binding) was unaltered by trypsin treatment. SDS-PAGE revealed a decrease in labeling of both 51K and 48K receptor subunits normally seen (approx. 20% remaining) and the appearance of a smaller receptor fragment that retains its recognition site for FNZM and is photolabeled subsequent to trypsin treatment (approx. 20% of control labeling). Thus, the reversible and irreversible binding measured by filtration following trypsinization of intact cells (approx. 40% of control) represents both intact BZD-Rs and a membrane associated trypsin-generated fragment of the receptor that retains the ligand binding site. We conclude that approximately 80% of BZD-Rs are susceptible to trypsin attack and may represent surface receptors while 20% are trypsin resistant and may represent membrane sequestered or intracellular receptors. The small fragment generated after trypsin treatment retains its recognition site for FNZM, as measured by both reversible and irreversible binding, and remains associated with the membrane. After saponin treatment of photolabeled cells, trypsin degrades the fragment, indicating that it has a cytoplasmic domain as well as an extracellular domain, as expected for a transmembrane protein. It appears that *in situ* trypsinization of the BZD-R may provide a way of probing the structural features of the receptor involved in the processes of receptor binding and receptor turnover.

80.11 PREGNENOLONE AND CORTICOSTERONE: POSSIBLE MODULATORS OF THE GABA/BENZODIAZEPINE RECEPTOR COMPLEX. M.D. Majewska*, P. Skolnick, S.M. Paul* (SPON: F.K. Goodwin). Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205.

We have previously reported that adrenal steroids potentiate [^{3}H] muscimol binding to CNS GABA receptors (Majewska et al., Brain Res., in press; Soc. Neurosci. Abstr. 1984). To further clarify the physiological significance of this phenomenon we investigated the effect of various steroids on benzodiazepine receptor binding carried out under a number of different incubation conditions. A number of naturally-occurring steroids potentiated [^{3}H]flunitrazepam binding to crude synaptosomes when the binding was carried out at 37°C. Two endogenous brain steroids, pregnenolone and its sulfate derivative were the most potent (EC_{50} = 0.4 nM) enhancers of [^{3}H]flunitrazepam binding with a maximal effect (25 - 48%) observed at concentrations from 1 to 10 nM. Other steroids such as cholesterol, testosterone, β-estradiol, dihydroepiandrosterone and dexamethasone enhanced specific binding at concentrations greater than 50-100 nM with maximal effects achieved at near micromolar concentrations.

The effect of corticosterone on [^{3}H]flunitrazepam binding was examined in control and adrenalectomized rats. In sham operated rats corticosterone only slightly potentiated (about 10%) [^{3}H] flunitrazepam binding (EC_{50} = 60-100 nM). In adrenalectomized animals (2 weeks post surgery) the dose response curve of corticosterone was shifted to the left (EC_{50} = 15 nM). The effect of corticosterone in enhancing [^{3}H]flunitrazepam binding was potentiated in the presence of depolarizing concentrations (50 mM) of KCl in a Ca^{2+} dependent manner while that of other steroids was not significantly affected by depolarizing conditions. Scatchard analysis of the effects of both pregnenolone and corticosterone on [^{3}H] flunitrazepam binding revealed that the effects of both steroids were due to an increase in the affinity of the benzodiazepine receptor for [^{3}H]flunitrazepam. Since the effects of both steroids in enhancing [^{3}H]flunitrazepam binding were abolished by picrotoxin, our data suggest that steroids interact with the GABA-benzodiazepine-chloride ionophore receptor complex, most probably in the domain of the Cl^- ionophore. Studies are in progress to determine whether the effects of these naturally-occurring steroids on [^{3}H] flunitrazepam binding occur under physiological conditions.

80.12 EFFECTS OF PENTYLENETETRAZOLE ON GABA/BENZODIAZEPINE/PICROTOXIN RECEPTOR COMPLEXES IN RAT BRAIN REGIONS. M. Ito*, T.H. Chiu and H.C. Rosenberg (Spon: H.J. Waller). Medical College of Ohio, Toledo, Ohio 43699

Evidence suggests that pentylenetetrazole (PTZ) causes convulsions by interacting with the GABA/benzodiazepine/chloride ionophore receptor complexes. The present study examined the effects of acute convulsive doses of PTZ on these complexes in various regions of rat brain.

Male rats (200-250 g) received subcutaneous PTZ (1 ml/kg, 90 or 100 mg/kg). Control rats were given the same volume of saline. Tonic-clonic convulsions were usually observed twice at 2-5 min and 13-17 min after PTZ injection. The mortality rate was 43-46%. Rats that survived were sacrificed 30 min after the injection. Brains were rapidly removed, dissected into 7 regions (cerebral cortex, hippocampus, midbrain, striatum, hypothalamus, medulla oblongata, and cerebellum), and stored at -70°C.

Brain regions were thawed and homogenized in 20 vol of ice cold 50 mM Tris-citrate buffer (pH 7.1) with a Polytron (PT 10, setting 6, 20 sec). The homogenate was centrifuged at 48,000 x g for 20 min, and the pellet was resuspended in ice-cold distilled water and recentrifuged. The pellet was washed 3 times with buffer and stored at -20°C for 16 hrs. After thawing, the pellet was washed once with Tris-citrate buffer and the final pellet was suspended in buffer for assay (protein concentration: 0.2-0.45 mg/ml). [^{3}H] Muscimol (2 and 80 nM) and [^{3}H]flunitrazepam ([^{3}H]FNP, 1 and 20 nM) binding were by filtration after incubation on ice for 30 min and 1 hr respectively. [^{3}H]GABA (4 and 100 nM) binding was by centrifugation after 1 hr incubation at 4°C. [^{35}S]t-Butyl bicyclophosphorothionate ([^{35}S] TBPS, 2 and 20 nM) binding was in buffer + 200 mM KBr for 2 hrs at room temperature followed by filtration.

PTZ caused a significant increase in striatal [^{35}S]TBPS binding; no change was found in other brain regions. Binding of the low concentration of [^{3}H]muscimol was decreased in cerebellum. No other changes in [^{3}H]muscimol, or in [^{3}H]FNP or [^{3}H]GABA binding were found.

	[^{35}S]TBPS binding		[^{3}H]Muscimol binding	
	2 nM	20 nM	2 nM	80 nM
Striatum (n=6-9)				
Control	27 ± 4	157 ± 20	254 ± 21	1486 ± 142
PTZ-treated	44 ± 5*	224 ± 24*	279 ± 23	1667 ± 174
Cerebellum (n=5-9)				
Control	90 ± 6	488 ± 31	1476 ± 52	6665 ± 340
PTZ-treated	71 ± 19	357 ± 92	1296 ± 75*	6827 ± 156

Values are mean+SEM (fmol/mg protein); *$p < 0.05$, two-tailed t-test

These results suggest that PTZ seizure selectively affects GABA/benzodiazepine/picrotoxin receptor complexes in certain brain regions. TBPS binding sites in striatum may be an important site of action of PTZ. Supported by RO1 NS16595 and RO1 DA02194.

80.13 INVERSE AGONISTS OF CENTRAL BENZODIAZEPINE RECEPTORS: "IN VIVO" VERSUS "IN VITRO" STUDIES IN THE RABBIT. L. Mele*, D. Lucantoni*, M.G. Caporali*, F.Gatta* and M. Massotti (SPON: R. del Carmine) Laboratori di Farmacologia and Chimica del Farmaco, Istituto Superiore di Sanità, 00161, Roma, Italy.

Inverse benzodiazepine agonists (IBA) induce effects opposite to those of the benzodiazepine (BDZ) agonists through an inhibition of GABA synaptic activity. Present work is aimed to assess whether a possible correlation can be found in the rabbit between the electroencephalographic (EEG) changes induced "in vivo" by IBA and their negative interaction with GABA receptor activity "in vitro".

The administration of IBA in the rabbit elicits EEG changes, characteristic enough to be chassified in three dose-related stages, similar to those observed after bicuculline,picrotoxin pentetrazol and Ro 5-3663: slow waves in the optic cortex, spike-and-wave complexes in the sensorimotor cortex, and grand-mal generalized seizures. These changes are antagonized by the administration of the BDZ antagonist Ro 15-1788 (0.4 mg/kg iv). No change of the electrical activity can be recorded in the spinal cord. The convulsant β-CCM (0.25-5 mg/kg iv) and DMCM (0.4-5 mg/kg iv) elicit all the three stages of EEG changes. The proconvulsant β-CCE (0.2-2 mg/kg iv) and FG 7142 (2-20 mg/kg iv) produce only the first two, while CGS 8216 (2-20 mg/kg iv) elicits only the first stage.

"In vitro" binding studies were aimed to show the ability of the various IBA to compete with ^{3}H-diazepam at BDZ receptors in presence and in absence of GABA. Membrane preparations from rabbit brain cortex frozen-thawed and incubated at 37°C for 30 min were used. The binding parameters of ^{3}H-diazepam were the following: K_D 32 nM; B_{max} 1.28 pmol/mg of prot. The various IBA inhibit ^{3}H-diazepam binding (1.5 nM) giving rise to the following values of IC_{50} (nM): DMCM, 21.2; β-CCM, 5.8; β-CCE, 24.2, FG 7142, 163.3; CGS 8216, 3.32. In the presence of GABA (10^{-5} M), the values of IC_{50} increased. The ratio between the values found in absence over those found in presence of GABA (GABA shift) was calculated for each IBA. The convulsant DMCM and -CCM show values of 0.20 and 0.14, respectively. The proconvulsant -CCE and FG 7142 show the values of 0.80 and 0.77, respectively, while CGS 8216 show the value of 0.94.

These data suggest that, in the rabbit, the extent of the GABA shift found with the various IBA parallels their ability to progress through the three stages of EEG changes characteristic of drugs which down regulate GABA synaptic activity by interfering with the function of GABA-BDZ-picrotoxin receptors oligomeric complex.

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80.14 INESCAPABLE TAILSHOCK STRESS DECREASES "PERIPHERAL-TYPE" BENZODIAZEPINE BINDING IN RAT KIDNEY. Robert C. Drugan, Anthony S. Basile, Jacqueline N. Crawley, Steven M. Paul, and Phil Skolnick. Clinical Neuroscience Branch, NIMH and Laboratory of Bioorganic Chemistry, NIADDK, NIH, Bethesda, MD 20205

Pharmacological and biochemical evidence suggests that central benzodiazepine receptors mediate the antianxiety actions of benzodiazepines and related compounds. Rats exposed to stressful or "anxiety"-producing situations show decreased numbers of benzodiazepine receptors (Lane et al., Eur. J. Pharmacol., 83:183-190, 1982; Braestrup et al., Psychopharm., 65:273-277, 1979; Lippa et al., Pharm. Biochem. and Behav., 9:853-856, 1978). Another pharmacologically distinct class of recognition sites for benzodiazepines can be characterized using the ligand [^{3}H]Ro5-4864 (4'-chlorodiazepam). These sites, in contrast to brain benzodiazepine receptors are found in both peripheral tissues (e.g. heart, lung, kidney) and the central nervous system. The physiological significance of these sites is unknown, although they appear to be under both neural and hormonal control.

We have investigated the effects of short and long sessions of inescapable tailshock stress on these "peripheral-type" benzodiazepine receptors and now report rapid changes in [^{3}H]Ro5-4864 binding sites following both short and long sessions of inescapable shock. Male Sprague-Dawley rats (250-350 gms) received either no shock, 20 5-second (1 mA) inescapable tailshocks or 80 5-second (incremented from 1-2 mA) inescapable tailshocks at a rate of approximately 1 per minute. Two hours after the end of the shock session, subjects were sacrificed for assay of [^{3}H]Ro5-4864 binding in whole kidney homogenates according to the methods of Weissman et al., J. Neurochem., 42:969-975, 1984). A significant decrease in the Bmax of [^{3}H]Ro5-4864 binding was observed following both short and long sessions of inescapable tailshock stress. Twenty shocks resulted in a 22% reduction in binding sites, while eighty shocks resulted in a 28% decrease in comparison to home cage controls. These results suggest that the "peripheral-type" benzodiazepine binding site may be important in the modulation of physiological responses to stressful stimuli.

80.15 [^{3}H]-FLUNITRAZEPAM BINDING IN BRAIN AND KIDNEY IS DISPLACED BY THIAZIDE AND THIAZIDE-LIKE DIURETICS. B.J. Ciliax, J.B. Penney, and A.B. Young. Depts. of Pharmacology and Neurology, University of Michigan, Ann Arbor, MI 48109.

[^{3}H]-Flunitrazepam ([^{3}H]-FLU) binds with high affinity to central/neuronal receptors (CNR) and to a peripheral binding site (PBS) located in a number of peripheral tissues (especially kidney). We tested the effects of a wide variety of diuretics on [^{3}H]-FLU binding to tissue sections from both brain and kidney.

Brains and kidneys were quickly removed from decapitated adult male Sprague-Dawley rats and frozen in dry ice. Frozen tissue sections 20 microns thick were cut and thaw mounted on microscope slides. The tissue sections were pre-washed in 50 mM Tris-HCl buffer pH 7.4 at 4°C for 3x5 minutes and then air-dried. The sections were incubated for 60 minutes in same buffer containing 5 nM [^{3}H]-FLU (74 Ci/mmol) in the absence and presence of increasing concentrations of the diuretics listed below. The drug vehicle was 1:1 dimethyl sulfoxide: ethanol present in the assay at a concentration of 0.1%. Sections were then rinsed for 5 min in ice cold buffer and quickly air-dried. LKB Ultrofilm-^{3}H™ was exposed to these sections for quantitative autoradiography. We tested prototype drugs from the following classes of diuretics: (a) thiazide/thiazide-like, (b) high-ceiling, (c) potassium-sparing, and (d) carbonic anhydrase-inhibitors. All drugs tested except those listed below displaced less than 30% of specific binding at concentrations as high as 1 mM. The inhibition constants (K_I) for the effective displacers were as follows:

Diuretics/Displacers	Inhibition Constants (K_I)	
	Brain	Kidney
1 Ethacrynic Acid	> 1mM	285uM
2 Hydrochlorothiazide	> 1mM	31uM
3 Trichlormethiazide	> 1mM	44uM
4 Metolazone	18uM	0.6uM
5 Quinethazone	295uM	> 1mM
6 Amiloride	340uM	> 1mM

The most effective drugs in displacing [^{3}H]-FLU from kidney (drugs 2-4) belong to the thiazide/thiazide-like class, which suggests that the PBS might be a site of action for this class of diuretics, whose mechanism of action remains unclear. The results suggest that the PBS may have, or be coupled to, an endogenous/pharmacological function, such as ion transport.

Interestingly these results may have clinical significance since metolazone displaces [^{3}H]-FLU from the CNR completely and this drug has been reported to aggravate convulsions (Br.Med. J. 1:1381-2, 1976).

80.16 LIGAND-INDUCED CHANGES IN GABA RECEPTOR BINDING KINETICS AND MODULATION BY BARBITURATES. Jane Yang and Richard W. Olsen. Department of Pharmacology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Both dissociation and association rates of [^{3}H]muscimol binding to GABA receptors were altered by agonist concentration in a manner suggesting negative cooperativity. This could be eliminated by pentobarbital, an effect consistent with enhancement of GABA-mediated inhibition as a mechanism of barbiturate action.

[^{3}H]Muscimol binding in freshly prepared mouse brain membrane homogenates has demonstrated the heterogeneity of γ-aminobutyric acid (GABA) receptor binding sites. The heterogeneity could be due to distinct receptor sub-types or to multiple conformational states of a single receptor. The explanation for binding heterogeneity and its possible functional relevance was investigated using both binding kinetics and tracer radioactive chloride flux assays with cortical slices. The kinetics of [^{3}H]muscimol binding showed a heterogeneity of rate constants. The overall dissociation rate was greatly increased as the concentration of [^{3}H]muscimol increased. Two measurable rate constants on the seconds to minutes scale adequately described the data at 5 nM ligand but three were required at ≥ 30 nM, with one portion too fast to measure (< 1 sec). The association rate (22°C) was decreased by increasing [^{3}H]muscimol concentration, with some very slowly associating sites evident. The changes in on-rate were greater than expected from differential occupancy of multiple populations by varying concentration and, together with the effects on off-rate, suggest ligand-induced interconversion.

Pentobarbital (0.3 μM), which synergistically enhanced muscimol (1 μM)-stimulated $^{36}Cl^-$ flux in brain slices, greatly enhanced the initial apparent association, and decreased dissociation rate. This eliminated the slowly associating binding seen at high muscimol concentrations.

We conclude that the GABA receptor exists in a resting, activatable state (fast on-rate, slow off-rate, Kd = 0.2-1μM). The binding of muscimol to the receptor induces a conformational change, probably involved in agonist activation of chloride channels, accompanied by negative cooperativity that results in the acceleration of muscimol dissociation. Pentobarbital enhances muscimol physiological activity by preventing the occurrence of negative cooperativity, thus prolonging the lifetime of the active state. These observations apparently do not relate to the phenomenon of desensitization which presumably occurs at higher ligand concentrations with even slower kinetics, or can be seen at 0°C corresponding to very high affinity sites (Kd = 1-50 nM).

Supported by NIH grant NS20704 and NSF grant BNS 83-18001.

80.17 PURIFICATION OF ENDOGENOUS BENZODIAZEPINE-LIKE MOLECULES FROM THE MAMMALIAN BRAIN. L. Sangameswaran* and A.L. De Blas. Dept. of Neurobiology & Behavior, State University of New York at Stony Brook, New York, 11794.

We have previously reported[1] the presence of endogenous benzodiazepine-like (BZD-like) antigenic determinants in the rat brain by using the anti-benzodiazepine monoclonal antibody (mAb) 21-7F9. This mAb is now used for the purification of an endogenous BZD-like substance from the soluble fractions of rat and bovine brains by immunoaffinity chromatography.

The immunoaffinity purified substance blocked the binding of the agonists [^{3}H] flunitrazepam and [^{3}H] diazepam, the inverse agonist [^{3}H] β carboline carboxylate ethyl ester, and the antagonist [^{3}H] Ro15-1788 to the neuronal-type benzodiazepine receptor (BZDR). However, it did not inhibit the binding of [^{3}H] Ro5-4864 to the peripheral-type BZDR. The inhibition of [^{3}H] Ro15-1788 binding to the BZDR by the endogenous substance was potentiated by 10^{-4}M GABA suggesting that the BZD-like substance is a receptor agonist. These binding properties were different from those of nicotinamide, adenosine, inosine, guanosine and hypoxanthine which have been proposed to be putative endogenous ligands for the BZDR. The endogenous molecules did not block the binding of the ligands for other transmitter receptors (adrenergic, GABA and adenosine receptors).

The endogenous BZD-like substance from bovine brain has been purified to homogeneity by the following sequential steps: i) immunoaffinity chromatography with mAb 21-7F9 coupled to Affigel G-10, ii) gel filtration on Sephadex G-25 and iii) two different HPLC steps. The purified substance appears between cyanocobalamin and bromophenol blue after gel filtration on Sephadex G-25 suggesting a M_w between 1355 and 670. The activity of the purified BZD-like substance is heat-stable and resistant to proteases.

[1]Sangameswaran, L., Cherwinski, H.M. and De Blas, A.L. (1984) Society for Neuroscience 10, 644.

80.18 EVIDENCE FOR A LINK BETWEEN CALCIUM CHANNELS AND PERIPHERAL BENZODIAZEPINE RECEPTORS IN A CULTURED PITUITARY CELL LINE. A. Doble*, M.L. Perrier*, M.C. Burgevin*, I. Pisseau*, J. Bénavidès*, A. Uzan, C. Guérémy* and G. Le Fur. PHARMUKA Lab., Groupe RHONE POULENC SANTE, 92231 Gennevilliers, France.

Radioligand binding to peripheral-type benzodiazepine binding sites (PBBS) and the effects of ligands specific for this site on various second messenger systems have been studied using the rat pituitary transformed cell line, GH3.

[^{3}H]PK 11195, a specific non-benzodiazepine ligand for the PBBS, binds with high affinity to a single saturable population of binding sites on intact GH3 cells. Their affinity and capacity are 3.3 nM and 243 fmol/10^6 cells respectively. The pharmacological characteristics of these sites are identical to those characterized previously in rat heart, brain or kidney. The binding is unaffected by chloroquine or cell lysis, consistent with binding to the outer surface of the cell membrane.

GH3 cells accumulate $^{45}Ca^{++}$ in response to depolarizing concentrations of potassium. This uptake can be blocked by dihydropyridines implicating voltage-sensitive calcium channels (VSCC) in this phenomenon. In fact, the VSCCs can be observed directly by the binding of [^{3}H]verapamil. The $^{45}Ca^{++}$ flux can be blocked by RO5-4864, a benzodiazepine selective for the PBBS. This effect is reversed by PK 11195, which will also reverse the stimulation of $^{45}Ca^{++}$ flux by the dihydropyridine BAY K-8644. These interactions of ligands acting at the PBBS with the VSCC are similar to previous findings in cardiac muscle. No interaction, however, could be demonstrated between the binding sites for [^{3}H]PK 11195 and [^{3}H]verapamil.

In contrast, the turnover of phosphoinositides (PI), which is thought to be related in some way to mobilization of intracellular calcium, was modified neither by RO5-4864 nor PK 11195. This was true both for basal PI turnover and for PI turnover stimulated by TRH. Neither have preliminary experiments demonstrated any effect of PBBS ligands on the pattern of protein phosphorylation in GH3 cells.

These results, obtained from a secretory cell, provide further support for the hypothesis, proposed on the basis of data obtained from cardiac cells, that the PBBS can regulate selectively the VSCC.

81.1 GAMMA-AMINOBUTYRIC ACID (GABA) ACTIVATES A PICROTOXIN-RESISTANT CHLORIDE CONDUCTANCE ON SPINY LOBSTER FOREGUT AND OPENER MUSCLE. J. Albert*, C.J. Lingle, E. Marder and M. O'Neil*. (Spon: J. Elam). Dept. Biol. Sci., Florida State University, Tallahassee, FL 32306 and Dept. Biology, Brandeis U. Waltham, MA 02254 .

GABA-activated chloride conductance increases in both invertebrate and vertebrate systems are known to be exquisitely sensitive to the convulsant alkaloid, picrotoxin (PTX). At the opener muscle of the walking leg of Homarus vulgaris, 50% block of a 40 micromolar dose of GABA occurs at less than 200 nM PTX (Constanti, 1978, Neuropharm. 17:159). In marked contrast, we have encountered a GABA-activated conductance change on the opener and GM6 muscles of the spiny lobsters, Panulirus interruptus and Panulirus argus, which is about 3 orders of magnitude less sensitive to PTX than are muscles of previously studied crustacean species. The experiments described here examine the properties of this PTX-resistant GABA response.

Most experiments were done on the GM6 muscle using standard two electrode current-clamp procedures. The threshold for the GABA-activated conductance increase is about 10-20 uM and the reversal potential of the response was close to the cell resting potential. The chloride-dependent nature of the response was verified by several experiments. Saline with 50% sodium or 0 calcium produced no change of the GABA-activated conductance change or reversal potential of the response. In contrast, shortly following perfusion of the muscle with a 50% chloride saline (proprionate substitution), a 12 mV depolarizing shift in the reversal potential for the response was observed. The threshold for PTX action on this GABA conductance was about 10 uM. 50% blockade of responses to 200 uM GABA required at least 500 uM PTX. The GM6 GABA response is mimicked by muscimol, but not baclofen. Bicuculline is a weak blocker, while beta-guanidino-proprionic acid produces weak agonist and blocking actions. Except for the relative insensitivity to PTX, in all respects the Panulirus GM6 and opener muscle GABA response appears similar to other crustacean GABA responses. In addition, we find that the GABA response of the GM6 muscle of Homarus is almost totally blocked by 1 uM picrotoxin.

Our results show that in Panulirus the classical neuromuscular GABA response has a reduced sensitivity to picrotoxin perhaps analagous to TTX-resistant sodium channels. Minimally, the results indicate that the binding site for picrotoxin can be altered without changing other functional properties of the GABA-activated conductance. (Supported by NIH NS-19139 to CL).

81.2 INTRACELLULAR STUDIES IN VITRO OF INHIBITORY SYNAPTIC EVENTS AND THE ACTIONS OF GABA AGONISTS IN THE DORSOLATERAL SEPTAL NUCLEUS (DLSN) OF THE RAT. D. R. Stevens, J. P. Gallagher, and P. Shinnick-Gallagher. Dept. Pharmacology, Univ. of Texas Medical Branch, Galveston, Texas, 77550.

The septal area has been proposed to function in the expression of anxiety and in learning of avoidance responses. γ-aminobutyric acid (GABA) has been suggested as an inhibitory neurotransmitter in this area. We have previously described the actions of GABA and the GABA-b agonist, (-) baclofen on principal neurons of the DLSN in a rat brain slice preparation (Gallagher, et al. Neuropharm. 23;825,1984) Here we report the results of further studies of the action of GABA and studies of inhibitory synaptic events in the DLSN.

The action potential of DLSN neurons is followed by an afterhyperpolarization (AHP) which is blocked by manganese (1 mM). The early phase of the AHP is depressed by low calcium-high magnesium treatment and by bicuculline methiodide (10 μM). This result indicates the presence of a synaptic contribution to the AHP which is mediated by GABA.

Local stimulation occasionally elicits an inhibitory postsynaptic potential (IPSP) with a latency of 2-3 milliseconds. The IPSP is depressed by picrotoxin (10 μM) and by low calcium treatment. The reversal potential of the IPSP is near -70 mV, a value similar to that of hyperpolarizing GABA potentials in these neurons.

Increasing the stimulus intensity results in the appearance of a late hyperpolarizing potential (LHP). This potential is of long duration (500 msec) and is associated with an increase in conductance. The LHP is insensitive to picrotoxin (10 μM) and has a reversal potential approximately 25 mV more negative than that of the IPSP. This value is comparable to the reversal potential of the baclofen hyperpolarization seen in these neurons.

Since baclofen is thought to act at a subtype of GABA receptor, we examined further the action of GABA on DLSN neurons. GABA produces a biphasic potential when applied by iontophoresis to these neurons. A hyperpolarizing response to gaba persists in the presence of picrotoxin (10 μM) or bicuculline methiodide (50 μM) when relatively large ejection currents are used. This hyperpolarization is the result of a conductance increase. The reversal potential of the picrotoxin-resistant GABA response is more negative than that of the ipsp and is comparable to the reversal potential of the baclofen response.

These results support the proposal that GABA is an inhibitory neurotransmitter in the DLSN. In addition, GABA activates a conductance which is comparable to that activated by baclofen. Finally, the ionic mechanisms of the hyperpolarizing baclofen response and that of the picrotoxin-resistant GABA response are similar to that of the LHP. It is possible that the the LHP is mediated by synaptically released GABA acting at a GABA-b receptor.

81.3 STEROID MODULATION AND MIMICKRY OF GABA-ACTIVATED CHLORIDE CONDUCTANCE IN CULTURED MAMMALIAN CENTRAL NEURONS. N.L. Harrison*, S. Vicini*, D.G. Owen* and J.L. Barker. Laboratory of Neurophysiology NINCDS, NIH, Bethesda, MD. 20205

The steriodal anesthetic alphaxalone (3 α-hydroxy 5α-pregnane 11,20-dione) enhances GABA-evoked Cl^--dependent responses in rat cuneate slices and stimulates [^{3}H]-muscimol binding to rat brain membranes (N.L. Harrison and M.A. Simmonds, Brain Res., 323:287-292). We have begun to study this interaction at the single cell level using conventional intracellular recording and whole-cell patch clamp techniques. Cl^--loaded hippocampal and spinal neurons depolarized from rest by pressure applications of GABA (20μM) were invariably potentiated by application of low (≤ 1μM) concentrations of alphaxalone. Higher steroid concentrations produced direct depolarizations that were accompanied by a fall in R_m and, like GABA-evoked responses, reversed at membrane potentials between -20 and -30mV. In neurons recorded with 3MKAc-filled electrodes GABA-evoked Cl^- currents were markedly potentiated by ≤ 1μM alphaxalone. Higher steroid concentrations (≥ 1μM) produced Cl^- currents in the absence of applied GABA. As is the case for pentobarbital, the peak current evoked by GABA could be increased 2-3-fold and the decay time for the current substantially prolonged (Fig. 1). The potentiating effect of the steroid following brief (2-5s) application was far more rapidly reversed than was the case for similar applications of diazepam. All steroid actions were insensitive to the benzodiazepine antagonist Ro15-1788. In view of the structural similarity between the steroid anesthetic and certain steroid metabolites, it is possible that endogenous steroid hormones modulate GABA-activated Cl^- conductance in the mammalian CNS.

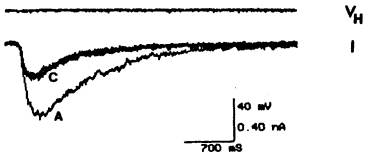

Fig. 1. Whole-cell patch clamp recording from a cultured rat hippocampal neuron voltage-clamped at -50mV (V_H) with a patch pipette containing 145mM KCl. 20μM GABA was applied by pressure (<2psi) to the cell body for 150 msec at a rate of 0.08Hz. Five control (C) current (I) responses are shown superimposed together with the first current response obtained after a brief (2s.) application of 1μM alphaxalone (A).

81.4 GABA-ACTIVATED CHLORIDE CHANNELS ANTAGONIZED BY PICROTOXIN SHARE KINETIC PROPERTIES WITH ONE FORM OF THE NATURALLY-GATED CHANNEL. M.G. Weddle and R.L. Macdonald. University of Michigan, Ann Arbor, Michigan, 48104-1687.

We have studied the actions of the γ-aminobutyric acid (GABA)-antagonist picrotoxin (PICRO) on GABA-activated single channel chloride currents, and have found similarities between channels in the presence of antagonist and one kinetic variation of the channel gated in its absence. Fetal mouse spinal cord neurons were maintained in dissociated cell culture for 2-6 weeks. For experiments, cells were transferred to a Hepes-buffered saline solution containing the glycine antagonist strychnine (100-500 nM.). Using the patch clamp technique, outside-out patches were obtained (intra-pipette solution contained the impermeable cation TRIS, a [Cl^-] similar to that of the bath and an EGTA-Ca^{++} buffer). Patches were clamped at positive-inside potentials (+50 to +80 mV.), and broken-tipped micropipettes were used to approach the patches and apply GABA and PICRO-containing solutions by diffusion. Some spontaneous currents were present in all of the patches used in this study, and GABA was frequently applied to increase the occurrence of this activity. The currents reversed at a membrane potential of 0 mV., and this reversal was shifted with a partial substitution of isethionate anions for bath chloride, indicating that Cl^- was the principal charge carrier producing these currents. Recorded activity was almost exclusively in the form of channel 'bursting': the channels tended to frequently, though briefly, close while in the open state. Less frequently, groups of discrete (non-bursting) openings were observed. First, the time distributions of channel openings and closures within channel bursts were analyzed. In the absence of PICRO, both distributions contained at least two populations of exponentially-distributed events. With increasing concentrations, the initial action of PICRO was the elimination of long open-time population(s), reducing the intraburst open-time distribution to a single exponential. The loss of one or more populations of long open-states in the presence of PICRO was not accompanied by a similar loss of closed-states. Higher concentrations of PICRO disrupted patterns of bursting, and further reduced the remaining open-times. Next, the time distributions of channel open- and closed-states during groups of nonbursting openings were analyzed. The longer intervals between these discrete events were represented in a shift of the closed-time distributions, and open-time distributions were of the form of single exponentials. These were quite similar to the intraburst open-state durations in the presence of low concentration PICRO. The data suggest that one action of PICRO may be to reduce the complexity of open-state kinetics in a way which can occur within the naturally-gating channel. Supported by Grant #NS 19613-01.

81.5 ETOMIDATE PROLONGS THE RECURRENT GABA-MEDIATED IPSP IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS IN VITRO. W.R. Proctor, M. Mynlieff and T.V. Dunwiddie. Dept. Pharmacol., Univ. Colorado Health Sciences Ctr. & VA Medical Research Serv., Denver, CO 80252

Etomidate is a sedative/hypnotic drug that has barbiturate-like anesthetic properties. Biochemical studies have shown that the (+) isomer of etomidate interacts with the GABA receptor-chloride ionophore complex (GABA/barbiturate/benzodiazepine receptors coupled to a chloride channel) in a barbiturate-like fashion, i.e., it enhances both benzodiazepine and GABA binding. The (-) isomer of etomidate is much weaker in this regard, but unlike many other stereoisomeric barbiturates, it does not appear to have excitatory properties. The present electrophysiological investigations were designed to examine the effects of the isomers of etomidate, and to compare their actions to those of barbiturates such as pentobarbital.

The effects of etomidate and pentobarbital were examined in the well-characterized recurrent GABAergic inhibitory pathway in the CA1 region of the in vitro hippocampal slice. Electrical stimulation of the alveus produces a hyperpolarizing Cl^--mediated IPSP in CA1 pyramidal cells, which is usually followed by a second inhibitory potential (late hyperpolarizing potential or LHP) of much longer duration that does not involve a chloride conductance. To examine the effects of etomidate and pentobarbital on each of these IPSP's independently, we recorded intracellular responses using bath superfusion with bicuculline methiodide (10 uM) to block the initial GABAergic IPSP, or with Cs^+-acetate filled electrodes to block the slower, presumably K^+-mediated IPSP.

Both pentobarbital (100 uM) and (+) etomidate (10 uM) markedly potentiated the duration of the initial GABA-mediated IPSP, increasing the duration from the usual 75-125 msec to as much as 400 msec in the presence of drug. In addition, there was occasionally a modest (10-20%) increase in the amplitude of this potential. However, no significant effects of either of these drugs were observed on the LHP. These responses showed a high degree of stereospecificity, in that (+)-etomidate was at least 10 times more potent than the (-) isomer, and approximately 10 times more potent than pentobarbital. These results correspond well with the relative as well as absolute potencies of these drugs in modulating the binding of GABA and benzodiazepines in in vitro binding assays. Although etomidate does not have the characteristic barbiturate structure as does pentobarbital, the similarity between the biochemical and functional effects of these drugs suggest that they act at a common stereoselective receptor site that is intimately associated with the GABA receptor and Cl^- ion channel.

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81.6 BLOCKERS OF GABA UPTAKE DIMINISH SPONTANEOUS IPSP SIZE IN MAMMALIAN DISSOCIATED CELL CULTURE. J.R. Buchhalter* and M.A. Dichter. Children's Hospital Medical Center, Boston, MA 02115.

Uptake of GABA into neurons and/or glia has been postulated to be a major mechanism of GABA inactivation. Thus, blockers of uptake would be expected to potentiate inhibitory postsynaptic potentials (IPSPs) as well as enhance the effects of exogenously applied GABA. We have examined these issues in rat cortical neurons which exhibited spontaneous GABA mediated IPSPs.

Rat cortical dissociated cell cultures were prepared as described previously (Dichter, MA. Br.Res. 149:279, 1978). Intracellular recordings were performed with potassium acetate filled electrodes utilizing standard methods. When an impaled neuron exhibited spontaneous IPSPs, it was pneumatically perfused with either a neuronal, glial or mixed blocker of GABA uptake in the indicated concentrations: diaminobutyric acid (DABA, 1mM; neuronal), beta-alanine (ALA, 1mM & 100uM; glial) or racemic nipecotic acid (NIP, 1mM; both glial and neuronal). DABA, ALA and NIP produced a decrease in the amplitude of spontaneous IPSPs in 17 of 20 neurons.

Experiments were performed to test the effect of the blockers on the postsynaptic hyperpolarization produced by exogenously applied GABA (5uM). Co-perfusion of DABA with GABA was compared with GABA and each blocker applied alone. NIP and DABA enhanced the effect of GABA in 9 of 21 and 2 of 7 cells, respectively. The effect of ALA was not interpretable as it produced significant hyperpolarization when perfused alone.

Our results are not consistent with the hypothesis that either neuronal or glial GABA uptake plays a role in limiting the size of IPSPs, at least for inhibitory synapses in culture. The mechanism by which the uptake inhibitors diminish IPSPs is likely to be presynaptic. They might block inhibitory interneuron action potentials, act on presynaptic receptors to decrease GABA release, or get taken up into axon terminals and released as "false transmitters." Some of these hypotheses should be testable by recording from pairs of neurons where both the pre and postsynaptic elements of the inhibitory synapse can be monitored.

81.7 HYPERPOLARIZING RESPONSES TO GABA AND ANALOGS ON PRIMARY AFFERENT FIBRES. G.M. Mitsoglou* and A.L. Padjen, Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Our previous studies indicated that GABA and analogs (baclofen; kojic amine; taurine) have dual effects on primary afferent fibres: a bicuculline/picrotoxin sensitive depolarization, and a hyperpolarizing response insensitive to the blockers (Bourne & Padjen, Abs. Neurosci. 1980; Padjen & Hashiguchi, Abs. Neurosci., 1982). In this study we have examined some properties of the hyperpolarizing response. Experiments were done at 11-12°C on isolated hemisected spinal cords of frog (R. pipiens) superfused with a Ringer's solution containing tetrodotoxin (.2 μM) to block indirect drug responses. Drug-evoked responses were recorded from the intramedullary part of dorsal roots (9th and 10th segment) using the sucrose gap technique. L-baclofen (0.005-0.5 mM) caused a long-lasting conc-dependent hyperpolarization of primary afferent fibres (2 mV at 0.5 mM, for up to 90 min in duration). D-baclofen was at least three times less potent in causing hyperpolarization. Kojic amine, GABA and taurine in low conc (<0.1 mM) were also able to hyperpolarize primary afferent fibres but this type of response was best seen in the presence of blockers (bicuculline, picrotoxin for GABA, kojic amine, taurine and 6-aminomethyl-3-methyl-4H-1,2,4-benzothiadizine in the case of kojic amine and taurine). Hyperpolarizing responses were $[K]_o$-dependent: in 0.1 mM K^+, the amplitude of L-baclofen (0.1 mM) response was increased by 150-200%, and in 4 mM K^+ decreased by 80%. At the same time, depolarizing responses to GABA (0.5 mM) and taurine (1 mM) were affected in the opposite sense: in 0.1 mM K^+, depolarization was decreased and often a hyperpolarizing component of the responses appeared. Addition of Ba^{2+} (0.5 to 5 mM) blocked hyperpolarizing responses to L-baclofen by up to 100%, while responses to other neutral as well as acidic amino acids were only depressed. On the other hand Mn^{2+}, Co^{2+} (both 2 mM) and Cd^{2+} (0.5 mM) were without effect on hyperpolarizing responses. Alleged antagonists of $GABA_B$ receptors (1 mM of delta-aminovaleric acid or delta-aminolaevulinic acid) had little effect on hyperpolarizing responses.

These results suggest that the hyperpolarizing responses to GABA and analogs, which appear to result from activation of $GABA_B$ receptors, are mediated by a "K^+ mechanism" and do not seem to be dependent on Ca^{2+} entry.

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81.8 EFFECTS OF BENZODIAZEPINES ON SINGLE UNIT ACTIVITY OF DOPAMINERGIC AND NON-DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA. D.P.O'Brien and F.J.White. Depts. of Veterinary Biosciences and Psychology, Univ. Illinois, Urbana, IL 61801

GABAergic pathways descend from the corpus striatum to the substantia nigra (SN) and from the nucleus accumbens to the ventral tegmental area (VTA). It has been suggested that these pathways form inhibitory feedback circuits,and that drugs which enhance this inhibition may be useful in disorders where excessive dopaminergic (DA) function is suspected, such as schizophrenia. Intrinsic GABAergic neurons are, however, present in both the VTA and SN. In SN, evidence suggests that the descending, GABAergic pathway inhibits other GABAergic neurons in the SN pars reticulata (SNR) which in turn inhibit DA cells in the SN pars compacta (SNC). Further, drugs which enhance GABA activity, such as the benzodiazepines, are more potent at the SNR than the SNC and thus consistently inhibit the non-DA SNR neurons while having variable effects, including excitation, on DA neurons in the SNC (Ross et al Life Sci 31:1025-1035, 1982; Grace and Bunney Eur J Pharm 59:211-218, 1979). The interactions of DA and non-DA neurons in the VTA are not as well understood. Since the VTA DA neurons are thought to be involved in the affective components of behavior as well as the pathogenisis of schizophrenia, the relationship between GABAergic and DA neurons in this area is of considerable practical importance. Therefore, we initiated experiments to determine the effects of benzodiazepines on DA and non-DA neurons in the VTA.

Extracellular single unit activity was recorded in chloral hydrate anesthetized rats. DA and non-DA neurons were distinguished by their waveforms, firing rates, and firing patterns.

Diazepam (DZ) administered iv exerted a potent inhibitory effect (usually >75%) on non-DA, SNR-like cells in the VTA at doses as low as 50μ_g/kg. The effects of DZ on DA cells was variable. Although most cells showed potent excitation (75% increase over basal rates) some showed moderate inhibition while others no effect. All the changes seen could be reversed by the benzodiazepine antagonist RO15-1788. Often the firing rate would rebound past the basal rate when the DZ effect was reversed by RO15-1788, although the latter had no effect when administered alone.

Preliminary microiontophoretic studies have shown that direct application of chlordiazepoxide also produces potent inhibition of non-DA cells in the VTA and moderate inhibition of some DA cells (but no excitation). Thus the inhibitory effects of benzodiazepines on these cells are probably mediated directly by benzodiazepine receptors on the cells. The extent to which the increase in firing in DA cells following iv DZ administration is dependent upon indirect mediation via SNR-like,non-DA neurons is presently under investigation.

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82.1 THE RELATIONSHIP BETWEEN THE AVAILABILITY OF GLUTAMINE AND THE RELEASE OF GLUTAMATE AND GABA FROM HIPPOCAMPAL SLICES. J.C.Szerb and P.A.O'Regan*. Dept. of Physiology and Biophysics, Dalhousie Univ. Halifax, N.S. B3H 4H7, Canada.

Glutamine (GLN) is present in the CSF in a high concentration of 0.5-0.6mM. It has been shown previously (Szerb and O'Regan, J.Neurochem. in press) that GLN added to the superfusion fluid in this concentration enhances the evoked release of glutamate much more than that of GABA. The smaller effect of GLN on GABA release could be the result of a greater contribution to release of GABA taken up by the terminals than of GABA freshly synthesized. Therefore, the effect of 0.5 mM GLN on GABA release was measured when GABA uptake was inhibited by 1 mM nipecotic acid. Nipecotic acid caused an increase both in the spontaneous and high K^+evoked release of GABA but GLN still had very little effect on GABA release while greatly enhancing both the spontaneous and evoked release of glutamate. Alternatively, GLN fails to increase GABA release because its synthesis is saturated at a lower concetration of GLN than that of glutamate. Therefore, rats were pretreated with methionine sulfoximine (MSO), a GLN synthetase inhibitor. This resulted in a 50% reduction in GLN efflux and a more rapid decline in glutamate release with prolonged depolarization. GABA release also declined in slices from MSO treated rats but much less than that of glutamate. Added 0.5 mM GLN fully reversed the effect of MSO pretreatment by increasing glutamate release twentyfold and doubling that of GABA. Since it is well established that labelled GLN is as readily incorporated into releasable GABA as is labelled glucose, lack of an effect of GLN on total GABA release suggests that releasable GABA synthesis is controlled by the enzyme GAD which can utilize glutamate derived from GLN or glucose interchangeably. In contrast, the rate of glutamate synthesis, which does not involve such a regulatory enzyme, depends more on substrate concentration.

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82.2 THE CALCIUM DEPENDENCY OF [3H]-GABA RELEASE EVOKED BY ELECTRICAL OR POTASSIUM STIMULATION. J. Peris*, R. A. Harris and N. R. Zahniser. Dept. Pharmacology, Univ. Colorado Health Sci. Ctr., Denver, Co 80262.

The stimulated release of cholinergic and adrenergic neurotransmitters from in vitro neuronal preparations is absolutely dependent on the presence of calcium ions (Ca); this is compatible with the Ca-dependent vesicular release hypothesis. However, the Ca dependency of the release of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in cortex, remains equivocal. Usually, either potassium (K) at high concentrations or electrical field stimulation (E) of high frequencies has been used to evoke GABA release. Although in some studies, K-stimulated GABA release was Ca independent, most reports conclude that a substantial proportion of this release is Ca dependent; in contrast, E-stimulated GABA release is mostly Ca-independent. In addition, Ca-dependent GABA release is occasionally only demonstrable when Mg levels are increased simultaneously with Ca removal. In order to determine the Ca-dependency of GABA release from rat cerebral cortex, we examined both E- and K-stimulated [3H]-GABA release from superfused cortical slices in the presence and absence of Ca (1.3mM) and elevated Mg (2.5 mM). We also measured the effects of a Ca-channel blocker (ruthenium red) on GABA release when Ca and Mg concentrations remained unchanged from normal Krebs' buffer. We found that K-stimulation and higher Mg concentrations led to a greater proportion of Ca-dependent release (50±0.6% of total stimulated release; X±SEM) compared to that found when slices were E-stimulated in the absence of excess Mg (20±19%). Intermediate proportions of Ca-dependent release were found when only one of these conditions (i.e., K-stimulation or excess Mg) was present. For example, Ca-dependent K-stimulated release was 40±8% of total stimulated release in the absence of excess Mg while Ca-dependent E-stimulated release was 30±5% when Mg was increased. Ruthenium red caused a dose-dependent decrease in E-stimulated GABA release with a maximum inhibition of 40% at 100 µM. Under all of these conditions, at least 50% of GABA release was not mediated by Ca-dependent mechanisms even under conditions favorable for Ca-dependent GABA release. It is possible that E- and K-stimulated GABA release are evoked from different neuronal pools, one of which is Ca dependent and the other Ca independent. The Ca-dependent component has traditionally been thought to be vesicular release while the Ca-independent component has been postulated to be a reversal of the Na-coupled transport system for GABA uptake. It is of interest that dopamine has been recently found to inhibit only Ca-independent GABA release from the retina (Kato, et al., J. Neurochem., 44, 893-899, 1985). We will determine whether drugs selectively alter the Ca-dependent and -independent release of GABA. This work was supported by grants NS 09199 (NRZ) and DA 07043 (JP).

82.3 GABAERGIC PRESYNAPTIC RECEPTORS IN TERMINALS IN SUBSTANCIA NIGRA COMPACTA BUT NOT IN NIGRA RETICULATA. B. Floran* and J. Aceves* (SPON: E. Stefani). Dept. of Physiology and Neuroscience. Centro de Investigación del IPN. 07000 México, D.F.

Arbilla et al.[1] suggested the presence of GABAergic presynaptic receptors in striatonigral terminals. However the GABAergic terminals going to the pars compacta seem to be different from those going to the reticulata: the first receives terminals mainly from globus pallidus (and from the reticulata itself through recurrent collaterals from neurons in the reticulata that project to thalamus, superior colliculus and reticular formation), while the latter receives most of the terminals coming from the neostriatum (globus pallidus also receives a large proportion of GABAergic terminals from neostriatum). We are studying the distribution of the GABAergic autoreceptors in the terminals of these GABAergic pathways, and here we report the results so far obtained.

Eventhough the natural ligand to activate the receptors is GABA itself, the exchange diffusion for GABA precludes the use of GABA to see the consequence of activation of the receptors on evoked release of tritiated GABA. Therefore muscimol was used instead of GABA. The experiments were done in slices of rat substantia nigra (either compacta or reticulata), globus pallidus and superior colliculus. The tissue samples were loaded with tritiated GABA in the presence of ß-alanine (1 µM) and the perfusion solution contained nipecotic acid (10 µM). Release was evoked by high potassium (15 mM), which was maintained elevated throughout the experiment. The release was Ca-dependent and TTX-sensitive. Muscimol (1 µM) totally inhibited evoked release from nigra compacta, but not from reticulata. Bicuculine (10 µM), which had not effect by itself, blocked the muscimol inhibition. Muscimol also inhibited evoked release from superior colliculus but not from globus pallidus slices. These results suggest that not all GABAergic terminals have presynaptic autoreceptors, but that there is a preferential location of the receptors on the terminals of certain GABAergic pathways. The physiological significance of this preferential location of the presynaptic GABAergic autoreceptors is difficult to assess. (Supported by PBBCCNA-020884 grant from CONACyT of México).

1. Europ. J. Pharmacol. 57: 211, 1979.

82.4 THE EFFECT OF ETHOSUXIMIDE ON BRAIN GABA CONTENT AND THE IN VIVO INTERACTION BETWEEN ETHOSUXIMIDE AND $GABA_a$ RECEPTOR AGONIST (THIP) IN MICE. E. Lin-Michell*, A.Y. Chweh, and E.A. Swinyard*. Dept. of Biochemical Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT 84112.

The effect of ethosuximide (an antiepileptic drug) on the GABA content in mouse brain was investigated. In addition, the effect of a $GABA_a$ receptor ligand, THIP, on the anticonvulsant activity and minimal neurotoxicity of ethosuximide was determined. Brain GABA content was estimated by a radioreceptor assay. Anticonvulsant activity and minimal neurotoxicity were measured by the pentylenetetrazol (PTZ, 85 mg/kg, s.c.) and rotorod tests, respectively. Administration of the anticonvulsant ED50 of ethosuximide (150 mg/kg, i.p.) did not increase brain GABA content (1.49 ± 0.07 vs 1.50 ± 0.06 µmol/g wet tissue). However, administration of the median minimal neurotoxic dose of ethosuximide (400 mg/kg) significantly increased brain GABA content (1.23 ± 0.02 vs 1.92 ± 0.14 µmol/g wet tissue, $p < 0.01$). Mice treated ten days with 500 mg/kg of ethosuximide exhibited no neurotoxicity; moreover, such treatment had no significant effect on brain GABA content (1.40 ± 0.05 vs 1.33 ± 0.03 µmol/g wet tissue). Administration of THIP with ethosuximide had no effect on either the anticonvulsant activity or the minimal neurotoxicity of ethosuximide. These observations suggest that GABA receptors are not directly involved in either the anticonvulsant or minimal neurotoxicity of ethosuximide; however, the increase in brain GABA content induced by toxic doses of ethosuximide contributes to its neurotoxicity. (Supported by NIH Contract No. NO1-NS-4-2361)

82.5 REGIONAL VARIATIONS IN THE IN VIVO EFFECTS OF GAMMA ACETYLENIC GABA ON GABA TRANSAMINASE AND GLUTAMATE DECARBOXYLASE ACTIVITIES IN RAT CNS: RETINA vs BRAIN. J.F. Cubells, J.S. Blanchard*, D.M. Smith*, and M.H. Makman*. Depts. of Neuroscience, Biochemistry, and Molecular Pharmacology. Albert Einstein College of Medicine, Bronx, NY 10461

4-amino, 5-hexynoic acid (gamma-acetylenic GABA; γ-AG) is a mechanism-based, irreversible inhibitor of several pyridoxal phosphate-linked enzymes, including GABA-transaminase (GABA-T), ornithine decarboxylase, and glutamic acid decarboxylase (GAD). We have shown previously that retinal GAD activity is more completely inactivated than striatal or nigral GAD activities in rats which have received 50 or 100 mg/kg γ-AG s.c. 4 hours prior to decapitation. We now report that decreases in both GAD and GABA-T activities which result from s.c. γ-AG administration vary according to the region of CNS in which they occur.

Male Long-Evans rats were given 2 ml/kg water or 1,5,10,25, or 50 mg/kg γ-AG s.c., and decapitated 4 hours later. The brains and eyes were removed rapidly. Retinae, substantiae nigrae, striata, olfactory bulbs and frontal cortices were dissected. One of each of these was frozen under nitrogen for GAD assay. The other samples were homogenized in ice-cold 0.32 M sucrose for immediate GABA-T assay.

At every dose, both GAD and GABA-T activities were markedly more diminished in the retinal samples. Frontal cortical GABA-T activity was more diminished than olfactory bulb, striatal or nigral GABA-T activities at 10,25, and 50 mg/kg. GAD activities in striatum and frontal cortex were more diminished at 25 and 50 mg/kg than those from olfactory bulb or substantia nigra. ID 50s for each enzyme were estimated from the dose response curves for each CNS region. They were as follows:

CNS Region:	Retina	F.Cortex	Striatum	O.Bulb	S.Nigra
Enzyme GAD:	8	43	52	>>50	>>50
GABA-T:	<1	28	60	60	>>50

In summary, GAD and GABA-T activities in retina are much more sensitive to γ-AG administered peripherally, in vivo, than they are in 4 other CNS regions. Regional variations in γ-AG induced loss of GAD and GABA-T activities also occur in the brain itself, but to a lesser degree than those observed between retina and brain. These regional differences may be due to differences in regional delivery of γ-AG within the CNS. The marked retinal sensitivity to γ-AG permits the in vivo study of this drug, and possibly other similar drugs, at low, nontoxic doses in an easily accessible region of the CNS.

γ-AG was a gift from Merrell Dow Research Institute. Supported by USPHS grants EY 04633, NS 09649, and T32GM7288.

82.6 IMMUNOCYTOCHEMICAL DEMONSTRATION OF GABA- AND SEROTONINERGIC ENDINGS AT THE LEVEL OF THE SECOND ORDER NEURONS OF THE ELECTROSENSORY PATHWAY IN MORMYRID FISH (TELEOSTEI, PISCES). J.P. Denizot°, S.Clausse°, K.Elekes°, K.Grant°, T.Szabo, M.Véron°. C.N.R.S., Lab. Physiologie Nerveuse, Dept. Neurophysiologie Sensorielle, 91190 Gif sur Yvette, France.

Several electrophysiological reports have demonstrated a strong inhibition in the fast electrosensory pathway of mormyrid fish at the level of the lateral line lobe nucleus (nLLL) (Steinbach & Bennett, 1971; Zipser & Bennett, 1976; Szabo et al., 1979; Bell, 1982). This inhibition appears as a result of the corollary discharge controled by the electric organ discharge (EOD) command system (Bell et al., 1983) and represents a gate at the level of the nLLL for the sensory impulses set up by the fish's own EOD. The nLLL is composed of several hundred, adendritic, 20um cells with a complex synaptology (Szabo & Ravaille, 1976): a small number of club endings with mixed synapses, originating from primary afferent electrosensory fibers (Szabo et al., 1983; Bell & Russell, 1978) contact the somatic membrane of each cell, while a very large number of synaptic boutons cover the rest of the somatic membrane as well as the initial segment. Ultrastructurally, two types of boutons can be distinguished.

Immunocytochemical treatment of 50um thick transverse sections with antisera dilutions of 1:2000 to 1:10000, developed using the PAP, IgG or Biotin/Avidin complex techniques, revealed the presence of the biosynthetic enzyme glutamic acid decarboxylase (GAD) and the inhibitory transmitter gamma aminobutyric acid (GABA) in a large number of bouton-like endings on the soma and on the initial segment of nLLL neurons. Labeled terminals in plastic embedded semi-thin (1um) sections showed that GABA containing boutons made synaptic contacts with nLLL neurons. This suggests that GABA is the inhibitory transmitter at this level of the fast electrosensory pathway.

Antiserotoninergic (5HT) (dilutions used 1:400 to 1:2000) immunoreactivity was demonstrated in numerous preterminal axons branching within the nLLL and in a small number of terminals within the neuropil contacting the soma of nLLL neurons.

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82.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF GAD IN RAT SUPERIOR CERVICAL GANGLION. S.L. Kenny* and M.A. Ariano (SPON: W.G. Bradley). Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

The superfusion of GABA in rat superior cervical ganglion (SCG) in vitro has been shown to produce depolarization of the resting membrane potential and an increased conductance to chloride ions in the principal, postganglionic neuron (Bowery & Brown, 1974; Adams & Brown, 1975). In an attempt to find the morphological substrate containing GABA in the SCG, we performed imunohistochemical localization for its synthetic enzyme, γ-amino butyrate decarboxylase (EC 4.1.1.15, GAD). Specific polyclonal sheep anti-GAD was a generous gift of I.J. Kopin (NIMH) and has been characterized previously (Oertel, et al, 1981).

Pairs of SCG were isolated from male Sprague-Dawley rats (200-250 gm), and frozen at -25°C in a cryostat. 8 μm sections were thaw-mounted onto chrom-alum coated glass slides. GAD antiserum was used at 1:1000 dilution in phosphate-buffered saline (PBS), incubated overnight at 4°C in a moisture box. PBS washes were used to remove unbound primary antisera. Secondary, fluorescein-conjugated antisera (Cappel Labs, West Chester, PA) was applied at 1:100 dilution in PBS for 1 hour at 4°C. Following a final PBS wash, sections were covered with 5% n-propyl gallate PBS-glycerine (1:9, Giloh & Sedat, 1982) and examined using ultraviolet optics (485 nm/520 nm; primary/secondary filter combination) on a Zeiss Photomicroscope. Alternatively, sections were incubated using the Sternberger-PAP technique (1979). Control experiments included omission of primary antisera or omission of secondary antisera. Sections were not reactive under these conditions.

The immunohistochemical localization of GAD was found to be present in intensely reactive, irregular annular structures of approximately 20 μm diameter. Safaranin-O counterstain showed these structures were not postganglionic neurons, capillary endothelia, or glial-satellite cells. Glyoxylic acid histofluorescence (De la Torre, 1981) staining of serial sections demonstrated that the GAD immunoreactive elements were not dopaminergic SIF cell neurons of the SCG. This preliminary data suggests that the GAD-immunoreactive cells may be a separate population of small-diameter neurons in the rat SCG.

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82.8 GAD^+ NEURONS FOR GABA-MEDIATED INHIBITION IN KAINATE-HIPPOCAMPAL EPILEPSY. C.J. Davenport[1]* and T.L. Babb[2]. Division of Neuropathology[1] and Department of Neurology[2], Brain Research Institute and Reed Neurological Research Center, University of California at Los Angeles, Los Angeles, CA 90024.

Previous in vivo and in vitro electrophysiological studies have suggested that kainic acid (KA) induced hyperexcitability in regions "remote" (ie, CA_1) from areas of cell loss (ie., CA_3 & CA_4) is due to a loss of GABA-mediated recurrent inhibition (Lancaster, B. and H.V. Wheal, Brain Res., 295:317, 1984; Wheal et al., Electrophysiology of Epilepsy, Academic Press, 1984). The present study quantitates KA toxicity on hippocampal principal cells and inhibitory interneurons proximal and remote to the KA injection.

0.5 - 1.5 μg KA/0.2 μl volumes were injected ipsilaterally into the posterior hippocampus of rats (n=15), with survival periods varying from 1-28 days. The control animals (n=10) received either a 0.2 μl saline injection or no injection ($saline^{\pm}$ = normal). Volumetric cell densities of anterior & posterior hippocampal subregions in Nissl stained preparations included FD upper & lower, CA_4, CA_3, CA_2, CA_1, prosubiculum, subiculum, parasubiculum, and medial & lateral entorhinal cortex. Glutamic acid decarboxylase (GAD) immunocytochemistry using sheep anti-GAD and the ABC technique was used to assess KA toxicity on hippocampal inhibitory interneurons in the hilar polymorph (superior hilus, hilar taper), CA_3 radiatum, CA_3 oriens, CA_1 radiatum and CA_1 oriens.

There was no significant (α=0.05) change in pyramidal or granule cell density in both the anterior & posterior hippocampus on the side contralateral (KA^-) to the KA injection when compared to controls. In the ipsilateral anterior ("remote" KA^+) hippocampus, only CA_3 and CA_4 subregions demonstrated significant cell loss when compared to the KA^- side and to controls. Ipsilateral posterior (injection site) cell densities were significantly reduced in all subregions examined. KA ($\geq$ 0.5 μg/0.2 μl) caused no significant change in hippocampal interneuron density in all the anterior ($KA^{\pm}$) or contralateral (KA^-) posterior hippocampal subregions examined when compared to controls. The effect of KA on principal cell and inhibitory interneuron density was not concentration or time dependent within the KA concentration range or survival times examined.

GAD^+ interneuron density remained unaffected in regions remote from the site of KA injection regardless of whether there was a significant (ie., CA_3 & CA_4) or nonsignificant (ie., CA_1) change of principal neuron density. Thus KA is not selectively neurotoxic for GAD^+ interneurons. These results suggest that CA_1 hyperexcitability is not due specifically to a loss or reduction of GABA-mediated inhibition.

83.1 EFFECT OF ANTI-CHOLECYSTOKININ ANTIBODY (AB) ON MORPHINE (MOR) INDUCED SUPPRESSION OF SPINAL NOCICEPTIVE TRANSMISSION. S.N. Suberg, E.S. Culhane*, G. Rosenquist*, E. Carstens & L.R. Watkins. Dept. Animal Physiol., Univ. of Calif., Davis, CA 95616.

Proglumide, the putative cholecystokinin (CCK) antagonist, has been shown to potentiate MOR induced suppression of spinal nociceptive transmission (Suberg et al., Soc. Neurosci. Abs. 10:106, '84). Our results indicated that endogenous CCK may modulate the pain suppressive effects of opiates. Because proglumide has not been shown to bind to CCK receptors the aim of our present study was to determine if CCK is indeed acting as an endogenous opiate antagonist and if the potentiation seen previously with proglumide is due to antagonism of CCK. To accomplish this we used a more specific antagonist, an anti CCK-antibody (capacity of AB to bind S-CCK-8 is 5 fm/ml).

An agar pool was constructed around the lumbosacral enlargement to allow drugs to be placed directly on the spinal cord. Tungsten microelectrodes recorded responses of single dorsal horn cells to noxious heating (48°-54°C, 10 sec/2 min) of the hind footpad of rats receiving continuous infusion of sodium pentobarbital to maintain constant anesthesia. The femoral vein was cannulated for administration of naloxone. Recordings of heat responsive neurons were made over time, to the application of MOR (.1 µg), AB and MOR+AB. MOR remained on the cord until heat evoked responses were 50% of baseline or for 30 min if no suppression occurred. AB remained on the cord for 40 min. MOR+AB remained on the cord between 30-60 min dependent upon the cell's prior response to MOR. Between application of MOR & AB the cord was rinsed with saline and recording was continued until responses returned to baseline. Between applications of AB and AB+MOR, 2 paradigms were tested: 1) AB was removed, the cord rinsed and recording was continued until responses returned to baseline or 2) AB was removed but the cord was not rinsed off.

AB alone did not effect baseline responsivity more than ±10%. In paradigm 1 (n=5), there was no potentiation of MOR inhibition when AB+MOR was applied (n=4). In one cell there was a slight potentiation of inhibition (~30%). In the 2nd paradigm, in 2 of 4 cells studied there was a potentiation of morphine induced inhibition with application of AB+MOR. In these cells inhibition was naloxone reversible (10 mg/kg, i.v.). In the remaining cells there was no difference between MOR inhibition and AB+MOR inhibition. Though the evidence as yet is inconclusive, these initial positive results indicate that CCK could be acting as an endogenous opiate antagonist and that the potentiation seen with proglumide may be due to antagonism of CCK. The results where AB+MOR did not potentiate MOR inhibition may be due to incomplete diffusion of AB (150,000 M.W.) to recording site. Supported by UCD grant DG #512 to S.N.S.

83.2 ANTINOCICEPTIVE AND MOTOR EFFECTS OF INTRATHECAL (IT) VASOPRESSIN (VAS). C.L. Thurston*, S.N. Suberg, E.S. Culhane*, E. Carstens, & L.R. Watkins (SPON: D.J. Mayer). Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616 USA

VAS has been suggested to have a role in pain modulation based on anatomical and behavioral evidence. VAS is located in the spinal cord dorsal horn as well as in brain regions thought to have a role in analgesia. Paralleling these anatomical findings, systemic, intracerebroventricular, and IT injections of VAS have been shown to produce non-naloxone reversible analgesia suggesting that VAS can inhibit pain at multiple levels.

The effects of IT VAS were characterized in 102 rats using 5 pain tests and analyzing motor function. Baseline values on tail flick (TF), tail shock vocalization (TSV), hot plate (HP), hind paw pinch (PP), and tail pinch (TP) tests were measured prior to drug injection. VAS was injected IT in doses of 0, .25, 2.5, and 25 ng dissolved in .5 µl saline. Rats were tested for responsiveness to pain using a blind procedure 5, 20, or 40 minutes post injection. Motor functions were tested 5 min post injection, 5 min prior to pain testing, and upon completion of the pain tests.

IT VAS showed dose-related effects on pain responsiveness and motor function. A prolonged dose-dependent decrease in response to pain was observed in the TF test with 25 ng and 2.5 ng VAS. A transient dose-related decrease in response to pain on the HP test was seen with 25 ng and 2.5 ng VAS. Transient suppression of the pain responses to TSV, PP, and TP was seen with 25 ng VAS while 2.5 ng had no effect. Dose-related transient effects on motor function including flaccidity of the hind quarters, splaying of the hind limbs, and to a lesser degree, a lack of grasping and righting reflexes were seen with 25 ng and 2.5 ng VAS. The lowest dose, .25 ng VAS, had no significant effect on any of the pain tests nor on motor function.

Although IT VAS can produce motor deficits, the deficits appear to be separable from the effects on responses to pain. First, for the TSV and HP tests, hind quarter motor suppression would not prevent the rat from responding. Second, the effects on pain responsiveness generally outlasted any observable motor deficits. Third, observations made during pain testing showed that rats responding least to pain showed no signs of pain perception (i.e., no orientation to pain, vocalization, or forebody movement to escape the pain). Thus, it appears that IT VAS can exert both motor and pain suppressive effects in a dose-dependent manner. Whether these antinociceptive effects are secondary to IT VAS induced hypertension is currently under investigation. Preliminary studies show that IT VAS does increase blood pressure and possible correlations with tail flick latency are being examined.

Supported in part by NIH grant NS20037 and UCD grant 2137.

83.3 EVIDENCE FOR A MODULATORY ROLE FOR SUBSTANCE P IN NOCICEPTION USING DESENSITIZATION TO SUBSTANCE P FOLLOWING REPEATED INTRATHECAL ADMINISTRATION. J. Sawynok and G.S. Robertson*, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7

There is considerable evidence that Substance P (SP) is a primary afferent transmitter for nociceptive sensory stimulation. However, there are a variety of situations in which there is a mismatch between alterations in SP function and sensory responses, and an alternative regulatory or trophic function has been proposed (SP in the Nervous System, Ciba Fdn. Symp. 91, 1982, p. 249). Recently, we demonstrated that the intrathecal injection of SP produced hyperalgesia in the rat tail flick test (control latencies 6-9 sec) and that this response desensitized progressively following repeated injection (Br. J. Pharmacol., 1984, 82:381). In the present study, we examined the effect of desensitization to SP on the antinociceptive effect of intrathecal morphine and baclofen to provide behavioural evidence for a role for SP in the spinal antinociceptive mechanism of action of these agents. Effects of desensitization on baseline values also were monitored because if SP is a transmitter for noxious thermal stimulation, desensitization should alter these values. All drugs were injected intrathecally.

Repeated injection of SP (7.5 or 15 µg) or pretreatment with two injections of these doses of SP produced desensitization to the hyperalgesic response to SP. Desensitization was dose-related with respect to degree and duration, and was relatively specific in that the hyperalgesic responses to methysergide, a serotonin receptor antagonist, and phentolamine, an adrenergic receptor antagonist, were much less affected than SP. Pretreatment with a desensitizing regimen of SP potentiated the antinociceptive effect of morphine and baclofen when these agents were injected immediately after the regimen. However, if a 30 minute delay was permitted before the injection of morphine and baclofen, an inhibition of their effects was observed. These results support the notion that the spinal antinociceptive effect of morphine and baclofen are due to interactions with SP mechanisms in the spinal cord, but the nature of such interactions may be more complex than is presently understood. Desensitization did not produce changes in baseline responsiveness in the tail flick test. This suggests either that the hyperalgesic response to SP is due to an action at a site other than the primary afferent synapse, or if the response is at this site (a) unspecified compensatory mechanisms occur or (b) SP is not the primary determinant of tail flick latency but may play a modulatory role. (Supported by MRC Canada).

83.4 EFFECTS OF HIGH DOSE INTRATHECAL OPIATES. Harty, G.J.* and Yaksh, T.L.* (SPON: Peter J. Dyck). Laboratory of Neurosurgical Research, Mayo Clinic, Rochester, MN 55905.

Intrathecal (IT) administration of morphine produces a powerful analgesia characterized by an action at an opioid receptor, e.g. characterized by a predictable structure-activity relationship, reversed by naloxone and cross tolerance to systemically administered opiates. At higher doses non-opiate receptor mediated phenomenon have been reported (Woolf, Brain Res. 209:491, 1981). We sought to examine these effects. Rats implanted with IT catheters were divided into groups and received drugs administered in 3 µl of saline. After a 1-2 min latency IT morphine (150 µg) resulted in a striking behavioral syndrome which consisted of: 1) spontaneous agitation (SA) characterized by intense biting and scratching of the body innervated by the portions of the cord segments proximal to the catheter tip, and 2) clear hyperesthesia where light stroking of the fur over the lower abdomen and flanks (but not the face or ears) would produce violent, coordinated effects to escape and/or attack of the probe, i.e. touch evoked (TE) agitation. Quantifying the SA behavior at 1-min intervals using a 3-point rating scale and the TE behavior at 5-min intervals using a 3-point scale, revealed a peak effect at 10-15 min and near absence at 50-60 min. The animals were examined on the hot plate (HP) and tail flick (TF) at 60 min.

IT Drug	SA score (Σ60 min)	TE score (Σ60 min)	HP (sec)	TF (sec)
Saline	0	0	18	2
Morphine (150 µg)	42	5	60	60
Morphine (30 µg)	0	0	60	60
Morphine (150 µg) + Naltrexone (30 mg/kg IP)	62	6	23*	2*
Morphine (150 µg) + Morphine pellets (150 mg/5 days)	49	5	33*	2*
Sufentanil (100 µg)	0	0	60	60
Methadone (150 µg)	0	0	60	60
Naltrexone (90 µg)	0	0	15	15
Morphine-3 glucuronide (3 µg)	40	4	19	2

*$p < 0.05$ as compared to morphine dose; n = 4 each group.

These results emphasize the observation that high dose morphine produces a phenomena which has a pharmacology totally unlike that observed with lower doses. Though the mechanisms are not clear, we note: 1) that the glucuronide produces the same syndrome at 0.02 x the dose, and 2) high dose of opiates may have an antiglycinergic action. (Funds from DA-02110 and the Mayo Foundation.)

83.5 INTRATHECAL BICUCULLINE IN RATS EVOKES HYPERALGESIA AND EXAGGERATES AVOIDANCE OF CONTACT BY OTHER RATS. S. Schwartz-Giblin and D.W. Pfaff. Rockefeller University. New York, N.Y., 10021.

Chronic catheterization of the spinal subarachnoid space was performed on 14 ovariectomized female rats (220-350 g) which had subcutaneous 5 mm silastic implants of estradiol. Gross inspection and dye studies showed the catheter tips to be between the lower thoracic and lumbar spinal segments. In 7/8 rats intrathecal injections of the GABA antagonist bicuculline methiodide in doses ranging from 1nM-4nM (0.5-2μg in 1μl-7μl) produced a syndrome of hyperalgesia and stereotyped behavior which was dose dependent in individual animals. The latency to onset was 1.5-3 min; the effect was maximal by 10 min and was usually over by 20-30 min. The onset was characterized by biting and scratching the flank. Subsequently, the animals avoided light touch or a weak air jet to the proximal hindlimbs and trunk by turning, rolling or jumping. These same stimuli were innocuous in the control period or following injection of saline. The rats vocalized to tactile stimulaton and displayed aggressive pawing and biting of the instruments of stimulation. At the higher doses, rats elicited spontaneous hyperkinetic behaviors such as persistent pushing of bedding material across the test cage, and intermittent jumping and kicking often related to scratching of the flank. Intrathecal picrotoxin at a threshold dose of 3.3nM elicited the same syndrome in 3/4 rats tested. Similar responses to intrathecal strychnine sulfate (5μg) have been reported (Beyer et al. Soc.Neurosci Abstr. 310.6, 1983).

The mating behavior of 4 females during 10 min tests with stud males was compared before and from 0-10 min after intrathecal bicuculline (1nM-4nM in 5μl), with or without subcutaneous progesterone. Females often vocalized and avoided male mounts by getting into the corner, turning over(T), kicking(K) and rearing(R). An avoidance index was calculated: T+K+R/Mounts+Attempted Mounts. From 0-10 min after drug, all females had a higher avoidance index compared to the control period. By contrast, at the same time, the Lordosis Quotient (lordoses/mounts with thrusting) was not reliably reduced by bicuculline.

Our data suggest: GABA modulates spinal mechanisms related to cutaneous signalling and pain, and may thus affect responses to rat-rat contact. But in mating tests, if the male can mount well, lordosis behavior may be prepotent even during bicuculline-induced hyperalgesia. (Supported by PHS HD 13795.)

MOTOR SYSTEMS: MOTOR AND SENSORY INTEGRATION

84.1 PRETERM HUMAN INFANTS: HEART RATE AND MOVEMENT CHANGES IN RESPONSE TO TACTILE STIMULATION. C. R. Almli and S. Lofsness*, Dept. Preventive Medicine, Program in Occupational Therapy and Neural Science, Washington University School of Medicine, St. Louis, MO, 63110.

Knowledge of the premature human infant's responses to sensory stimulation is important for an understanding of the relation between development of the nervous system and behavioral ontogeny, and for an understanding of the effects of early neuropathology on behavioral development. The present study investigated the preterm infant's motor movements and heart rate changes in response to 3 modes of tactile stimulation.

Preterm infants, born at 26 to 32 weeks estimated gestational age (EGA) were tested at 1 and 3 weeks postnatal age. Heart rate and respiration were recorded (polygraph and FM tape recorder) and movements were simultaneously videotaped. While supine in their beds, punctate, brush and pressure tactile stimuli (10 grams) were applied to the left and right perioral and abdominal regions (3 trials each, 5 sec stimulation, 20 sec interstimulus internal). Heart rate and movement data were averaged over trials into 5 second bins.

Analysis of heart rate revealed that preterm infants responded with cardiac acceleration to punctate or brush modes of stimulation, and with cardiac deceleration to pressure stimulation. The motor movement data paralleled the heart rate data with increased movement to punctate or brush stimulation and decreased movement in response to pressure stimulation. These responses were obtained on tests at 1 and 3 weeks postnatal. The major difference between tests was a large increase in movement for the test at 3 weeks of age. Infants with diagnosed neuropathology (e.g., intraventricular hemorrhage) displayed complex reactions to the tactile stimulation.

These results indicate that preterm infants: (1) can discriminate punctate, brush and pressure modes of tactile stimulation; (2) may display an "orienting-like" response to certain modes of sensory stimulation (e.g., pressure); and (3) show integrated autonomic and skeletal nervous system response to sensory stimulation (in contrast to previous reports). The results also indicate that pre-term infants with and without intraventricular hemorrhage may be discriminated on the basis of movement and heart rate changes in response to sensory stimulation. (Supported by Grant USHRSA-51998)

84.2 MALDEVELOPMENTS OF VISUAL MOTION PROCESSING IN HUMANS WHO HAD STRABISMUS DURING INFANCY. L. Tychsen* and S.G. Lisberger. (SPON: J.L. Bixby) Div. Neurobiol., Dept. Physiol., U.C.S.F., San Francisco, CA.

Strabismus during the critical period is known to cause deficits in information processing in the visual cortex. We have used pursuit eye movements, and psychophysical judgements of target velocity, as probes to determine if these deficits include motion processing in humans.

The initiation of pursuit was studied in 7 adult subjects who had strabismus in infancy (concomitant esotropia, "latent" fixation nystagmus, and dissociated vertical deviation). Subjects were non-amblyopic, i.e. optotype acuity was normal in both eyes. Small targets were presented in individual trials of ramp motion. Eye position was recorded using a scleral coil. Eye acceleration, averaged over the first 100 msec of smooth eye velocity, was used to estimate the open-loop response of the pursuit system.

Strabismics had a naso-temporal asymmetry in the initiation of pursuit. Viewing monocularly, strabismics initiated pursuit weakly for temporally-directed target motion, and normally for nasally-directed target motion. The movements of the two eyes were always conjugate, and the asymmetry flipped direction when viewing changed from right to left eye. Maximum temporally-directed eye acceleration was 2-8X lower than maximum nasally-directed acceleration. Strabismics often initiated pursuit in the wrong direction. When the target moved temporally, they accelerated nasally. By starting the target at different locations in the visual field, we were able to map the topography of this defect; wrong-way pursuit was most pronounced for targets 12 deg or more eccentric in the nasal visual field. Strabismics also had up-down asymmetries of pursuit initiation. For targets in the lower visual field, downward pursuit acceleration was 2-6X lower than upward pursuit acceleration.

To determine if these defects were due to abnormal visual processing, we used the method of S.P. McKee and L. Welch (J Opt Soc Amer 2:243, 1985) to test foveal velocity discrimination in 2 strabismics. Targets appeared in individual trials of horizontal ramp motion, at one of five unpredictable velocities (range 9-19°/sec). The subject judged whether each trial was faster or slower than the perceived mean of the range. Strabismics, like normals, were able to divide the velocities into slow or fast categories, but viewing monocularly, they judged temporally-directed targets to be moving 15-35% slower than nasally-directed targets that were actually moving at the same velocity. The psychophysical error, and the pursuit defect, correlated with the magnitude of the strabismus.

We conclude that maldevelopments of visual motion processing are closely related to strabismus. The lack of binocularity during the critical period, however, makes it difficult to assign cause and effect. (Supported by Fight for Sight Inc. of NYC, Nat. Children's Eye Care Foundation of Wash DC, and NEI grant EY05431).

84.3 **SENSORY GATING DURING STEREOTYPED BEHAVIOR IN THE RAT.** R.T. KNIGHT and S. BRAILOWSKY. Dept. of Neurology, Univ. of Calif., Davis, VAMC, 150 Muir Rd., Martinez, Calif., 94553

The effects of stereotyped behavior on sensory transmission were investigated with auditory evoked potential and EEG power spectra techniques. Chronically implanted, free-moving F344 rats (n=8) were recorded while alert and immobile and during grooming episodes. In each behavioral state, brainstem auditory potentials (BAEP, 100-3,000 Hz bandpass, 11/sec stimulation rate) and longer latency auditory potentials (AEPs, 1-300 Hz bandpass, 0.5/sec stimulation rate) were recorded from a vertex electrode to free field clicks (0.1 msec, 55 dB above BAEP threshold). EEG power spectra (0-32 Hz) was simultaneously recorded from the same electrode. Behavioral state had no effect on the amplitude or latency of BAEP waves I,II-III, IV or the following negativity (4.6 msec). In the alert and immobile animal a myogenic N7-P12 potential was recorded which was associated with a startle response at higher intensities. Following the myogenic response, prominent (25 - 90 uV) neurogenic N17, P23, N38 and N50 AEPs were recorded in all animals. In contrast to the BAEP, the myogenic P12 and all neurogenic AEPs had significant amplitude reductions during grooming periods (P12 , $p < 0.01$; N17 , $p < 0.05$; P23 , $p < 0.001$; N38 , $p < 0.002$; and N50 , $p < 0.001$). These attenuations were accompanied by a theta peak in the power spectra (7.17 +/- 0.33 Hz) which was absent in the alert, immobile animal. The unaltered BAEP during grooming indicates intact lemniscal conduction up to the level of the inferior colliculus. The suppression of the startle associated N7-P12 potentials may be mediated at sub-collicular levels. Further data on the intracranial sources of the behavior dependent neurogenic AEPs is necessary to determine the locus of their inhibition. The association with a prominent theta rhythm, however, supports involvement of hippocampal structures.

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84.4 SINGLE UNIT RESPONSES IN THE MEDULLARY ELECTROSENSORY NUCLEUS IN THE LITTLE SKATE. J.G. NEW .Dept. of Biology, Wesleyan University Middletown, CT 06457

Single unit responses to localized electric fields were recorded in the dorsal octavolateralis nucleus (DON) to determine the response characteristics of different cell classes. Little skates, Raja erinacea, were anesthetized during surgery, then decerebrated, and immobilized with tubocurarine chloride (1mg/kg). A concentric bipolar stimulating electrode was placed in the lateral mesencephalic nucleus (LMN); the principal midbrain target of ascending DON efferents. E fields were presented as DC step uniform fields or small localized fields from a dipole surface.

Several different classes of cells could be distinguished by responses to E field presentation and antidromic responses to LMN shock; afferent fibers of the anterior lateral line nerve (ALLN), DON efferent cells ascending to the LMN, and local DON interneurons. ALLN fibers possessed very small excitatory receptive fields (RFs) at threshold stimulus intensities which could usually be isolated to the area of a single ampullary pore. These responded to cathodal stimuli with increased tonic activity, increasing in frequency with increasing stimulus intensity. Ascending efferent cells, identified by antidromic responses to LMN shocks, possessed larger ipsilateral RFs encompassing several pores with no evidence of an inhibitory surround. Efferents responded to cathodal fields with an initial burst of spikes followed by an elevated rate of firing. The frequency of the burst spikes increased with increasing stimulus intensity as the response latency decreased. Three classes of interneuron response could be discerned: 1) Cells that exhibited activity indistinguishable from the efferent cells but that were not activated by LMN stimulation. A minority (<3%) possessed only RFs contralateral to the DON. 2) Cells that possessed larger and more diffuse RFs and responded to increasing stimulus intensity with tonic activity of increasing duration and spike frequency. 3) Cells with very large and very diffuse RFs that responded to cathodal stimuli with an elevated bu irregular firing rate with variable latency and temporal pattern.

All of the cells recorded within the DON were excited by presentation of a cathode and inhibited by presentation of an anodal field. The small RFs of the DON efferents reflect limited convergence of ALLN fibers in ascending pathways, and the uniform cathodal excitability of these cells and other DON neurons differs from previous studies in different fish species.
(Supported by a Sigma Xi Grant-in Aid of Research.)

84.5 DIFFERENTIAL PROJECTION OF THALAMIC NEURONS UPON THE NUCLEUS OF THE BASAL OPTIC ROOT AND PRETECTAL NUCLEUS LENTIFORMIS MESENCEPHALI. K. V. Fite, L. Bengston* and N. Montgomery. Division of Neuroscience & Behavior, University of Massachusetts, Amherst, MA.

Utilizing both anterograde and retrograde transport of HRP, we have shown that neurons which are postsynaptic to the retinorecipient thalamic neuropil of Bellonci project to a region of the ventral mesencephalon which includes the nucleus of the basal optic root (nBOR). In addition to the primary projection from the contralateral retina, nBOR thus appears to receive second-order optic input from the ipsilateral anterior thalamus (Montgomery, et al, J. comp. Neurol., 203: 595-612).

Recently, we have further observed a second population of thalamic neurons which project to the pretectal nucleus lentiformis mesencephali (nLM). Following microinjections of crystalline HRP into nLM, retrogradely HRP-filled neurons were seen in the ventrolateral nucleus and in the most caudal portion of the "lgn" and "nucleus rotundus" cell groups (terminology of Frontera, 1952). Many of these neurons extend long dendrites laterally into the second major thalamic optic neuropil, corpus geniculatum. A differential retinal distribution of ganglion cells also exists which distinguishes the first-order optic projection to nBOR vs that to nLM. The peripheral retina appears to be more substantially represented in the retinofugal projection to nBOR, while the central retina predominates in the retinal projection to nLM. These neuroanatomical relationships suggest that major differences exist in the organization of both first and second-order visual projections to these extratectal, mesencephalic optic nuclei which may be related to central vs peripheral regions of the visual field and, functionally, to the initiation and/or modulation of compensatory oculomotor behaviors. (Supported by NSF BNS 8209026)

84.6 TECTO-RETICULO-SPINAL NEURONS HAVE PHASIC DISCHARGE RELATED TO HIGH BUT NOT LOW VELOCITY SACCADIC GAZE SHIFTS. D.Munoz and D.Guitton. Montreal Neurological Institute, Dept. Neurology and Neurosurgery, McGill Univ. Montréal, Québec, H3A 3B4, Canada

Tecto-reticulo-spinal neurons (TRSNs) are normally silent in the alert cat looking spontaneously about the laboratory. However, when the hungry cat is presented with a food target, TRSNs can show gaze related discharges that are both sustained and phasic. We believe that the sustained discharge maintains cells, at a particular collicular site, active until the saccade associated with that site is finally triggered. (Brain Res 1985 in press). The phasic discharge is discussed below. Methods: 22 TRSNs were recorded in the superior colliculus (SC) of 4 alert cats whose heads were either fixed or free. Cells were antidromically identified by stimulation of either the spinal cord at C1 or the predorsal bundle rostral to the abducens nucleus. Eye and head movements were recorded with the magnetic search coil technique. EMG activity of dorsal neck muscles was monitored. Results: When observed, the movement-related phasic bursts were well correlated to the amplitude and direction of the gaze shift and the accompanying bursts of neck EMG activity. A characteristic feature of the phasic bursts was their inconsistent presence even if the amplitude and direction of the gaze shift was optimal for that cell. This was particularly evident in a paradigm where the cat oriented to either a visible or an imagined food target. In this task, the target, visible to one side of a rectangular opaque barrier, was fixated by the cat. The target then disappeared behind the barrier and either reappeared on the other side, in the TRSN's visual receptive field, or remained hidden behind the barrier. The cat predicted the reappearance of the target and oriented irrespective of whether the target reappeared (visible target) or not (imagined target). Gaze shifts to both the visible and the imagined targets were preceded by a sustained discharge. When the target was visible, the cell also discharged a high frequency burst of spikes whose onset was tightly correlated to that of the gaze shift. This phasic component was usually absent when the cat oriented to the imagined target. In this condition, the gaze shifts were generally slower than those to the visible target. However, in those few trials where high velocity gaze shifts were directed at the imagined target, TRSNs had a phasic burst. Conclusions: The phasic bursts preceded a population of saccadic gaze shifts whose directions and amplitudes (vectors) defined a movement field. But the cat was capable of generating saccadic gaze shifts along the optimal vector (head-fixed or head-free) without these bursts in the TRSN pathway. Bursts in the TRSN pathway were observed when the target was a very "arousing" stimulus and coincided with an increase in the velocity of the orienting movement. (Supported by MRC, Canada.)

84.7 HOW ARE SONG SYLLABLES REPRESENTED IN THE BRAINS OF AWAKE SONGBIRDS? D. S. Vicario and F. Nottebohm. The Rockefeller University, New York, NY 10021

We studied the functional organization of a nucleus in the vocal control system of songbirds: the hyperstriatum ventralis, pars caudale (HVc). HVc projects to nuclei in the motor pathway for song and lesion experiments have shown that it is essential for normal song production. Recordings made in awake songbirds have shown that some HVc neurons have song-related activity, and this activity can be specific to the syllable produced (McCasland, 1983). Interestingly, HVc's volume is correlated with the size of the individual bird's learned song repertoire. Could this mean that particular syllables are associated with sub-regions of HVc? HVc has also been shown to receive auditory input and birdsong appears to be an especially effective stimulus (Margoliash, 1984). How is the auditory input from particular syllables distributed to neurons with specific motor associations?

To address these questions, recordings were made in both anaesthetized and awake, freely-moving zebra finches and canaries. In order to carry out a spatial analysis of sensory and motor activity, 4-7 fine wire electrodes were chronically implanted in the left and/or right HVc. Signals were led off through a flexible cable and multichannel commutator. These enabled directly comparable recordings to be made simultaneously at multiple sites.

Auditory responses to artificial (tone and noise bursts) and natural (song and song fragments) stimuli were studied in both awake and anaesthetized birds. Auditory evoked potentials with a short, stable latency, often accompanied by multi-unit activity, were seen at all HVc locations. The pattern of response depended on the stimulus, the recording site, and the bird's state. For instance, when song or syllable pairs extracted from song were presented to an anaesthetized bird, responses to the second and later syllables were weak or absent; however, in the awake bird, there was a response to each syllable. Single units had either irregular bursting or very low spontaneous activity and variable auditory responses, as others have reported. We are attempting to define factors contributing to this variability, which seems greater in the awake preparation.

We also recorded in awake birds during active song with the same electrode arrays. "Silent" units with no spontaneous or auditory activity were often recruited during song. Some features of single and multi-unit activity were similar to those noted by McCasland (1983), such as burst patterns related to the pattern of song syllables being produced. Most interestingly, simultaneous recordings from electrodes in different sites showed differences in timing and patterns of song-related activity. This may be evidence that subregions of HVc contribute particular influences to the motor pathway. (Supported by NS17991, MH39319, MH15125)

84.8 ACOUSTIC RESPONSE PROPERTIES OF MOTONEURONS INNERVATING THE STAPEDIUS MUSCLE OF THE CAT. J.B. Kobler, S.R. Vacher*, and J.J. Guinan. Dept. of Elec. Eng. & Comp. Sci., M.I.T., and Eaton-Peabody Lab., Mass. Eye & Ear Infirmary, Boston, MA 02114.

Retrograde transport studies have shown that a large number of motoneurons (>1100) innervate each cat stapedius muscle, and that they are located in a number of different perifacial and periolivary areas (Shaw, M.D. and Baker, R., J. Comp. Neur. 216:10, 1983; Joseph, M.P. et al., J. Comp. Neur. 232:43, 1985). Stapedius EMG responses following partial lesions of the stapedius motoneuron pool suggest that stapedius neurons in different locations might have different inputs and response properties (McCue, M.P. and Guinan, J.J. Soc. Neurosci. Abst. 9:1085, 1983). We have now recorded the responses of stapedius motoneurons to acoustic stimuli; the results show that the stapedius motoneuron pool does have functional subdivisions.

Cats were anesthetized with ketamine or decerebrated (both preparations produced similar results), and were paralyzed with gallamine to prevent muscle movement. The dorsolateral surface of the stapedius muscle and adjacent facial nerve were exposed surgically. Single fibers in the facial nerve fascicles which innervate the stapedius were impaled with glass micropipets.

Stapedius units exhibited high thresholds (>75 dB SPL) to acoustic stimuli and responded with regular discharge patterns characteristic of motoneurons. Units were divided into four groups based on their responses to sound: 1) "Binaural-Or" units (47% of 116 units) responded when sound stimulated either the left or the right ear and often showed summation when both ears were stimulated simultaneously; 2) "Binaural-And" units (12%) responded only when both ears were stimulated; 3) "Ipsilateral" units (17%) responded selectively to ipsilateral stimuli (showing little or no responses to contralateral stimuli); and 4) "Contralateral" units (24%) responded selectively to contralateral sounds. Ipsilateral and contralateral units frequently showed lowered thresholds and higher discharge rates in response to binaural stimuli.

Frequency tuning curves show that most stapedius motoneurons are maximally sensitive to frequencies between 1 and 2 kHz. All were broadly tuned, and, for levels of 105 dB SPL, many responded throughout the frequency range from less than 1 to greater than 30 kHz. Spontaneous activity was found in 10% of the units; 75% of those with spontaneous activity were of the "binaural-or" response type and had relatively low thresholds.

We conclude that the recruitment order of stapedius motoneurons will be strongly influenced by the spatial position and spectral characteristics of sounds eliciting the acoustic reflex. Thus, in this motoneuron pool, recruitment order is not solely a function of intrinsic motoneuron properties such as size or surface area.

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84.9 SOMATOSENSORY MODULATION OF JUVENILE PLAY IN THE RAT. S.M. Siviy and J. Panksepp. Dept. Psychology, Bowling Green State University, Bowling Green, OH 43403.

Rough-and-tumble social play in the juvenile rat is best characterized as an energized level of social activity, including chasing, jumping, rough grooming and wrestling. Therefore, this activity involves the processing of a multitude of sensory information. In the following work, we evaluated the involvement of somatosensory input in the play of juvenile rats at various levels of neural integration. A social deprivation paradigm was used, with pups housed individually and observed for 5 minute periods on test days (see Panksepp & Beatty, Behav. Neural Biol., 1980, 30, 197).

Anesthetization of the dorsal body surface of juvenile rats with xylocaine resulted in a concentration-dependent reduction in the frequency of pinning, an indicator variable of play, with a 2.0% concentration reducing pinnning by 36%. Play motivation was not impaired by this concentration of xylocaine. We next sought to analyze the central modulation of somatosensory involvement in play by examining involvement at several points along the neural hierarchy. Electrolytic lesions placed within the ventrolateral aspects of the brain stem resulted in a 51% reduction in the frequency of pinning, while having no reliable effect on play motivation. Electrolytic lesions of either the parafascicular area of thalamus or posterior thalamus reduced pinning by 64% and 62%, respectively. Lesions of similar size placed within the ventrobasal complex of the thalamus had minimal effects on pinning. While lesions of the parietal cortex resulted in a 65% reduction in frequency of pinning, generalized activity was also increased among these animals by 123%, suggesting that any play deficits may have been due to a generalized enhancement of activity and/or impaired attentional abilities.

It is concluded that somatosensory information, traveling via ventral brain stem pathways and terminating in parafascicular and perhaps posterior thalamus is involved in mediating the elaboration of juvenile play in the rat. Although not yet conclusive, the data do not suggest a role for parietal cortex in the modulation of play, supporting past work (Soc. Neurosci. Abst., 1984, 10, 612).

84.10 ORGANIZATION OF THE FIFTH SOMATIC SENSORY CORTEX(SV) IN THE CAT. A Mori*, N Matsuura*, K Kagaya*, T Seki*, H Hiraba* and R Sumino*. (SPON:Y Shinoda) Dept. of Physiol., Sch. of Dent., Nihon Univ., Chiyoda-ku, Tokyo 101, Japan.

We examined the physiological properties of the neuron in the anterior suprasylvian sulcus (ASSS) and suprasylvian gyrus of the cat's cortex. Under ketamine anesthesia a chamber was installed over the skull surrounding the SI, SII and SIII. A single tungsten in glass microelectrode was inserted into the SI, SII, SIII and ASSS, and was used for intra-cortical microstimulation (ICMS), and also for recording unitary spikes from the cortex. The motor effects were examined either by recording EMG or observing movements. The receptive fields of neurons were examined by using natural somesthetic stimulation.

A new somatic sensory area of the body surface in the cat was found in the rostro-caudal part of the medial bank of the ASSS by recording unit activites responding to tactile stimulation. This area was isolated from SI-SIV.This region was located in area 5. We have designated this area as the Fifth Somatic Sensory Cortex (SV). Cells with receptive fields on the face formed the rostral aspects of the ASSS. The tail and hindlimb areas are situated in the caudal aspect of the medial ASSS. The trunk area was located in the medial bank of the ASSS between the fore- and hindlimb regions. The majority (68%) of somatic cells studied in this region responded to a light touch of the skin. The other somatic cells responded to movement of the hair by a gentle stream of air (32%). In addition, a few neurons responded to a light touch of the skin and visual stimuli. However, neurons of the SV did not receive input from receptors in the deep structure of the face and the body. Neurons in the SV have large receptive fields from the contralateral side of the body and face. The receptive field of neurons in the trunk area was larger than in the other areas of the SV.

ICMS of the face region in the medial bank of the ASSS produced eye blinking or whisker movement on the contralateral side of the face using 10μA or less. The minimum threshold current for movement of eye blinking was 2μA. The threshold current that produced eye blinking was lower than that which produced whisker movement. However, ICMS of the limbs or trunk regions in the SV did not show any movement using 20μA or less. The latencies of the EMG responses in the face muscles, measured from the start of ICMS, ranged from 13-17ms (average:15ms). Neurons in the motor effective area received afferent input from receptors in the skin structures in the region of the face which was related to that movement. However, ICMS of the first somatic sensory area (SI) did not show any movement using 20μA or less.

84.11 THE ORGANIZATION OF TECTAL PROJECTIONS TO THE VENTRAL MIDBRAIN IN RANA PIPIENS. T. Masino and P. Grobstein. Dept. of Anatomy., Univ. of Chicago, Chicago, ILL. 60637

The role of direct descending projections from the optic tectum has been the focus of much previous work on the neuronal basis of orienting behavior in the frog. Recent studies from this lab suggest that indirect descending tectal projections, involving relays in ventral midbrain tegmental nuclei, may also be important in orienting behavior. Cells in the nucleus of the medial longitudinal fasiculus (nMLF), the anterodorsal tegmental nucleus (AD), and the nucleus profundus lateralis (LP) all have descending axons in a ventral white tract which when lesioned unilaterally abolishes orienting turns in one direction (Kostyk and Grobstein, submitted). That each of these nuclei themselves receive tectal input is suggested by several general studies of tectal efferent organization, but there has been no specific description of the organization of tectal projections to the ventral mesencephalon. To provide such a description, we have studied patterns of anterograde labelling following horseradish peroxidase injections into the optic tectum.

Following multiple HRP injections distributed widely across one tectal lobe, prominent collateral branching from the tectal efferent bundles was evident throughout their midbrain trajectory, both contralateral and ipsilateral. Dorsally, collateral branches and associated finer fibers with bouton-like swellings defined a rostro-caudally oriented columnar terminal zone lateral to the compact tegmental grey matter. Ipsilaterally, this zone extended from the rostral to the caudal end of the midbrain; contralaterally, it was less extensive caudally. Rostrally, this dorsal terminal zone overlies the LP on both sides of the brain, confirming earlier reports of a bilateral tectal projection to this structure. More caudally, it overlies cells identified by some workers as a continuation of the LP and by others as the magnocellular nucleus of the torus. Medial to the dorsal columnar terminal zone, branched fibers bearing swellings reached the anterodorsal nucleus on both sides of the brain. Ventrally, prominent collaterals were associated with the nMLF, more dramatically ipsilaterally, but also contralaterally. These findings confirm earlier reports of tectal projections to these nuclei and indicate that these projections too are bilateral. Collaterals were also apparent bilaterally in the area of the isthmic reticular nucleus. Prominent collaterals into the vicinity of the oculomotor and trochlear nuclei were also evident, as noted by some but not by all previous workers. We were unable to confirm the suggested existence of a projection into the ventrally located interpeduncular nucleus. In addition to the more prominent projections, our material also suggested the existence of a more diffuse terminal zone, occupying much of the ventrolateral tegmentum, adjacent to the anteroventral, posterodorsal, and posteroventral nuclei. Labelling patterns following single HRP injections placed either rostrally or caudally were quite similar to each other and, in the distribution of labelling, to the described patterns following multiple injections.

In general, our findings indicate the existence of quite substantial projections from the tectum into more ventral midbrain structures. These projections could certainly provide a basis for indirect descending tectal outflow paths, and may indicate that a significant amount of the post-tectal information processing involved in orienting occurs in the ventral midbrain, rather than in more caudal structures. Our findings also provide further evidence that the efferent projections of different tectal regions are quite similarly distributed.

84.12 SHORT AND LONG TERM EFFECTS OF UNILATERAL VESTIBULAR LESIONS ON POSTURE AND ORIENTING MOVEMENTS IN THE FROG. C. Comer, J. Schotland* and P. Grobstein. Dept. Biol. Sciences, Univ. of Illinois at Chicago, Chicago, Il. 60680.

Frogs (Rana pipiens) capture prey with orienting movements of the head and body and a rapid flick of the tongue. Previous research (Comer and Grobstein, J. Comp. Physiol., 142:141, 1981) has suggested that once these movements are triggered by a sensory stimulus, they can proceed to completion without the use of sensory feedback concerning the position of the stimulus. We have been interested in determining if proprioceptive feedback participates in the control of these orienting movements.

To characterize the extent of vestibular involvement in orienting behavior, we have analyzed the visually and tactually elicited movements of frogs with selective unilateral lesions of the VIIIth nerve. In a group of eight adults we cut either the anterior or posterior branch of the left VIIIth nerve and then measured their orienting behavior using standard perimetric techniques. Lesions of the posterior branch of VIII (innervating the post. vertical canal, auditory papillae and lagena) produced a slight postural asymmetry, but had little or no effect on vestibular reflexes and orienting movements. Abnormalities in posture disappeared with 1 week.

Lesions of the anterior branch of VIII (innervating the ant. vertical and horizontal canals, sacculus and utriculus) produced marked postural asymmetry, disrupted vestibular reflexes and caused a clear abnormality in visual and tactile orienting behavior. These frogs responded readily to prey items but their orienting and snapping responses (to both visual and tactile stimuli) usually overshot their target. Though the metrics of movement were thus abnormal, the amplitude of orienting turns still varied systematically with target position. In addition, the degree of overshooting was much greater for movement toward the side of the lesion than for movements away from it. Within 2-4 weeks, the animals compensated for the postural and reflex deficits. However, the abnormality in orienting movements persisted for as long as the animals were tested (5 months).

These results indicate (1) unilateral loss of vestibular afference can produce abnormalities in orienting movements which are not directly dependent on changes in posture or vestibular reflex function. (2) Circuitry related to the control of orienting movements possesses a much different potential for vestibular compensation than that related to posture and reflex control. (3) The results suggest that movements of the head and body of the sort used in orientation may not be entirely centrally generated and that proprioceptive signals may play a role in specifying the final position of the response.

84.PO COMPARISON OF HUMAN AND PRIMATE PERFORMANCE IN A SUSTAINED ATTENTION TASK

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The performance of 3 humans and 4 monkeys were compared in two tasks, one using tactile and the other using auditory stimuli. Both tasks were designed to avoid responses to predictable stimuli while requiring sustained attention of the subject for maximum performance. The subjects were cued that the tactile task could be performed by the presence of a green light which was turned on at the beginning of the task and remained on throughout the task. Random vibratory stimuli of variable amplitude and duration were then delivered to the glabrous skin of the right hand. The left hand was used to press a lever for response which was allowed to occur up to 1 sec after the start of the stimuli. Humans were instructed that detection of a 10 Hz stimulus would result in a high score which was the object of the task, and the monkeys were rewarded with a few drops of fruit juice for correct responses.

The performance (% score) of humans and primates were charted as a function of stimulus durations at various stimulus amplitudes. Both humans and monkeys only need 100 msec duration threshold vibratory stimulus (10 Hz) to perform at 80%. Below 100 msecs the performance dropped severely. Both humans and monkeys were then required to not only detect but to discriminate between frequencies of randomly occurring vibratory stimuli (10 Hz vs 100 Hz).

Again the results show that both humans and monkeys need only 100 msecs of threshold stimulus to detect and discriminate among stimuli. However the reaction times were prolonged by 250 msecs as compared to the case when only detection was needed for a correct score. A similar task using auditory stimuli demonstrated a similar detection and discrimination response.

We conclude that human and primate performance is very similar in the task of our paradigm. Humans and primates require 100 msecs of threshold stimulus to detect and discriminate randomly occurring auditory and vibratory stimuli. However, the reaction time increased by 250 msecs when they were required to discriminate. We argue since humans report that they must decide whether the stimulus is 100 Hz or 10 Hz after detection that discrimination must occur in the period beginning at 100 and ending at 350 msecs after the onset of the random stimuli in this paradigm.

84.PO NEURAL ACTIVITY IN THE PERIARCUATE REGION DURING VARIOUS EPOCHS OF MONKEY BEHAVIOR IN A GO NO-GO PARADIGM

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The object of this report is to describe our exploration of the functional properties of neurons in the periacuate region of the monkey cortex. The monkeys were trained in a paradigm as described in the previous paper to make decisions about arriving stimuli and to respond only to contingent stimuli. Previous studies by us and others have shown that ablation of the periarcuate region causes severe deficits in paradigms such as we are using. Thus in monkey this region plays some crucial role in this type of paradigm. As shown in the previous paper the performance scores and timing of humans and monkeys were found to be very similar. Verbal reports of humans suggests that there is a temporal order to the behavorial events in the paradigm. The sequence can be characterized by: 1) a state of attentiveness in awaiting the stimulus arrival, 2) a detection of stimulus presence, 3) a discrimination phase to determine whether the stimulus is contingent, 4) a decision period of whether or not to act, and finally, 5) the action if the stimulus is contingent.

While the monkeys were performing these tasks the single unit activity of neurons in the periarcuate region of the cortex was observed. Neurons were found in this region that were excited or inhibited for short periods after the time that the stimulus was presented. The various periods during which these neurons were active could be associated with various epochs of the information processing steps. In particular some neurons were found that had activity related to the detection of the stimulus onset. These neurons were active for both the rewarding and the non-rewarding stimuli. Other neurons were found that were active during the time period in which discrimination must occur. These neurons were active only when the rewarding stimulus occurred. Other neurons were found that were active about 200 msecs before the onset of movement. The verification of these associations were made by shifting the time during which the named epoch can occur while noting the corresponding shift in timing of the neural activity.

The results show that neurons can be identified in the periarcuate that play an important role in go no-go tasks with regard to the decision making required.

85.1 SEX DIFFERENCES IN ETHANOL SENSITIVITY AND METABOLISM IN THE SYRIAN HAMSTER. H.B. Moss*, R.J. Salin-Pascual*, P.R. Giri*, D. Goldman*, and L. Tamarkin*, (SPON: G. Merriam). Laboratory of Clinical Studies, DICBR, NIAAA and Clinical Psychobiology Branch, NIMH, Bethesda, MD 20205.

Syrian hamsters preferentially select ethanol over water in a free-choice situation, with female hamsters showing a lower ethanol preference than males. We have evaluated sex differences in both ethanol sensitivity and liver alcohol dehydrogenase activity to determine how these correlate with preference patterns. Adult male and female Syrian hamsters (n=6/group) were administered by IP injection one of four different doses of ethanol (10% w/v). Duration of loss of righting reflex (LRR) was measured. Recovery of righting reflex (RRR) was defined as the time at which the hamster was able to right itself twice in one minute. At RRR a blood sample was obtained by cardiac puncture for measurement of blood ethanol concentration (BEC). Determination of liver alcohol dehydrogenase activty was performed on samples from alcohol-naive male and female hamsters using an established assay. At the three highest dosages, males had a significantly longer duration of LRR. Although BEC upon recovery was higher in females at each dosage level, significance was achieved only at the 3.5 gm/kg dose.

Dose: (gm/kg)	Sex:	%LRR:	DURATION LRR: (MIN.)	BEC AT RRR: (MG./100 ML.)
4.0	F	100	60.43±19.0*	223.8
	M	100	145.2±74.0	196.6
3.5	F	66.6	45.45±12.4**	301.1 #
	M	83.3	151.38±75.5	285.5
3.0	F	66.6	23.1±9.0***	240.1
	M	100	70.76±10.0	221.5
2.7	F	33.3	12.2±4.2	265.1
	M	50	23.1±4.0	237.0

*p<.05, **p<.006, ***p<.0001 #p<.005

Liver alcohol dehydrogenase activity of female hamsters was approximately twice that of males and about three times that of the male Fisher rat. The results indicate that female Syrian hamsters are more resistant to the ataxia-producing effects of ethanol probably on the basis of more rapid metabolism. The BEC data upon recovery suggests that there may also be a differential CNS sensitivity between male and female hamsters. It appears that in this animal model, sex specific preference for ethanol correlates with greater sensitivity and less rapid metabolism.

85.2 CEREBRAL NEUROTRANSMITTER RECEPTORS IN RAT LINES SELECTED FOR ALCOHOL-RELATED BEHAVIORS. E.R. Korpi* (SPON: L. Sellin). Research Laboratories of the Finnish State Alcohol Company (Alko Ltd), SF-00101 Helsinki, Finland.

As a part of ongoing research on the neurochemical differences in the central nervous system between rat lines selectively bred for low and high voluntary alcohol consumption or sensitivity to ethanol-induced motor-impairment, the dopamine and opiate receptors of these lines have been studied. Naive male rats of about 250 g were used. After decapitations, the brains were quickly excised, striatae and olfactory tubercles dissected for dopamine D-2 receptor assay and forebrain hemispheres for delta-opiate receptor assay. The tissue pieces were stored at -70°C in plastic containers. The D-2 receptor numbers and affinities were determined with [^{3}H]spiperone as a ligand (20 to 800 pM), (-)-sulpiride (3 μM) defining the specific binding in the presence of physiological extracellular sodium concentration. The delta opiate receptors were assayed using [^{3}H]-D-Ala^2-D-Leu^5-enkephalin as a ligand (0.1 to 20 nM), unlabelled D-Ala^2-D-Leu^5-enkephalin (1 μM) defining the specific binding in the presence of morphiceptin (3 μM) to block mu-receptors in the absence of ions. The brain membranes were incubated with ligands for 15 min at 37°C, then filtered on glass fiber discs under vacuum, and washed three times with ice-cold incubation fluid. The spiperone binding displayed a single binding site in striatum and olfactory tubercle as deduced from straight Scatchard plots and Hill coefficients close to 1. No differences in D-2 receptor numbers or affinities were observed between the rat lines. In vitro, ethanol was also a poor inhibitor of D-2 receptor binding, causing only 20 % decrease of [^{3}H]spiperone (50 pM) binding at the concentration of 1000 mM. Analysis of delta-receptor binding revealed a single binding site in cerebral hemispheres with a K_D of about 4 nM and B_{max} of about 5 pmol/g original tissue weight. Our preliminary experiments on alcohol drinking (AA) and non-drinking (ANA) rat lines suggest that in the alcohol drinking rat line there are fewer delta-opiate binding sites with higher affinity towards the ligand than in the non-drinking line. Recently, ethanol has been shown to inhibit opiate binding in vitro, especially that by delta receptors, thus warranting further studies on the possible line difference in these receptors.

85.3 REGIONAL BRAIN SEROTONIN CONTENT IN ALCOHOL-PREFERRING AND -NON-PREFERRING RATS FROM THE N/Nih HETEROGENEOUS STOCK. J.M. Murphy, W.J. McBride, L. Lumeng* and T.-K. Li*, Dept. Psychiatry, Biochemistry and Medicine, Inst. Psychiatric Research, Indiana Univ. Sch. Medicine, Indianapolis, IN 46223.

Many studies have attempted to establish a relationship between brain serotonin (5-HT) and alcohol-drinking behavior, but findings have been equivocal. We have shown previously that rats of the selectively-bred alcohol-preferring P line exhibit lower levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral cortex, hippocampus, thalamus and hypothalamus when compared with the alcohol-nonpreferring NP line (Murphy et al., Pharmacol. Biochem. Behav. 16: 145, 1982). Moreover, 5-HT uptake inhibitors have been found to reliably decrease alcohol consumption in the P rats (Murphy et al., Alcohol, in press). The present investigation was designed to test the generality of this relationship by examining brain monoamine contents of rats from the N/Nih heterogeneous stock which differ in alcohol preference. The N/Nih rats exhibit a wide range of individual differences in alcohol-drinking behavior when tested with food, water and a 10% (v/v) ethanol solution freely available. After testing, ethanol was withdrawn from those selected for study for at least three weeks. Animals with high preference (HP; N=13; 6.8 ± 0.2 g ethanol/kg body wt/day) and low preference (LP; N=15; 0.2 ± 0.1 g/kg/day) were killed by near-freezing in liquid nitrogen. The brains were dissected at -20°C into the cerebral cortex, corpus striatum, hippocampus, thalamus, hypothalamus and midbrain. The brain regions were stored at -70°C until assayed for monoamine contents by HPLC with electrochemical detection. Compared with the LP rats, the HP animals had significantly lower contents of 5-HT in the thalamus (5.88 ± 0.17 vs. 6.39 ± 0.17 nmoles/g) and hypothalamus (7.20 ± 0.26 vs. 7.87 ± 0.18 nmoles/g). The HP rats also had significantly lower levels of 5-HIAA in the thalamus and hypothalamus, and had lower contents of dopamine (DA) and norepinephrine (NE) in the thalamus. No other regional brain differences between the HP and LP groups were observed for 5-HT, 5-HIAA, NE, DA, and the DA metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid. Taken together with the previously observed differences between the NP and P lines in the regional brain contents of 5-HT and 5-HIAA, the present findings with the N/Nih rats suggest a possible role for 5-HT neuronal systems of the hypothalamus and thalamus in the mediation of preference for alcohol. (Supported by AA 03243).

85.4 HUMAN CEREBROSPINAL FLUID CONTAINS Protein III, A SYNAPTIC VESICLE-ASSOCIATED PHOSPHOPROTEIN. M.D. Browning, E. Perdahl*, G. Sedvall*, and P. Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University. New York, N.Y. 10021.

Protein IIIa and Protein IIIb are two homologous phosphoproteins (collectively referred to as Protein III) that, together with synapsin Ia and synapsin Ib, constitute a family of synaptic vesicle-associated phosphoproteins. Protein III has been shown to be phosphorylated in response to electrical stimulation, potassium depolarization, or exogenous cAMP in intact nerve cell preparations. These and a variety of other data have led us to hypothesize that Protein III may play some role in modulating synaptic vesicle function.

In rat brain, Protein IIIa migrates as a 74,000 M_r band on SDS-gels while Protein IIIb migrates as a 55,000 M_r band. In a recent study, we demonstrated that Protein III was present in human brain. In this material, which was obtained at autopsy, three M_r variants in both Protein IIIa and Protein IIIb were detected in some of the autopsy samples examined. While one of the variants of Protein IIIa and Protein IIIb in human brain comigrated with the rat forms of these proteins, the two other variants of Protein IIIa and Protein IIIb migrated more slowly than the rat forms of Protein IIIa and Protein IIIb. Interestingly, although the more slowly migrating variants were rarely seen in control material (only 2 of 17 individuals), in material obtained at autopsy from alcoholic individuals who died sober (no detectable blood alcohol levels) 12 of 12 samples examined exhibited the more slowly migrating variant forms of Protein III. Moreover, in material obtained at autopsy from alcoholic individuals who died while intoxicated (blood alcohol levels >50mg/100 ml), the more slowly migrating variant forms of Protein III were seen in only 4 of 17 cases. A high incidence of the more slowly migrating Protein III variants was also detected in a number of individuals who had suffered from a variety of neuropathological conditions (e.g. Alzheimer's disease).

In an attempt to follow up on these observations in material that can be obtained from living subjects, we have examined human cerebrospinal fluid (CSF) for the presence of Protein III. We have determined by immunochemical and peptide mapping studies that both Protein IIIa and Protein IIIb are present in human CSF. A quantitative radioimmunochemical assay has been used to determine the amount of Protein III in the CSF of alcoholics. These studies have provided preliminary evidence that there is an increase in Protein III in the CSF of abstinent alcoholics compared to non-alcoholic controls. Work is currently under way to determine whether this increase in Protein III represents an increase in one or in more of the variant forms of this protein.

85.5 Differential Enhancement of Benzodiazepine Binding, by GABA, in Mice Selectively Bred for Sensitivity to Ethanol. R.J. Marley* and J.M. Wehner* (Spon: J.R. Sheppard). Inst. for Behav. Genetics and School of Pharm., Univ. of Colo., Boulder, CO. 80309.

Previous observations have indicated that CNS effects of ethanol may result from an interaction with the GABA-benzodiazepine (BZ) system. The exact nature of this interaction has not been determined, but it has been suggested that ethanol potentiates GABAergic functions at a site other than the GABA-BZ receptor complex (Greenberg, D.A., et al., J. Neurochem, 42:1062, 1984). We investigated both the binding of ^{3}H-flunitrazepam (FNZ) and the allosteric enhancement of FNZ binding by GABA among females from two lines of mice, short-sleep (SS) and long-sleep (LS), selectively bred for sensitivity to ethanol. Each experiment represents from three to five assays conducted at 37°C. The allosteric enhancement of ^{3}H-FNZ binding (1_3 - 3 nM), by increasing concentrations of GABA (10^{-7}M - 10^{-3}M), was investigated initially in extensively washed membrane preparations from whole brain. Significant differences in GABA's enhancement of FNZ binding were observed between LS and SS mice at all concentrations of GABA employed in the study. The binding of ^{3}H-FNZ was enhanced to a greater degree in SS mice than in LS mice. Similar differences in GABA enhancement were also observed in crude membrane preparations of whole brain from these two lines of mice, however, the effect was much more robust in well-washed preparations. To further investigate this phenomenon, brains were dissected into seven major regions (cortex, midbrain, hindbrain, cerebellum, hippocampus, hypothalamus, and striatum) and the specific binding of ^{3}H-FNZ in the presence and absence of 10^{-5}M GABA determined following extensive washing of the membrane preparations. FNZ binding was enhanced, by GABA, to a greater degree in SS mice, than in LS mice, in cerebellum, hypothalamus, and striatum. No difference was observed in the other four regions, with the possible exception of cortex where a small difference was observed. No differences were observed in either ^{3}H-FNZ binding affinity (11.3 nM for SS and 10.5 nM for LS) or binding capacity (1.08 and 1.25 pmol/mg protein for SS and LS, respectively) when saturation assays were conducted on membranes prepared from whole brain. There were no differences observed when single-point binding assays were conducted on the various brain regions using a concentration of approximately 1.5 nM ^{3}H-FNZ. In these binding assays, the various membrane preparations were washed in the same manner as in the enhancement studies. These results suggest that the differences in ethanol sensitivity observed for these two lines of mice may be related to differences in some factor responsible for modulation of the GABA-BZ receptor complex. (Supported, by AA-03527 and an NSF Graduate Fellowship).

85.6 NEUROCHEMICAL EVIDENCE FOR THE DEVELOPMENT OF NEURONAL TOLERANCE IN THE ALCOHOL-PREFERRING P LINE OF RATS. W.J. McBride, J.M. Murphy, M.B. Waller, L. Lumeng* and T.-K. Li*, Depts. Psych. & Med., Inst. Psychiatric Res. and Regenstrief Institute, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

The objective of the present study was to provide neurochemical evidence for the development of neuronal tolerance after chronic alcohol treatment. Adult, male rats of the alcohol-preferring P line were divided into 4 groups (N=6 each). Groups A and B were maintained on H_2O and normal rat chow ad lib. Group C was given a liquid diet containing 5% ethanol and group D received a control liquid diet without ethanol. As measured by a jumping-platform test (Waller et al., Pharmacol. Biochem. Behavior 19: 683, 1983), P rats in group C demonstrated behavioral tolerance to an i.p. injection of 2.5 g ethanol/kg after 8 days of chronic ethanol consumption (10 ± 1 g ethanol/kg/day). Rats in group D, which were given the liquid diet without ethanol, did not show this behavioral tolerance to i.p. ethanol. Groups A and B were not tested and hence remained ethanol naive until the day of killing. One week after the observed development of behavioral tolerance by group C, all 4 groups were killed by the near-freezing technique. Prior to killing, group A received i.p. saline while groups B, C and D received 2.5 g ethanol/kg. One hour after the injection of either saline or ethanol, the rats were killed and the frontal cortex (FCTX) and nucleus accumbens (ACC) were dissected at -20°C. These two limbic regions were assayed by HPLC for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). There were no differences in the levels of DA, DOPAC and HVA in the FCTX and of DA in the ACC of the ethanol injected groups (B, C and D) in comparison to the values for the saline injected group (A). However, in the ACC, the contents of the DA metabolites were higher in groups B (6.6 ± 0.2 nmol/g for DOPAC; 3.0 ± 0.1 for HVA) and D (6.6 ± 0.3 for DOPAC; 2.9 ± 0.1 for HVA) than were found in the saline-injected group (5.6 ± 0.4 for DOPAC; 2.1 ± 0.2 for HVA). Similarly, the levels of 5-HIAA in the ACC were greater in groups B (4.36 ± 0.05 nmol/g) and D (4.36 ± 0.12) than in A (3.65 ± 0.07). Higher levels of 5-HIAA were also observed in the FCTX of groups B (2.60 ± 0.09) and D (2.90 ± 0.11) than in A (2.36 ± 0.06). On the other hand, the ethanol-tolerant rats (group C) did not demonstrate increased levels of DOPAC (5.6 ± 0.4), HVA (2.2 ± 0.1) and 5-HIAA (3.55 ± 0.13) in the ACC or of 5-HIAA (2.50 ± 0.12) in the FCTX following i.p. ethanol. The results suggest that ethanol-tolerant P rats do not respond to an i.p. dose of ethanol with increased release and/or metabolism of DA and 5-HT in the ACC and of 5-HT in the FCTX as do the nontolerant groups. These specific regional DA and 5-HT systems may represent neuronal substrates for the demonstrated behavioral tolerance to ethanol in the P rats. (AA-03243)

85.7 Synaptosomal Phospholipid Acyl Composition of Mouse Strains Differing in Ethanol Sensitivities. L. Lee and T. L. Smith. Veterans Administration Medical Center, Tucson, AZ 85723 and the Dept. of Pharmacology, University of Arizona Medical Center, Tucson, AZ 85724.

Through studies with inbred rodents, it is evident that initial neurosensitivity to ethanol is under strong genetic influence. Yet, the neurochemical basis for differential neurosensitivity to ethanol among various mouse strains is poorly understood. For example, synaptic cholesterol/phospholipid ratios observed in different mouse strains are not correlated with behavioral sensitivity to ethanol (Smith, J. Pharmacol. Exp. Ther. 232:702, 1985). It is known, however, that an increase in fatty acyl saturation results in an increase in membrane microviscosity which, in turn, has been related to alterations in tissue sensitivity to ethanol. Thus, it was of interest to determine and compare fatty acid composition of synaptosomes from cerebral cortex and cerebellar tissues of RIIIS and AKR/J mouse strains which differ in their initial behavioral sensitivity to ethanol. Mice from both strains were injected with ethanol (I.P., 4.0g/kg body wt.) and blood alcohol conc. (B.A.C.) upon awakening was determined. In a separate group of animals which were not previously treated with ethanol, synaptosomes were prepared and the fatty acyl composition determined. RIIIS and AKR/J mice had B.A.C.'s (mg %) of 306±20 and 197±35, respectively. Fatty acyl compositions of RIIIS and AKR/J cerebral cortical synaptosomes were essentially identical. Similarly, synaptosomes from cerebellum which is more directly associated with ethanol-induced ataxia were also analyzed. The fatty acid compositions of cerebellar tissues from these two mouse strains were also identical. Interestingly, in both mouse strains significant differences in the % composition of 16:1 were observed when comparing fatty acid profiles of cortex with that of cerebellum. It is concluded that the synaptosomal fatty acyl composition is not a determinant for differences in initial neurosensitivity to ethanol observed in RIIIS and AKR/J mice. Supported by a medical research grant from the Veterans Administration.

85.8 GENE FREQUENCY FOR A BRAIN POLYPEPTIDE VARIANT DIFFERS BETWEEN SELECTIVELY-BRED MOUSE LINES PRONE (WSP) AND RESISTANT (WSR) TO ETHANOL WITHDRAWAL SEIZURES. D. Goldman* and J.C. Crabbe. (M. Meikle, Spon.) Lab. Clin. Stud., NIAAA, Bethesda, MD 20205 and Ore. Hlth. Sci. Univ. and VA Med. Ctr., Portland, OR 97201.

A substantial proportion of individual differences in ethanol withdrawal severity are genetically determined in mice. For example, inbred strains of mice differ widely in the severity of ethanol withdrawal after a standard chronic treatment regimen. One of us (JCC) has selectively bred two mouse lines prone (withdrawal seizure prone: WSP) to withdrawal seizures following cessation of chronic ethanol inhalation. Two lines have been bred for ethanol withdrawal seizure resistance (WSR) and two non-selected control lines (WSC) are maintained. The WSP lines exhibit 10-20 fold more severe withdrawal seizures than the WSR lines after identical chronic ethanol exposure. Little inbreeding irrelevant to the selected characters has occurred in these lines, so any fixed genetic differences should be markers or determinants of the selected trait.

Whole brain homogenates from eight mice from each line were typed by two dimensional gel electrophoresis at Generation S_{13} for eight brain polypeptides which show genetic variation between inbred strains of mice. One variant, a highly abundant, 71 kilodalton pI 5.4 polypeptide, was genetically fixed in both WSR lines (0/16 individuals exhibited the basic allele). Allelic frequencies in the two WSP lines were .125 and .21, and in the WSC lines .08 and .06. The differences in allele frequency among the lines were significant ($p < .05$) by chi square and probability analysis.

This variant brain polypeptide may be a genetic marker for ethanol withdrawal severity, and/or may be a functional determinant of withdrawal.

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85.9 NEONATAL MSG LESIONS REDUCE NEUROSENSITIVITY TO ETHANOL-INDUCED HYPOTHERMIA IN MICE. J.C. Crabbe, A. Kosobud*, B.R. Tam*, E.R. Young*, L.D. Keith*, J.D. McSwigan*, and D.M. Dorsa. Ore. Hlth. Sci. Univ. and VA Med. Ctr., Portland, OR 97201, and Univ. Wash. and GRECC, VA Med. Ctr., Seattle, WA 98108 (DMD).

A number of studies have indicated a relationship between brain peptide activity and sensitivity to the behavioral effects of ethanol. Specifically, it has been suggested that ethanol effects are mediated by changes in the endogenous opioid peptides derived from the proopiomelanocortin (POMC) precursor. Most cell bodies containing brain POMC-derived peptides are found in the arcuate nucleus of the hypothalamus. Neonatal administration of monosodium glutamate (MSG) has been reported to destroy cell bodies of the arcuate nucleus. We treated WSC strain mice on postnatal Day 4 with a single sc injection of 4 mg/g MSG or saline. When adult, mice were challenged with 3.5 g/kg ip ethanol and reduction in body temperature was assessed 45 min later. Blood ethanol concentration (BEC) was measured and the hypothalamic content of ß-endorphin like immunoreactivity (ß-EP) was determined by radioimmunoassay.

ß-EP was reduced 79 percent in females and 50 percent in males by MSG treatment. MSG-treated animals of both sexes showed significantly less ethanol-induced hypothermia than controls. BEC was higher in MSG-treated animals of both sexes than in controls, so the differences were not due to ethanol pharmacokinetics. ß-EP was generally lower in males.

These data suggest that ß-EP cell bodies in the arcuate nucleus of the hypothalamus mediate neurosensitivity to ethanol-induced hypothermia in mice.

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85.10 REPRODUCTIVE BEHAVIOR OF FEMALE RATS TREATED POSTNATAL DAYS 1-8 OR 8-14 WITH ETHANOL. T.B. Sonderegger, B. Mesloh,* A. J. Ritchie,* A. Tharp,* and M. Spencer,* Dept. of Psychol., Univ. of Nebraska-Lincoln, Lincoln, NE 68588-0308.

The studies reported here examine the effects of known quantities of ethanol administered during postnatal weeks 1 or 2 upon the reproductive behavior of female rats. In study one, ethanol in a 30% Sustagen (Mead Johnson) vehicle was administered twice daily on postnatal days 1-7 (early) or 8-14 (late) using an intragastric intubation procedure designed to counteract the effects of underfeeding (Sonderegger et al., Neurobehavior. Tox. and Teratol. 4:477, 1982). Doses were tapered to reach a maximum of 4 g/kg on treatment day 4. In study one, 25 litters of Charles Rivers CD albino rats (10 pups per litter) were used in a split-litter design. On day 1 after birth pups were assigned to a treatment group: ethanol (E), Sustagen (S), pair-underfed (P), or handled (H). S pups received comparable volumes of isocaloric (sucrose) vehicle. Unhandled (U) animals were obtained from additional litters.

Body weights of all animals were comparable on day 21 and thereafter. Animals were weaned and housed in same-sex pairs with ad libitum food and water.

On day 90, females were bred in groups of 3 (E, S, and P littermates) with one proven sire; 42 females from early and late groups. If pregnancy did not occur within two weeks, subsequent two week trials with other proven sires were given. Ratios of animals that became pregnant for early and late groups, respectively, were E = 3/8, S = 5/6, P = 2/6; E = 3/10, S = 5/6, P = 5/6. Estrous cycles of all animals appeared to be typical. However, the nonfertile females when in estrous, did not exhibit lordosis in a standard mating test. Litter sizes and sex ratios of pups of females that became pregnant did not differ among treatment groups.

In study two, the procedure was repeated using animals from 14 litters. All treatment groups had comparable body weights on day 21. Findings from this study will also be described.

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85.11 PRENATAL PHENOBARBITAL EXPOSURE ALTERS THE DEVELOPMENT OF BEHAVIORAL TOLERANCE IN ADULT RATS. C. Stamper, D. Braget* and J. Diaz. Dept. of Psychology, University of Washington, Seattle, WA 98195.

Prenatal exposure to phenobarbital has been shown to have long-term effects on hormonal systems, CNS morphology, drug metabolism and behavior. The purpose of the present study was to investigate the effect of prenatal exposure to phenobarbital on the development of behavioral tolerance to barbiturates in adulthood. Behavioral tolerance has been described as a decrease of the behavioral effects of a drug after it has been administered for a number of times.

Phenobarbital was administered to pregnant dams in the drinking water from days 9-18 of gestation. The mean serum phenobarbital level on the last day of drug administration was 101.6+26 ug/ml. Beginning on postnatal day 180, the offspring were trained on an automatic treadmill device which assessed the animal's motor performance. Following the training period, the animals were tested for performance on the treadmill after the administration of pentobarbital (10 mg/kg) for a period of five days.

There were no significant differences in the preweanling growth of the offspring. On day 90, the control group offspring were significantly heavier than the drug group offspring ($T=2.23, p<.05$). There were no significant differences between the groups in the learning of the treadmill motor task. There was a significant group effect ($F(1,4)=5.9$, $p<.05$) and a significant time effect ($F(1,4)=21.7$, $p<.001$) for the development of tolerance segment of the study. The following table summarizes the results:

	Seconds of Error on the Treadmill				
	day 1	day 2	day 3	day 4	day 5
Control	53.2	39.4	39.2	34.7	17.1
Barb	48.4	36.6	21.0	7.8	3.4

These data indicate that there were significant differences in the development of behavioral tolerance to pentobarbital between animals exposed to phenobarbital in utero and control group animals. There is a great deal of variation in the response of individuals to various drugs, which is not explained by gender, size or age. Perhaps early (even prenatal) exposure to certain drugs is responsible, in part, for determining the response to a variety of drugs throughout life.

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85.12 THE EFFECTS OF THE IN UTERO ADMINISTRATION OF ALCOHOL ON GAMMA GLUTAMYL TRANSPEPTIDASE FROM RAT HIPPOCAMPUS. E. Reyes, D. Sandoval*, K. Garcia* and J. Wolfe*. Department of Pharmacology School of Medicine The University of New Mexico, Albuquerque, NM 87131.

The membrane bound enzyme, gamma glutamyl transpeptidase (GGTP), has been shown to be elevated by alcohol treatment in brains of adult rats. Several different brain areas have been shown to be significantly higher in GGTP activity following alcohol treatment (Reyes, 1978,85). Recently we have shown that the in utero administration of alcohol produces an elevation of GGTP in specific brain areas. It was the intent of the present study to determine if the increase in GGTP activity observed in Fetal Alcohol Syndrome (FAS) rats is due to an alteration in the isoenzyme distribution or to alterations in their kinetic properties. Animals from alcohol treated mothers and from pair-fed control mothers were sacrificed by decapitation at 30 days of age. The brains were immediately excised and dissected according to the method described by Glowinski & Iverson (1966) and homogenized in 0.01M Tris buffered saline to yield a 20%(w/v) suspension. GGTP from the hippocampus was purified according to the method described by Reyes & Barela (1980). In the hippocampal area the total GGTP activity for the FAS group was found to be 1.66 units and that for the control group was 0.866 units. The 30-70% ammonium sulfate enzyme preparations were applied to a DEAE cellulose column and eluted with a linear NaCl gradient. Two distinct peaks of GGTP activity (I,II) were produced from the FAS enzyme preparation while only one peak (I) was found for the control. Fractionation of peak I from the FAS and control groups on a concanavalin A-sephrose 4B affinity column showed that the peak I was composed of the same 3 isoenzyme groups. Peak II from the FAS group was also fractionated into isoenzymes with varying affinities for concanavalin A. Approximately 7 times more enzyme was recovered from the FAS preparation than from the control preparation. The isoenzymes were characterized with respect to molecular weights and Michaelis constants for the substrate γ-glutamyl-p-nitroanilide. This work was supported by MBRS program grant #081-39 and New Mexico Child Health Care 73/665.45.32/022.

85.13 BEHAVIORAL EFFECTS OF PRENATAL ETHANOL IN RAT PUPS. C.S. Sebastian*, M. Herkert* and S.I. Dworkin (SPON: G. Trapp). Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Sebastian, et al (Federation Proceedings, 43:286, 1984) found no differences in performance between prenatally ethanol or dextrose exposed rat pups in a radial arm maze. This was attributed to factors including a low dose (5 mg/kg), pups' age when tested (70 days), an insensitive test and the direct effect of intubation. However, an additional study using a higher dose (7 mg/kg) and younger pups (25 days) also failed to show differences. Therefore, a behavioral procedure that was more sensitive to the effects of prenatal-drug exposure was evaluated. An interresponse time schedule of liquid food presentation was used to study the effects of prenatal ethanol administration. This schedule requires the animals to pause a specified period of time between each response and has been used to assess the effects of many pharmacological agents. Three groups of pregnant rats were studied. One group (N=3) received ethanol (7 mg/kg) and the second group was given (N=3) isocaloric dextrose by gavage during third week of gestation. The third group (N=2) was untreated.

The offspring of ethanol-treated (N=16) and untreated (N=11) rats were studied. Sessions were run Monday-Friday and were terminated after 15 minutes. A nose-poke response was used and the reinforcer consisted of liquid milk. Response-independent food presentations were presented an average of every 3 min. Furthermore, each response that was spaced one sec after the previous response resulted in additional food presentations. The response-indpendent schedule was first eliminated and then the IRT value was raised to 5 sec during additional sessions. The pups exposed to ethanol had higher response rates and received fewer food deliveries during all sessions.

These pups were tested at a younger age and a behavioral baseline that was more sensitive than in prior experiments. They showed constant behavioral differences from untreated pups. While the effect of stress from intubations to these findings could not be assessed in this study, it is noteworthy that the rats were dosed with ethanol only during the third week of gestation instead of the entire gestation as in previous experiments, thus reducing the stress considerably.

85.14 PRENATAL ALCOHOL EXPOSURE DOES NOT INFLUENCE SEXUAL DIMORPHISM OF SACCHARIN PREFERENCE IN C57 MICE. H.C. Becker, C.L. Randall and R.F. Anton. VA Medical Center and Medical University of SC, Charleston, SC 29403.

Maternal alcohol consumption during pregnancy has been shown to produce a number of physical and behavioral anomalies in human and animal offspring (e.g., Abel, 1984; Randall et al., 1982). Most recently, McGivern et al. (1984) reported that prenatal exposure to alcohol in rats eliminated the expression of gender differences in behaviors that are typically sexually dimorphic in nature (e.g., saccharin preference, maze learning). The purpose of this study was to investigate whether prenatal alcohol exposure influences the sexual dimorphic nature of saccharin preference in the mouse. We have previously found C57 mice to be susceptible to both the morphological as well as behavioral teratogenic properties of ethanol (Randall and Taylor, 1979; Middaugh and Randall, 1985).

Pregnant C57 mice were fed liquid diets containing either 25% or 0% ethanol-derived calories (EDC) from Gestation Day 6-18. The 0% EDC group was pair-fed to the 25% EDC animals. A third control group received lab chow ad libitum throughout gestation. Parturition was noted and litters were culled to six of equal sex, when possible. Offspring were weaned at 25 days and then left undisturbed.

At 50 days of age, one male and one female offspring from each of the three prenatal treatment groups was given two bottles containing either water or 0.1% saccharin. After four days, the saccharin concentration was increased to 0.2% for four additional days. Food was freely available for the duration of the preference test. Mice were weighed every other day and the bottles were read daily, at which time position of the tubes was alternated.

Results indicated that relative saccharin intake (ml per 100 g body weight) was reliably greater for females than males for both concentrations of saccharin ($F(1,74)=22.35$, $p< 0.01$). This main effect of sex, however, was not influenced by prenatal treatment (sex x treatment interaction, $p< 0.1$). Thus, contrary to findings in the rat, prenatal exposure to alcohol does not appear to influence the sexual dimorphism of saccharin preference in C57 mice. Whether this discrepancy is related to differences in species or some other methodological issue remains to be determined.

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85.15 PRENATAL ALCOHOL EXPOSURE FACILITATES FEMININE SEXUAL RESPONSIVITY WITHOUT DISRUPTING MASCULINE SEXUAL BEHAVIOR IN MALE RATS, A. Lumia[1]*, P. Kleber*, S. Vanderwoude*, J. Flynn*, and J. Broida* (SPON: B.Sachs). Psychology Department, Skidmore College, Saratoga Springs, NY 12866.

Perinatal exposure to testosterone is important for display of male sexual behavior. its absence increases the potential for the display of feminine behavior. Prenatal exposure to alcohol, which inhibits release of testosterone, should have a deleterious effect upon the organization of masculine copulatory behavior. We examined the effects of prenatal exposure to alcohol on expression of both male and female sexual responsivity in adulthood.

Pregnant female rats were given a liquid diet containing 36, 18 or 0 percent alcohol derived calories on days 5 through 20 of gestation. Additional females received standard laboratory chow. Litters were culled to 8 pups and cross-fostered to a separate group of non-alcohol exposed dams on the day of birth, and male offspring were castrated 3 days later.

At 60-80 days of age, males were screened for mounting in response to sexually receptive females prior to testosterone replacement therapy. These males were then given daily injections of 500ug testosterone proprionate and paired with sexually receptive females every other day until they mounted.

Additional males were given two injections of estrogen (6ug), 48 and 24 hours prior to exposure to a sexually vigorous male. These animals also received a single injection of progesterone (500ug), 4 hours prior to pairing, when their lordotic behavior was assessed. Lordosis was assessed twice, the second test one week after the first.

Prenatal exposure to alcohol did not alter on latency to mount, However, animals whose mothers received 36% ethanol during pregnancy had higher LQs (75) than did those whose mothers received 18% or 0% ethanol or lab chow controls (39, 20, and 12, respectively), Differences in body weight did not account for this differential sensitivity to estrogen and progesterone. Thus prenatal exposure to alcohol was found to disrupt defeminization without affecting masculinization.

[1]Supported by a Skidmore Faculty Development Grant and a grant from the Gerald Freed Foundation to ARL.

86.1 EXCITATORY EFFECT OF ETHANOL ON MOTOR ACTIVITY AND ROTATION MEASURES IN MICE. P.B. Silverman and H.L. Altshuler. Neuropsychopharmacology Section, Texas Research Institute of Mental Sciences and Neuroscience Program, University of Texas Graduate School of Biomedical Sciences, Houston, Texas 77030 USA

The long term objective of the work begun here is to gain understanding of the mechanisms involved in the excitatory effect of ethanol. The euphoriant, excitatory effect of a drug is generally considered an important indication of abuse liability. The excitatory effect of ethanol, especially after repeated administration, is a relatively unexamined phenomenon despite its probable role in alcohol abuse. The work presented here was intended to examine the excitatory effect of ethanol as a function of the schedule and context of administration and to assess the effects of acute and chronic ethanol in a behavioral model of dopamine function.

The specific aims were to determine if tolerance or sensitization to the locomotor stimulant effect of ethanol in mice develops upon repeated administration, to determine the contribution of intertreatment interval and context (setting) on any such changes in the locomotor stimulation induced by successive ethanol treatments, to determine if changes in ethanol-induced stimulation can be attributed to changes in blood and brain levels of ethanol in a well established whole animal model of central dopamine function, i.e, the unilaterally 6-hydroxydopamine-lesioned mouse rotation model.

Results to date show a dose dependent increment in locomotor activity in DBA and Swiss-Webster mice in response to 1 to 3 g/kg ethanol. No striking change in locomotor response to repeated ethanol treatments in this dose range has been found in either strain of mice despite testing with a number of different intertreatment intervals. This lack of demonstrable tolerance or sensitization may be the result of only a limited number of treatments (5 or 6) having been given. More treatments than were initially anticipated may be required to demonstrate a change in locomotor response.

In the rotation experiments, mice lesioned by intrastriatal administration of 6-hydroxydopamine were screened for rotation in response to the dopamine agonists, apomorphine and (+)-amphetamine, which resulted in contralateral and ipsilateral rotation, respectively. They were then tested for rotational response to ethanol. Surprisingly, ethanol induced contralaterally directed rotation in a dose responsive manner up to 3 g/kg. In our initial studies, this effect of ethanol was significantly antagonized by 10 mg/kg naloxone in Swiss-Webster mice.

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86.2 FACTORS INFLUENCING DEPRESSANT-INDUCED NARCOSIS IN THE SHORT-SLEEP AND LONG-SLEEP MICE. T.D. McIntyre and H.P. Alpern. Behavioral Neuroscience Program, Dept. of Psychology, University of Colorado, Boulder, CO 80309.

We have argued that mechanisms responsible for sleep time differences in mice selectively-bred for short and long ethanol-induced sleep times (SS/LS) are not alcohol specific. Evidence indicates that differences between these lines are due to differences in more general CNS mechanisms (McIntyre and Alpern, 1985), because, LS are more sensitive to soporific effects of ethanol, t-butanol, methanol, enflurance, isoflurance, paraldehyde, chloral hydrate, trichloroethanol, gamma-butyrolactone, chlordiazepoxide, adenosine, pentobarbital, barbital, phenobarbital and thiopental. Under certain conditions, however, SS appear to be more sensitive to pentobarbital. To explain this discrepancy we examined the experimental designs of reports dealing with pentobarbital-induced narcosis, and believe that factors such as dose, sex, age, and periodicity may have significantly impacted the results. In only one of the five reports which fail to find LS more sensitive to pentobarbital is more than one dose employed. Further, all mixed both sexes, used animals at different ages and tested at different times. Thus, our experiments examined the effects of each of these variables on depressant-induced narcosis.

In the first study several doses of pentobarbital were given to both sexes of LS and SS at three different times. The results are complex in that there were interactions among all variables. In general, LS were affected by time of day and SS were not, and at 2400h line differences disappeared. Further, females tended to sleep longer than males, although female scores fluctuated over time of day. To show that these variables are important for other depressants, thiopental was given to both sexes of the lines at three ages. Again, there were interactions among all variables. Males of both lines gave stable results at all ages, but females displayed variability over the age factor. In Experiment 3 chlordiazepoxide was given to both sexes of the lines at three ages. LS animals regardless of sex or age had longer sleep times than SS. An age effect was not apparent for SS, but young SS had significantly lower sleep times than older SS.

Clearly, interpretation differences may arise when experimental variables are not adequately controlled.

86.3 ROLE OF PAVLOVIAN CONDITIONING IN THE DEVELOPMENT OF TOLERANCE AND CROSS-TOLERANCE TO THE HYPOTHERMIC EFFECT OF ETHANOL AND HYDRALAZINE. A.D. Lê*, J.M. Khanna and H. Kalant*. Dept. of Pharmacology, University of Toronto, and Addiction Research Foundation of Ontario, Toronto, Canada M5S 2S1.

The development of tolerance to the hypothermic effect of ethanol and of cross-tolerance to hydralazine, was investigated. In the first study, the influence of Pavlovian conditioning on tolerance was examined. Two groups of rats (n = 12) were treated on alternate days with 2 or 4 g/kg of ethanol (i.p.) in a room made distinctive (DR) by dim light and static noise from the radio. On the non-alcohol days, they were injected with saline in their home room (HR). A control group (n = 12) received saline in both environments. Tolerance to the hypothermic effect of ethanol (2 g/kg i.p.) was demonstrable in the DR in both the 2 and 4 g/kg treatment groups. Tolerance, however, was observed in the HR only in the 4 g/kg treated group. Cross-tolerance to the hypothermic effect of hydralazine (5 mg/kg i.p.) was observed for both ethanol treated groups in the DR but not in HR. In the second study, ethanol treatment was carried out by daily intubation with 6 g/kg of ethanol in the home cage. Tolerance to ethanol-induced hypothermia was demonstrated either in the home cage or in a novel environment. This treatment, however, failed to confer cross-tolerance to the hypothermic effect of hydralazine.

The results suggest that when high ethanol treatment dosage was employed, tolerance could be manifested in the absence of classical conditioning. Conditioning, however, plays a predominant role in the tolerance produced by low ethanol treatment dosage. The data also suggest that conditioning might be a separate component in tolerance development, which is of special importance for "effect-specific" rather than "drug-specific" tolerance.

86.4 SEASONAL VARIATION OF THE EFFECTS OF ALCOHOL ON AGGRESSIVE BEHAVIOR AND GONADAL HORMONES IN HIGH-STATUS SQUIRREL MONKEYS. J.T. Winslow*, K.A. Miczek, and J. Ellingboe. Dept. of Psychology, Tufts U., Medford, Ma 02155 and Alcohol and Drug Abuse Research Ctr., McClean Hosp.-Harvard Med.Sch., Belmont, Ma 02178.

Gonadal hormones such as testosterone (T) and luteinizing hormone (LH) may mediate the effects of alcohol on primate social behavior. We invesigated the relationship between endocrine processes, sensitivity to alcohol, social behavior within groups of squirrel monkeys. Consistent behavioral differences between dominant and subordinate monkeys were quantitatively measured using computer assisted, continuous measurement techniques. Dominant monkeys exhibited higher frequencies of aggressive behavior than subordinate monkeys, but the amount of time allocated to social behavior accounted for less than 10% of an individuals behavior. In contrast, social behavior was a significant determinant of general behavioral comportment and endocrine activity. Blood samples collected during breeding season revealed 267 ng/ml of T in dominant monkeys and 57.9 ng/ml T in subordinate monkeys. We previously determined that alcohol produced biphasic changes in the aggressive behavior of dominant but not subordinate monkeys, with low doses (0.1, 0.3, 0.6 g/kg) increasing the the frequency of threats, grasps and displacements and a higher dose (1.0 g/kg) decreasing these behaviors in dominant monkeys. Subordinate monkeys were not affected at any dose. We have studied the effects of alcohol on blood levels of T, LH and concurrent behavioral changes. Preliminary data suggest that changes in aggressive behavior produced by alcohol may be related to corresponding changes in T, however both of these effects are dependent on seasonal variations of endocrine activity. We are studying seasonal changes in T and LH, and sensitivity to alcohol in an effort to clarify how status-dependent drug effects are related to blood levels of gonadal hormones.

86.5 RENTENTION OF LEARNED ALCOHOL AVERSIONS FOLLOWING GUSTATORY NEOCORTEX AND OLFACTORY BULB ABLATIONS. C. W. Metzler*, S. W. Kiefer and G. J. Lawrence. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506.

Ablation of the gustatory neocortex (GN) in rats disrupts retention of learned aversions utilizing basic tastes (E.g., sucrose) but does not disrupt the retention of learned alcohol aversions. It was suggested that alcohol is a complex stimulus and that olfactory cues may mediate the normal aversion retention in GN rats. The present experiment tested the effect of gustatory neocortex ablations as well as olfactory bulbectomies on the retention of learned alcohol aversions.

Fifty one naive rats were placed on a schedule of restricted fluid access. All rats were trained to avoid both a 5% alcohol solution and a 0.1 M sucrose solution using LiCl illness (3% bw of 0.15 M LiCl). Following training, animals were divided into four surgery groups: normal controls (NC, N=15), GN ablations GN, N=15), olfactory bulbectomy (OB, N=10), and a combination GN and OB ablation (GNOB, N=11). As much as possible, rats were grouped according to training scores (amount consumed) and order of aversion training. Three weeks of post-operative recovery were followed by a return to the restricted fluid schedule. Tests for aversion retention were then done; sucrose and alcohol were presented in alternate test trials, each separated by two days of water access.

All animals acquired aversions to both sucrose and alcohol solutions in one or two trials. Postoperatively, the animals with GN lesions and GNOB lesions consumed more sucrose solution than animals with NC or OB lesions ($p<.01$). The means (and SEMs) for the sucrose solution consumption (ml) were: GN=13.8 ($\pm$1.7), GNOB=15.3 ($\pm$2.1), OB=2.6 ($\pm$1.5), NC=0.1 ($\pm$.1). These data indicate that the GN ablations were complete and included the critical gustatory area. Although rats lacking GN failed to retain the sucrose aversion, these rats displayed normal retention of the alcohol aversion. In contrast, the olfactory bulbectomies disrupted alcohol aversion retention. Groups OB and GNOB consumed significantly more alcohol solution than the NC and GN groups ($p<.01$: GNOB=9.3 ($\pm$1.5), OB=8.8 ($\pm$2.3), GN1.4 ($\pm$.6), NC=0.0 ($\pm$0.0).

The results indicate that alcohol aversion retention is not disrupted by GN lesion but is disrupted by olfactory bulbectomy. This suggests that odor is a potent cue in learned alcohol aversions and that the odor can mediate the aversion when the salience of the taste is degraded by GN ablation.

(Supported by Grant NIAAA 05898)

86.6 **STUDIES OF STRESS-INDUCED FREE CHOICE CONSUMPTION OF ETHANOL BY RATS. I. A POSSIBLE ROLE FOR THE HYPOTHALAMUS-PITUITARY-ADRENOCORTICAL AXIS.** **J. F. Nash, Jr.# and R. P. Maickel. Dept. of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.**

Previous experiments from this laboratory (Nash and Maickel, Fed. Proc. 43: 782, 1984) have demonstrated the induction of ethanol consuming behavior in adult, male Sprague-Dawley rats exposed to stressful stimuli (immobilization or isolation) on an unpredictable/irregular schedule for two weeks. The animals had continuous access, in a choice situation to two consummatory fluids: 0.1% aqueous (w/v) saccharin and 10% (v/v) ethanol in 0.1% aqueous (w/v) saccharin. Twenty-four hour consummatory volumes were recorded. In an attempt to assess the role of the hypothalamus-pituitary-adrenocortical axis in this consummatory behavior, two sets of experiments have been performed. In one set, repetitive doses of crystalline ACTH were administered intravenously over a two week period at irregular intervals corresponding to the times of immobilization or isolation stress previously used. The dosages used were 20-80 mU/rat; these provided plasma corticosterone response patterns similar to those seen with 1-4 hours of stressful stimuli (Smith, et al., J. Pharmacol. Exptl. Therap. 139: 140, 1963). The effects of this treatment were reflected in alterations in the consummatory behavior of the rats during and following the treatment with ACTH. In a second set of experiments, sustained release pellets containing dexamethasone (15 mg, release rate 0.71 mg/24 hours; 5 mg, release rate 0.24 mg/24 hours) were implanted, subcutaneously in the dorsal neck region, 7 days prior to the start of the two week period of immobilization or isolation on the irregular/unpredictable schedule. Animals implanted with the high dose of dexamethasone were sensitive to the stress; virtually all died in 7-10 days, with increased consumption of saccharin and markedly decreased body weight. The consumption of ethanol by these animals was minimal. The results suggest that adrenocoortical function may play a role in stress-induced consumption of ethanol (Supported in part by USPHS grant GM-07095 and by a grant from Innovative Research of America).

86.7 ACUTE TOXICITY OF COMBINATIONS OF ETHANOL AND FLURAZEPAM. R.J. Reiffenstein and W.Y. Hu* (SPON: G.B. Baker), Department of Pharmacology, Faculty of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Concurrent consumption of ethanol is said to greatly increase the effects of benzodiazepines and vice versa, but the severity of this interaction in humans or animals is really unknown. We assessed the acute interaction between flurazepam and ethanol in male ICR (outbred) albino mice.

The drugs were given i.p. either alone, or in combination, to groups of 6-24 mice per dose combination. Endpoints observed were death (at 18 h), and acute anesthesia, loss of righting reflex (motor coordination), heavy sedation (hypnosis) and light sedation. Probit analysis was used to determine ED_{50}'s for each of these endpoints for each drug alone and in combination with constant doses of the other drug, and these and their 95% confidence limits were plotted as 50%-response isobolograms (Gessner, P.K. & Cabana, B.E., J. Pharmac. exp. Ther., 174: 247, 1970).

Lethality of either compound was not significantly affected by the other (except near the LD_{50} of the other). The drugs were also less than additive for anesthesia. Loss of righting reflex was additive for ethanol up to 1.5 g/Kg, however there was mutual potentiation at higher doses of ethanol. The drugs were also synergistic for heavy sedation above 0.8 g/Kg of ethanol, and for light sedation at and above 0.5 g/Kg of ethanol.

For comparison purposes one can equate the mouse LD_{50} for ethanol (4 g/Kg) to the average human lethal blood level (400 mg/dl). Likewise, the human hypnotic dose of flurazepam (30 mg) is about equivalent in effect to 100 mg/Kg in the mouse. On this basis one could predict that one hypnotic dose of flurazepam, taken while blood ethanol is above 150 mg/dl, is likely to produce total incapacitation or coma. Likewise, 80-150 mg/dl of ethanol, combined with the residual flurazepam 24 h after a single hypnotic dose, is likely to produce extreme drowsiness or sleep. Even low levels of flurazepam and very low ethanol levels are likely to produce unexpectedly severe sedation and loss of alertness. On the other hand, it is apparent from the lethality data that the combination does not greatly increase the likelihood of death due to overdose.

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86.8 TEMPERATURE DEPENDENCE OF ETHANOL DEPRESSION MEASURED BY ROTAROD PERFORMANCE IN MICE. R.L. Alkana, M. Bejanian, B. Jamieson*, B. Jones*, G.G. Galleisky, P.J. Syapin and D.A. Finn*. Alcohol and Brain Research Laboratory, Institute for Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA 90033

Previous findings in our laboratory indicate that ambient temperature during intoxication influences body temperature and ethanol sensitivity as measured by sleep-time, wake-up blood and brain ethanol concentrations and lethality in mice and rats. The current study extended this investigation to sub-hypnotic ethanol doses. Rotarod-trained, adult, male, drug-naive C57BL/6J mice were injected i.p. with 1.5 or 2.5 g/kg ethanol or an equivalent volume of normal saline and exposed to ambient temperatures of 15, 22 or 36°C. Ethanol induced a significant, dose and ambient temperature related effect on body temperature. Rotarod performance decreased with increased ambient and resultant body temperatures in the intoxicated mice. The time course for the return of rotarod fucntion was significantly faster in the intoxicated mice exposed to 15°C than in mice exposed to 36°C. The enhanced performance in the cold versus warm exposed groups could not be attributed to temperature related changes in the elimination of ethanol, since blood ethanol concentrations did not differ between ambient temperature groups at 60, 90 and 120 minutes post-ethanol injection, or to the effects of temperature on sober performance. The results indicate that temperature dependence of ethanol sensitivity occurs following sub-hypnotic ethanol doses and can be measured using the rotarod task. Further, these results support the hypothesis that brain sensitivity to ethanol depression varies with body temperature in accordance with membrane perturbation theories of anesthetic drug action. (Supported by RO1 AA05234, National Institute on Alcohol Abuse and Alcoholism, ADAMHA).

86.9 HYPERBARIC EXPOSURE ANTAGONIZES THE DEPRESSANT EFFECT OF ETHANOL ON LOCOMOTOR ACTIVITY IN MICE. P.J. Syapin (1), J. Chen*, D.A. Finn* and R.L. Alkana. Alcohol and Brain Research Laboratory, Insitute for Toxicology, Univ. South. Calif. School of Pharmacy, and (1) Department of Neurology, Univ. South. Calif. School of Medicine, Los Angeles, CA 90033.

Previous studies have shown that hyperbaric exposure antagonizes the acute behaviorally depressant effect of hypnotic ethanol doses in rodents, precipitates and exacerbates withdrawal in ethanol-dependent mice, and reduces ethanol-induced inhibition of in vitro ATPase activity. The present study extends these investigations to include the sub-hypnotic dose range. C57BL/6J, adult, male, drug-naive mice were given two treatments, 2.5 g/kg ethanol and 0.9% saline, spaced one week apart according to a balanced cross-over design. Following injection, animals were individually exposed to 1 atmosphere absolute (ATA) air or to 1 ATA or 12 ATA helium-oxygen (HeOx) inside 18 liter hyperbaric chambers. Locomotor activity was measured continuously, beginning 10 minutes after injection, and was recorded at prescribed intervals for 60 minutes. Chamber temperatures were adjusted to offset ethanol-induced hypothermia and the cooling effect of helium, when applicable.

Exposure to 12 ATA HeOx significantly reduced locomotor activity in saline-injected mice compared to 1 ATA saline-injected controls ($p < 0.001$). Consequently, within subjects comparisons between saline and ethanol performance were used to evaluate the effect of hyperbaric exposure on ethanol intoxication. These comparisons indicated that 2.5 g/kg ethanol induced a significant reduction in locomotor activity for the first 30 minutes post-injection in the 1 ATA exposed mice ($p < 0.01$), with no significant differences occurring in the second 30 minute period. In contrast, the activity of ethanol-injected mice exposed to 12 ATA HeOx did not change significantly with respect to their activities following saline injection for the first 30 minutes after injection. Over the next 30 minutes, locomotor activity after ethanol injection was significantly increased in the 12 ATA exposed mice ($p < 0.01$). These results indicate that hyperbaric exposure antagonized the acute depressant effect of ethanol on locomotor activity. Blood ethanol concentrations were not significantly different 70 minutes post-injection in mice treated with 2.5 g/kg ethanol and exposed to either 1 ATA air, 1 ATA HeOx, or 12 ATA HeOx, thus making a pharmacokinetic explanation of these results unlikely. These results are consistent with, and extend, previous evidence suggesting that hyperbaric exposure antagonizes ethanol's action at the membrane level. (Supported by PHS grant RO1-AA03972, National Institute on Alcohol Abuse and Alcoholism, ADAMHA).

86.10 CHRONIC LITHIUM TREATMENT INCREASES BOTH WATER AND ALCOHOL DRINKING IN THE RAT. E.C. Critcher* and R.B. Mailman (SPON: H.Tilson). Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Enhanced water intake is a common side effect of chronic lithium treatment and an effect which can be reliably produced in the laboratory animal. We have recently reported that lithium increases the voluntary consumption of alcohol in the rat in a dose-dependent manner when water and a 5% solution of alcohol are offered concurrently (Critcher,E.C. and Mailman,R.B., Alcohol, in press, 1985). Alcohol intake increased significantly during treatment with 2 mmol/kg of LiCl, when compared with the control treatment of 2 mmol/kg of NaCl. A dose of 1 mmol/kg of LiCl produced an intermediate level of alcohol intake which was not significantly different from the other two treatment groups. The daily dose of lithium (2 mmol/kg) which is effective in increasing alcohol and water drinking produces plasma levels comparable to therapeutic levels in humans (i.e., 0.6-1.2 mmol/kg). During the course of treatment, animals follow a normal growth pattern and exhibit no overt signs of toxicity. Although both water and alcohol intake increase with chronic lithium, the time course for their development differs substantially. Water intake is maximal within 6 to 10 days of treatment, whereas an increase in alcohol intake appears only after 18 to 30 days.

It has been shown that water polydipsia induced by lithium requires an intact nigrostriatal dopaminergic system (Mailman,R.B., Psychopharmacol.80:143-149, 1983). In regard to this finding, we now report that haloperidol (0.5mg/kg i.p.) attenuates water, but not alcohol, drinking. In addition, naloxone and atropine, each in a dose of 1 mg/kg, fail to alter either water or alcohol intake. When increasing concentrations of alcohol (7-15%) were offered, the g/kg intake of alcohol and total fluid intake remained unchanged, whereas water intake increased. This finding suggests that under these experimental conditions alcohol may be consumed for its pharmacological action(s). Work is continuing to characterize the neurochemical basis of these observations. However, these data do suggest that the neuronal substrates of lithium-induced increases in water consumption are different than those for the increased consumption of alcohol. (Supported in part by NIAAA Grant #AA-05192).

86.11 THE ROLE OF CENTRAL ACETALDEHYDE IN THE MEDIATION OF ETHANOL-INDUCED NARCOSIS. C.M.G. Aragon*, K. Spivak* and Z. Amit (SPON: L. Switzman). Center for Studies in Behavioural Neurobiology, Department of Psychology, Concordia University 1455 de Maisonneuve Blvd. W., Montreal, Quebec, Canada H3G 1M8.

This investigation presents evidence for the oxidation of ethanol in the brain via the peroxidatic activity of catalase and the oxidation of acetaldehyde via brain aldehyde dehydrogenase. In addition, it provides evidence for the role of central acetaldehyde in the narcotic effect produced by ethanol administration in rats. Pretreatment with the catalase inhibitor, 3-amino-1,2,4-triazole shows an attenuation in the length of sleep after an ethanol dose of 3 g/kg as compared with control animals pretreated with saline. Simultaneous pretreatment with 4-methylpyrasole (alcohol dehydrogenase inhibitor) and sodium cyanamide (aldehyde dehydrogenase inhibitor)significantly increased the time to regain the righting reflex as compared with the two previous groups. Peripheral blood and acetaldehyde levels were similar in animals regardless of pretreatment in the course of the experiment. Correlations between the enzymatic activity of brain catalase and aldehyde dehydrogenase and the observed behavior were performed. The results suggest a role for central acetaldehyde in the mediation of ethanol-induced sleep time. In addition these data increase the support for the notion that acetaldehyde is produced and degradated directly in the brain.

86.12 ETHANOL AND 1,4 BUTANEDIOL IN LIVER : INTERACTIONS AND IMPLICATIONS. Flavio Poldrugo, O. Carter Snead, III, Steven Barker, F. Mallardi, M. Basa. Department of Pediatrics, Pharmacology, Chemistry, and The Neurosciences Program. The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama and Department of Anatomy, The University of Trieste School of Medicine, Trieste, Italy.

1,4 butanediol (BD) is a hepatotoxic aliphatic diol which occurs naturally in mammalian brain and liver and which has been shown to potentiate the behavioral effects of ethanol (ETOH). The object of these experiments was to pursue this BD-ETOH interaction in terms of toxicology, pathology, and biochemistry. These were two groups of experiments. For the first, rats were administered large doses of BD and ETOH either alone or in combination. The animals were observed behaviorally for sedation, catalepsy, ataxia, and loss of righting reflex. Blood ETOH and liver BD levels were measured. Mortality was assessed at 24 hours and histopathologic studies of liver and kidney performed. The second group of experiments were designed to ascertain the effect of acute and chronic ETOH administration on endogenous concentrations of BD in liver. These studies were prompted by the observations of Rutstein et al that other aliphatic diol compounds are elevated in the serum of chronic alcoholics (Lancet 2:534, 1983). Rats were treated acutely or chronically (5 days) with ETOH, sacrificed and liver concentrations of BD determined.

The combined administration of ETOH and BD resulted in a significantly increased mortality over either alone. ETOH also potentiated the hepatotoxic effects of BD. Marked fatty infiltration and necrosis of hepatic parenchyma were seen in the livers of animals treated with both BD and ETOH.

Acute ETOH administration had no effect of liver BD; however, chronic ETOH treatment resulted in a two-fold increase in endogenous liver BD concentrations.

These data showing potentiation of the hepatotoxic effect of BD by ETOH and elevation of liver BD by chronic ETOH, presumably by competition for alcohol dehydrogenase (Poldrugo and Snead, Neuropharmacology 23: 109, 1984), raise the possibility that BD may play a role in the pathogenesis of liver disease in chronic alcoholics.

86.PO THE EFFECTS OF ALCOHOL, A MINOR TRANQUILIZER, AND AN ANTI-HYPERTENSIVE AGENT ON THE CONDITIONED SUPPRESSION OF BEHAVIOR. M.A. Caplan, Dept. of Psychol., Adelphi Univ., Garden City, NY 11530

Subjecting an animal to various kinds of aversive stimulation can result in an increase in the voluntary consumption of an alcohol solution by animals that initially show an aversion to it. This increased alcohol intake has been attributed to the reinforcing pharmacological properties of alcohol, that it reduces tension induced by stressful stimulation. The definition of tension however, has never been verified independently of the increased alcohol intake following the application of some form of aversive stimulation. One way to accomplish this would be to compare the anti-suppressant properties of an anti-anxiety agent and an anti-hypertensive drug with the behavioral disinhibiting properties of alcohol.

The conditioned emotional response paradigm has been standardized as a prototypical behavioral analogue of conditioned anxiety, or tension, and is used as a standardized baseline for the study of anti-anxiety drugs. According to this procedure, a neutral stimulus is paired with shock while the organism is responding for positive reinforcement on a free-operant schedule. Following a limited number of such stimulus-shock pairings, the stimulus acquires conditioned aversive properties and results in suppression of the free-operant behavior when it is presented.

This procedure was used in the present experiment to compare the possible anti-suppressant effects of propanolol (Inderal), a clinically effective anti-hypertensive agent, and alcohol on this conditioned suppression of behavior. Albino rats received tone and uncontrollable footshock pairings in an aversive conditioning chamber, and were then transferred to an operant test chamber where they had been conditioned to press a lever for sweetened condensed milk. The tone was randomly presented during the session and resulted in an average of 50% response suppression. Two doses each of propanolol and alcohol were administered over a 2-3 week period; since none of the injections resulted in a reinstatement of the appetitive behavior when the tone was presented, two doses of chlordiazepoxide (Librium) were administered. This drug did restore appetitive responding during the tone presentations. Alcohol thus cannot be said to have the kind of tension relieving properties that are characteristic of anxiolytic drugs. The respective drug effects must be appropriately distinguished, and "tension" must be more precisely defined in order to delineate the psychological and behavioral effects of alcohol.

NEURAL BASIS OF BEHAVIOR: ALCOHOL III

87.1 CHRONIC ALCOHOL FAILS TO ALTER BRAIN GANGLIOSIDES. W. R. Klemm and D. M. Foster*, Dept. Vet. Anat., Texas A&M Univ., College Station, TX 77843 and Lab Svc., Olin E. Teague Veterans' Center & Dept. Med. Path. (Texas A&M Univ.), Temple, TX 76501.

We recently reported (Fed. Proc. 44 (4): 1089, 1985) that a single dose of alcohol (3 gm/kg), given to alcohol-naive rats, markedly decreased all species of brain gangliosides to less than 1/2 of normal at 4 hours post injection. Here, we evaluated the effect of chronic alcohol, given via liquid diet for 35 days, beginning at 30 days of age. The same analytical methods were used as in our previous acute study; namely, brains were extracted with chloroform-methanol and purified through sequential steps of DEAE chromatography, saponification, reverse-phase chromatography, and silicic acid chromatography; data were quantified with TLC densitometry and integrating recorder.

Daily alcohol consumption ranged from 13.8 to 15.8 gm/kg for each rat, and mean blood alcohol at sacrifice (0900 hrs) was 49.1 mM/l. Brain weights of alcohol rats were 7.8% less than the pair-fed controls, but that was accounted for by smaller total body weight. No significant differences were noted between alcohol-treated and control rats in any of the ganglioside bands (GT_{1b}, GD_{1b}, GD_{1a}, GM_1, GM_3). The total ganglioside levels in controls averaged 97.25% that of their alcohol-treated pair mates. The lack of effect is attributed to the relative neurological maturity of the rats when first exposed to alcohol and to a "protection" afforded by tolerance development (alcohol in the diet was increased gradually during the first week). Three prior reports that chronic alcohol decreases sialic acid are explained by methodological differences and by the fact that the effects were small and could have been largely due to a glycoprotein origin of the sialic acid.

Because acute alcohol reduces gangliosides, it suggests a transition period during chronic exposure in which brain cells develop a capacity to resist damage; the time course could parallel the resistance to membrane "fluidization" that develops during chronic exposure to alcohol. Thus the issue is raised: are ganglioside levels merely an expression of tolerance, or does ganglioside metabolism help to create membrane-level tolerance?

87.2 SYSTEMIC INDOMETHACIN OR ARACHIDONATE DO NOT AFFECT ETHANOL STIMULATED CORTICOSTERONE PRODUCTION. R.F. Anton, C.J. Wallis, H.C. Becker and C.L. Randall. VA Medical Center and Medical University of SC, Charleston, SC 29403.

Acute ethanol exposure stimulates adrenal corticosteroid production in rodents and man. Some of ethanol's neurobehavioral effects in mice can be inhibited by pretreatment with prostaglandin synthesis inhibitors, such as indomethacin, suggesting a role for eicosanoids as mediators in this process. Additionally, eicosanoids are involved in the regulation of corticotropin releasing factor (CRF) and ACTH release. We wished to examine the effect of the in vivo pharmacologic manipulation of eicosanoid production on ethanol-induced corticosterone release in mice.

C3H/He male adult mice were group housed (N=5) with free access to water and lab chow on a 12 hr light cycle with lights on at 0600. Between 0800 and 1000 hrs daily for seven days, the animals were handled and on four of those days given i.p. injections of saline to acclimate them to the stress of the procedure. On day eight in three separate experiments, animals (at least 5/group) received injections of study drugs or controls in a balanced design at defined times prior to an i.p. injection of ethanol (1.6 g/kg) or control saline. Animals were decapitated 30 min after ethanol or control injection and trunk blood was obtained for plasma corticosterone and blood alcohol level determination. Corticosterone was measured by a competitive protein binding assay and ethanol by a spectrophotometric assay. In Experiment 1, indomethacin (I) (5 mg/kg) or buffer control (B) was given i.p. 30 min prior to ethanol (E) or saline control (S). Corticosterone (ug%) levels (X±S.E.M.) for B-S, I-S, B-E, and I-E were 5.8±3.0, 5.1±1.4, 54.7±13.0, and 53.6±8.4, respectively. There was a main effect of E on plasma corticosterone ($p < 0.01$) but no main effect of I or interaction between I and E. In Experiments 2 and 3, the eicosanoid precursor arachidonate (A) as the sodium salt (50 mg/kg) or buffer (B) control was given s.c. at either 1 hr (Exp. 2) or 24 hr (Exp. 3) prior to injection with ethanol (E) or saline (S) control. The corticosterone levels for B-S, A-S, B-E, and A-E in Exp. 2 were 8.4±3.3, 7.8±1.8, 23.0±1.9, and 28.6±4.1 and in Exp. 3 were 3.5±1.4, 1.2±0.6, 20.2±2.9, and 17.9±2.3, respectively. In both experiments there was a main effect of E ($p < 0.01$) but no main effect of A or interaction between A and E. The blood ethanol concentration (X±S.D.) achieved across all experiments was 150±13.3 with no difference between treatment groups.

These data support previous findings that acute i.p. ethanol increases plasma corticosterone in mice. There was no effect of systemic pharmacologic manipulation of the prostaglandin system on either basal or ethanol stimulated corticosterone production. (Supported by the Veterans Administration.)

87.3 ACUTE ETHANOL ALTERS FIRING PATTERN AND SYNAPTIC INPUT OF PURKINJE NEURONS IN CULTURE. C.L. Franklin* and D.L. Gruol (SPON: S. Henriksen). Div. of Preclin. Neurosci. and Endocrin., and The Alcohol Research Center, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The Purkinje neuron (PN) of the cerebellum is a target of ethanol (EtOH) action when EtOH is administered systemically to rats. In this experimental paradigm, alterations in PN activity may result from direct actions of EtOH on the PN or via changes in afferent input from sensitive cerebellar interneurons or extracerebellar targets that regulate cerebellar activity. To identify actions of EtOH that are intrinsic to the cerebellum and direct to the PN we have developed a cerebellar culture system that is limited to only the five neuronal types present in the cortical region. We have previously shown that interneuronal associations and synaptic events reflective of the known circuitry in vivo are established in culture and that the cultured cerebellar neurons display many of the physiological and pharmacological characteristics typical of cerebellar neurons in vivo (Gruol, Brain Res. 263, 1983). In the present studies, the effect of acute EtOH (20-50 mM) on the spontaneous activity of cultured PNs was examined using extracellular and intracellular recording techniques and computer analysis of data. The majority of PNs studied (N=25) displayed a regular (10-20 Hz) simple spike (SS) firing pattern similar to that characteristic of PNs in vivo. The remainder exhibited mixed or bursting patterns. Intracellular recordings from the PNs revealed that the regular SS firing pattern is endogenously generated by ionic mechanisms intrinsic to the PN. In PNs exhibiting the regular SS firing pattern, acute EtOH, added to the recording medium (final concentration of 20 to 50 mM), increased the firing rate (1-5 Hz) and regularity of the SS pattern in a dose-dependent manner. In neurons exhibiting bursting patterns, acute EtOH (20-50 mM) changed the bursting pattern to a simple spike pattern. This change in pattern was usually accompanied by a small decrease in firing rate (1-3 Hz). In preliminary experiments intracellular recordings were used to examine the effect of acute EtOH on firing rate and spontaneous synaptic activity. EtOH (20-50 mM) increased the SS firing rate and frequency of synaptic input in these experiments. These data suggest EtOH can alter the activity of PNs by two mechanisms: 1) an action on the voltage-sensitive conductances generating the endogenous activity and 2) an action at cerebellar interneurons providing the synaptic input. Studies using intracellular recordings will examine the postsynaptic sites of EtOH action in more detail and extracellular recordings will be used to identify the sensitive presynaptic neurons. (Supported by NIAAA grant AA 06420)

87.4 POSTSYNAPTIC EFFECTS OF ETHANOL AT A CRAYFISH NEUROMUSCULAR JUNCTION. R.N. Friedman* and G.D. Bittner. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Intracellular recordings of potential changes in the membranes of single, opener muscle fibers in the propodites of crayfish (Procambarus simulans and clarkii) first walking limbs were used to assess the postsynaptic effects of ethanol (EtOH). Earlier studies showed that EtOH at concentrations that depressed excitatory junctional potentials (EJPs) had no presynaptic effect on nerve terminal potentials (R.N. Friedman and G.D. Bittner, Subst. Alc. Act. Misuse, in press).

As in the previous studies, EJPs were evoked by inducing short-term facilitation with superthreshold pulse trains of 15 to 40 Hz. Single, surface muscle fibers were impaled with two microelectrodes spaced less than 100 um apart to pass constant current pulses and to record resulting changes in membrane potential. Current-voltage relationships were plotted and effective membrane resistances (R_{eff}) in control and 500mM EtOH solutions were compared. Following each R_{eff} determination, EJPs were evoked and measured after 10s of stimulus application.

The results show a 24% decrease in R_{eff} of the muscle fiber membrane accompanying a 40% depression of EJP amplitude. Following the observation of the effects of 500mM EtOH, the preparation was again bathed in control (van Harreveld's) solution. EJP amplitude was increased to a level greater than that of the pre-treatment control. There was a concommitant increase (35%) in R_{eff}. The resting potential (-77mV) remained stable for the duration of the experiment. The membrane time constant varied with the changes in R_{eff}.

The results indicate that EtOH-induced EJP depressions that occur without change in nerve terminal potential, as previously observed, are most likely due to conductance increases in the muscle fiber membrane. The EJP enhancement on returning the preparation to the control bathing medium may be due to residual EtOH which has been shown to increase EJP amptitudes in this preparation at a 50mM concentration in our previous studies (R.N. Friedman and G.D. Bittner, Soc. Neurosci. Abstr. 10:195, 1984).

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87.5 **DECREASES IN MEDIO-BASAL HYPOTHALAMIC SEROTONIN IN RATS CONSUMING ETHANOL IN A LIQUID DIET**. Felten, S.Y. and D.L. Felten. Dept. of Anat., Univ of Rochester, Sch. of Med., Rochester, NY. 14642

Numerous attempts to correlate chronic ethanol exposure with changes in brain catecholamine and serotonin levels have led to contradictory findings, depending on dose of ethanol, route of administration, nutritional state, length of exposure, sex of the animal, and area of the brain examined. In an attempt to resolve some of these contradictions, age and weight matched female Wistar rats were pair-fed isq-caloric liquid diets (AIN 76) for 20 weeks. The alcohol diet contained the same levels of vitamins, minerals, protein, and fat as the control diet, but 36% of the calories were derived from ethanol. The control diet had the same number of carbohydrate calories, but in the form of maltose dextrin. Animals receiving the alcohol diet were allowed to consume as much as they wanted in a 24 hr period. This amount was recorded and given to the control animal of the pair the next day. This type of pair-feeding is designed to control for nutritional differences between ethanol treated and control animals, and in addition, minimizes the amount of animal handling necessary. At sacrifice there was no difference in weight between groups. The dose of ethanol averaged 11.26 g/kg body weight.

After 20 weeks of ethanol consumption animals were sacrificed by decapitation, brains were rapidly removed, and discrete areas were microdissected. Each sample was placed in 0.1M perchloric acid sonicated for 10 sec, and centrifuged at 11,000 g for 10 min. The supernatant was removed and injected onto a C18 reverse phase HPLC column for separation of the monoamines which were subsequently detected with electrochemical detection (Brain Res Bull 13:437). Monoamines were measured in 9 areas: paraventricular nucleus, dorsal raphe, medio-basal hypothalamus, 3 regions of hippocampus (CA 1, CA 2 + CA3, CA 4), striatum, parietal cortex, and cerebellar cortex. Serotonin and its metabolite 5-hydroxyindoleacetic acid were reduced in the medio-basal hypothalamus and dorsal raphe in animals consuming ethanol. Norepinephrine was increased in the CA 1 region of the hippocampus but decreased in the CA 2 - CA 3 region.

The reduced serotonergic input to medio-basal hypothalamus may be related to the altered endocrine state of animals consuming alcohol, particularly in the hypothalamo-pituitary axis related to gonadotropins and ACTH. In the hippocampus, rather than decreased serotonin levels, such as those found in the genetic strain of alcohol preferring rats (Pharm Biochem Behav 16:145), these alcohol fed rats showed alterations in NE. The difference in levels among different regions of the hippocampus suggests that alcohol consumption results in a redistribution of noradrenergic fibers within the hippocampus. Whether this is a primary effect or the result of neuronal alterations in the target neurons of the hippocampus is being explored with other techniques.

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87.6 CHOLECYSTOKININ OCTAPEPTIDE: EFFECT ON THE ETHOGRAM OF ETHANOL CONSUMPTION AND BLOOD ETHANOL LEVELS. P. J. Kulkosky*, M. R. Sanchez* and G. W. Glazner* (SPON: P. Schnur). Dept. of Psychology, Univ. of Southern Colorado, Pueblo, CO 81001.

Recent experiments show that peripheral injection of sulfated cholecystokinin octapeptide (CCK-8) specifically inhibits consumption of ethanol solutions in the rat. This effect of CCK-8 was characterized by constructing an ethogram of ethanol consumption and measuring blood ethanol levels. Ten naive, male Wistar rats were deprived of water for 23 hours. Rats were injected with i.p. saline and allowed access to 5% w/v ethanol for 45 minutes daily, followed by 15 min access to water. During daily ethanol access, behavioral observations were made with the technique of Gibbs et al. (Soc. Neurosci. Abstr., 6: 530, 1980). Observations of behavior were made at tone-cued 1 min intervals and classified into categories which included ethanol drinking, feeding, standing, grooming, resting, and a category for all other behaviors. After an initial adaptation period of 6 days, rats were randomly assigned to receive injections of either saline or CCK-8 (4 µg/kg). Injection of CCK-8 significantly reduced ethanol consumption, $F(1, 9)=20.17$, $p < 0.05$. Examination of the observational data revealed a significant difference only in total observed feeding behavior after CCK-8. Analysis of these data in 5-min blocks showed a significant decrease in feeding 15 min after ethanol presentation. No other differences were found in any other category of behavior. These rats were then adapted to a schedule of 30 min access to 5% ethanol followed by 30 min access to water. Rats received i.p. injections of CCK-8 (4 µg/kg) or saline 10 min prior to ethanol presentation. At the conclusion of ethanol access, a blood sample was taken from the tail of each rat and assayed for ethanol content as described by Cornell and Veech (Anal. Biochem., 132:418, 1983). CCK-8 significantly reduced both ethanol consumption, $F(1, 8)=24.05$, $p < 0.05$, and blood ethanol level, $F(1, 8)=9.18$, $p < 0.05$. Mean ethanol consumption and blood ethanol level were 14.2 ml and 46.3 mg/dl after saline, and 5.6 ml and 19.1 mg/dl after CCK-8. Blood ethanol level was significantly correlated with ethanol consumption, $r(8)=0.88$, $p < 0.05$. These experiments indicate that CCK-8 reduces ethanol consumption and blood ethanol levels. The accompanying behavioral display differs from control only in feeding behaviors. These results are consistent with the possibility that CCK-8 may act endogenously as a factor in ethanol satiation. (Supported by NIH Grant No. RR-08197).

87.7 ETHANOL INHIBITION OF NOREPINEPHRINE, GLUTAMATE, AND POTASSIUM BUT NOT CARBACHOL STIMULATED INOSITIDE HYDROLYSIS IN BRAIN. F.T. Crews, C. Theiss* and R.A. Gonzales. Dept. of Pharmacology, University of Florida Medical School, Gainesville, FL 32610.

A variety of neurotransmitters have been shown to stimulate the breakdown of inositides in rat brain slices. Evidence suggests that this biochemical event represents the initial step in a receptor activated cascade-amplifier mechanism used in the process of neurotransmission. We have investigated the effects of ethanol in vitro on stimulated inositide hydrolysis in brain. Cortical slices were prepared from male Fisher 344 rats and incubated with [^{3}H]inositol to label membrane inositides. The accumulation of [^{3}H]inositol phosphates stimulated by various agonists ± ethanol was determined in the presence of 8 mM lithium as measured by Dowex-1 chromatography. Ethanol (500 mM) had marked inhibitory effects of the production of [^{3}H]inositol phosphates elicited by 100 µM norepinephrine, 20 mM KCl, and 1 mM glutamate. No significant effects were observed on inositide hydrolysis stimulated by carbachol, 5-hydroxytryptamine (5-HT), or A23187. Ethanol's inhibitory effect on norepinephrine stimulated inositol lipid hydrolysis was concentration dependent and was significant at 100-500 mM. In addition, the effect of ethanol appeared to be exerted on the maximal effect and not on the ED_{50} for norepinephrine. KCl stimulated inositide breakdown was also decreased by ethanol in a concentration dependent manner. The inhibition by 500 mM ethanol was approximately 80% at 20 mM KCl but only 20% at 70 mM KCl. These results suggest that agonist stimulated inositide hydrolysis is differentially sensitive to the effects of ethanol with the order of sensitivity as follows: norepinephrine > KCl > glutamate with no effect on carbachol, 5-HT, or A23187. The lack of effect of ethanol on A23187 stimulation of inositide hydrolysis indicates that the inhibitory effect seen with other agonists is due to factors other than calcium influx into the cell. Ethanol is known to have membrane disordering effects at the concentrations used in these studies. The different agonist sensitivities to ethanol suggest that the membrane transduction systems from agonist-receptor complex to activated phospholipase C have different membrane order requirements. In addition, the changes in receptor stimulated inositide breakdown caused by ethanol for certain receptor systems and not others may contribute to some of the pharmacological and/or toxic effects of ethanol. (Supported by NIAAA grant AA06069).

87.8 PURINERGIC INVOLVEMENT IN THE BEHAVIORAL INTERACTION OF ETHANOL AND ACUTE AND CHRONIC METHYLXANTHINES IN MICE. M. Saeed Dar and Wallace R. Wooles, Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27834

The effect of acute and chronic administration of the methylxanthines, caffeine, theophylline and isobutylmethylxanthine (IBMX) on acute ethanol-induced motor incoordination has been investigated in mice. In acute methylxanthine studies, a significant potentiation of ethanol-induced motor incoordination was observed at 20, 45, and 75 min after a test dose (1.5 g/kg i.p.) of ethanol in mice pretreated with 62.5 mg/kg i.p. of caffeine and 12.5 mg/kg i.p. of IBMX. Pretreatment with 40 mg/kg i.p. of caffeine potentiated ethanol-induced motor incoordination only at 20 min after ethanol. The ethanol-induced motor incoordination was unaltered by theophylline (50 mg/kg i.p.). The results of methylxanthine pretreatment on ethanol-induced motor incoordination were similar regardless of a shorter (10 min) or a longer (75 min) pretreatment time. The methylxanthines produced no effect on motor coordination or behavior when administered in the same doses alone. In chronic (10 day) studies involving IBMX, the same test dose of ethanol produced a significantly greater motor incoordination in mice receiving either 40 or 65 mg/kg/24 h in the drinking water when compared to control mice which received tap water. Ethanol-induced motor incoordination was greater at 1 and 24 h after the withdrawal of IBMX. This motor incoordination was associated with increased binding of ^{3}H-L-PIA in whole brains of mice treated with IBMX. At 1 h of withdrawal, binding in the group receiving 40 mg/kg/24 h of IBMX was 333.17±23.85 vs 205.67±23.02 fmole/mg protein in the control group and at 24 h binding was 368.17±36.94 fmole/mg protein in the IBMX group and 264.16±22.11 fmole/mg protein in control mice. Binding was increased as the dose of IBMX was increased and in mice given 65 mg/kg/24 h whole brain binding at 1 h of withdrawal was 453.53 ±40.70 vs 201.47±24.75 fmole/mg protein in the control group. At 24 h of withdrawal from this dose of IBMX binding was 315.85 ±27.44 vs 198.33±18.25 fmole/mg protein in the control. In experiments involving chronic administration of 60 mg/kg/24/h of caffeine similar results were obtained; there was an increase in ethanol-induced motor incoordination and ^{3}H-L-PIA binding was increased from 202.44±21.44 fmole/mg/protein in control mice to 381.49±32.66 fmole/mg protein in caffeine-treated mice at 24 h withdrawal. The increased ^{3}H-L-PIA binding associated with increased ethanol-induced motor incoordination further supports our previous study showing that adenosine is involved in some of the CNS effects of ethanol.

87.9 REM-SLEEP DEPRIVATION-INDUCED INCREASE IN ETHANOL INTAKE: ROLE OF BRAIN MONOAMINERGIC NEURONS. K. Kiianmaa* and J. Aalto* (SPON: European Neuroscience Association). Research Laboratories of the Finnish State Alcohol Company (Alko Ltd), SF-00101 Helsinki, Finland.

The ethanol intake of Long-Evans male rats was recorded before, during and after rapid eye movement (REM) sleep deprivation produced with the flowerpot technique modified by using a cuff pedestal and electrified grid floor instead of water. Ethanol intake increased significantly during REM-sleep deprivation. A rebound decrease in ethanol drinking was then observed during the REM-rebound phase immediately after the termination of REM-sleep deprivation.

Since REM-sleep deprivation has been reported to impair the function of central monoamine neuronal systems, which some studies also have implicated in the control of voluntary ethanol intake, we studied whether different monoamine uptake blocking agents could antagonize the REM-sleep deprivation-induced increase in ethanol intake. After three days of REM-sleep deprivation, rats were given two daily injections (5 mg/kg/d, i.p.) of either citalopram, GBR 12909, or talsupram (uptake blocking agents for 5-hydroxytryptamine, dopamine, and noradrenamine, respectively). Citalopram and GBR-12909 did not modify the increased level of ethanol intake. In contrast, talsupram caused a decrease in ethanol intake to levels seen prior to deprivation, and during the REM-rebound phase an even further decrease was found. These effects of talsupram could be antagonized by blocking alpha-1-adrenoreceptors with prazosin (1 mg/kg/d, i.p.). Prazosin alone tended to increase ethanol consumption. These findings suggest that functional alterations in central noradrenergic neurons during REM-sleep deprivation may contribute to the concurrent increase in ethanol intake. This supports an earlier view indicating the importance of these neuronal systems in the regulation of voluntary ethanol consumption.

87.10 ALTERATIONS IN 2-DEOXYGLUCOSE UPTAKE IN BRAIN PRODUCED BY DIAZEPAM IN ETHANOL WITHDRAWING RATS. C.A. Marietta*, K. L. Zbicz, M. J. Eckardt, E. Majchrowicz* AND F. F. Weight. Laboratory of Preclinical Studies and Laboratory of Clinical Studies, DICBR, National Institute on Alcohol Abuse and Alcoholism. Rockville, MD 20852

Diazepam is commonly used to treat the ethanol-withdrawal syndrome. It has been previously shown that regional uptake of glucose, as measured with radiolabeled 2-deoxyglucose (2-DG), is altered during withdrawal from ethanol dependence (Campbell et al., Brain Res. 237: 517, 1982). We investigated the effects of diazepam (DZP) administration on 2-DG uptake in ethanol-withdrawing rats. Male, Sprague-Dawley rats (200-300g) were rendered physically dependent upon ethanol by the oral administration of 20% v/v ethanol (8-11g/kg/day) in Sustacal. Fractional doses were administered over a period of 4 days using a previously published intubation technique (Majchrowicz, Psychopharmacologia, 43:245, 1975). On the day following termination of ethanol administration, the rats were evaluated hourly for the signs of withdrawal (tremors and other behavioral signs of CNS hyperactivity). After 4-6 consecutive hours of withdrawal signs (usually beginning 12-18 hours after the final dose of ethanol), rats were prepared for the determination of local brain glucose uptake using the 2-DG method of Sokoloff et al. (J. Neurochem. 28:897, 1977). 2-DG was injected 30 min after the IP injection of 5 mg DZP/kg of body weight. This dose was selected because it was shown to alleviate the behavioral signs of withdrawal 30 min after injection. Control rats were similarly withdrawing from ethanol but received an IP injection of vehicle 30 min prior to the injection of the 2-DG. A generalized increase in 2-DG uptake was observed in the withdrawing controls, as well as columnar areas of increased uptake in the frontal sensorimotor cortex, ovoid areas of increased uptake in the cerebellar vermis, and increased uptake in various thalamic nuclei. These results are similar to those reported by Campbell et al. (Brain Res. 237: 517, 1982). Compared to the vehicle-injected withdrawing controls, the autoradiographs of withdrawing rats administered DZP revealed a generalized decrease in uptake, a lack of columnar areas of increased uptake in the frontal sensorimotor cortex, and a lack of ovoid areas of increased uptake in the cerebellar vermis. Our observations suggest a correlation between the effects of DZP on the behavioral signs of alcohol withdrawal and 2-DG uptake in the frontal sensorimotor cortex and cerebellar vermis. Further detailed analysis is necessary to determine whether the activity of other brain structures in the withdrawing rat is influenced by DZP.

We thank Hoffman-LaRoche for their gift of diazepam.

87.11 ACETYLCHOLINE (ACh) AND MUSCARINE REDUCE CA1 FIELD POTENTIALS IN THE HIPPOCAMPAL SLICE: POTENTIATION BY ETHANOL. George R. Siggins and Samuel Madamba, Div. Preclinical Neuroscience and Endocrinology, and Alcohol Research Center, Research Institute of Scripps Clinic, La Jolla, CA 92037.

We reported previously that systemic ethanol potentiates the excitatory effects of iontophoretically applied ACh on hippocampal pyramidal neurons (Mancillas et al, Soc. Neurosci. Abst. 10: 968, 1984). However, several concerns arise from such in vivo studies: 1) the anesthetic used (halothane) may have influenced the results; 2) the concentrations of ACh were unknown; 3) understanding the mechanism of this effect requires intracellular recording, a difficult procedure in vivo. Therefore, we have repeated these studies in vitro, using the rat hippocampal slice preparation.

Such slices were prepared by standard procedures (Siggins and Schubert, Neurosci. Lett. 23: 55, 1981) and totally submerged and superfused at the rate of 2-5 ml/min with carbogenated artificial CSF (ACSF). Stratum radiatum was stimulated with a double-pulse paradigm and evoked field potentials were recorded in the CA1 pyramidal layer. Only slices with population spikes of 5-12 mV were used. The potentials were digitized, averaged, stored and analyzed by computer. Drugs were introduced in defined concentrations into the recording chamber, without altering ACSF flow, by means of a manifold-valve system.

In apparent contrast to the excitatory effects of ACh iontophoresis in vivo, ACh superfusion (10-100 μM) consistently reduced the size of both the primary and secondary population spikes recorded in the CA1 pyramidal layer. This effect occurred at 2-4 min after entrance of ACh into the bath and usually persisted for 10-15 min with little evidence of tachyphylaxis. Enlargement of the field potentials was rarely seen. Lower ACh concentrations had no detectable effect. Muscarine, a "pure" muscarinic receptor agonist, gave comparable results at 1-10 μM, suggesting involvement of muscarinic receptors. The 10 μM dose often totally abolished the synaptic field potential and the population spike of both primary and secondary fields, whereas 0.1 μM or less had no effect. Superfusion of ethanol in low concentrations (10-22 μM, equivalent to 50-100 mg%) had little direct effect on the field potentials. However, these concentrations markedly potentiated the effect of threshold amounts of ACh and muscarine in reducing the field potentials.

These results suggest that the ethanol potentiation of ACh effects in vivo does not result from the use of anesthetics or unphysiological concentrations of ACh. However, the mechanisms behind this potentiation and the apparent inhibitory effect of superfused ACh (see also Valentino and Dingledine, J. Neurosci. 1: 784, 1981) in our slice preparation remains to be resolved by intracellular recording. (Supported by USPHS grant AA-06420)

87.12 A DIRECT CORRELATION BETWEEN ETHANOL-INDUCED LOSS OF RIGHTING REFLEX AND ENDOGENOUS AMINO ACIDS RELEASED IN THE MEDIAL SEPTUM AS MEASURED BY IN VIVO DIALYSIS. T.J. McCown and G.R. Breese. Biol. Sci. Res. Ctr., UNC Sch. of Med., Chapel Hill, NC 27514.

It has been proposed that ethanol exerts its depressant effects on the CNS by increasing the fluidity of neuronal membranes. As the neuronal membrane become more fluid, normal functions become impaired. Therefore, ethanol's alterations of a specific behavior would result from the impairment of neuronal function in the CNS sites which mediate the behavior. Identification of such CNS sites could be accomplished by establishing site specific interactions with ethanol. For example, we have previously shown that antagonism of ethanol-induced loss of righting reflex by thyrotropin-releasing hormone (TRH) occurs most potently in the medial septum (McCown and Breese, Alcohol 1:168, 1984), while increasing GABAergic activity in the medial septum potentiates this measure of ethanol-induced depression. If ethanol does alter activity in the medial septum, do endogenous neurochemical changes parallel the behavioral changes?

In order to answer this question, rats were implanted under ether anesthesia with a 2 mm long dialysis fiber (Amicon Vitafiber) in the medial septum. Following a 3 hr perfusion period (2 ul/min., Ringers solution), samples were collected every 20 min., and the concentrations of histidine, glutamine, glutamate, serine, glycine and taurine were determined in the dialysate. At no time was the GABA concentration large enough to quantify. When animals were injected with 3.5 g/kg ethanol (i.p.), the righting reflex was lost and the amino acid concentrations were significantly decreased. The time at which the animals regained their righting reflex coincided with a significant increase in amino acid concentrations towards the control baseline. If the animals received a 5 mg/kg dose (i.p.) of TRH 40 min. after the ethanol treatment, the time necessary for the animals to regain their righting reflex was significantly shortened, and the reversal of ethanol-induced decreases in amino acid concentrations parallelled the return of the righting reflex. In contrast to these data from the medial septum, dialysis of the frontal cortex revealed a totally different pattern of change. Ethanol administration caused a significant increase in taurine concentration, which coincided with the lost of the righting reflex.

From these results, we hypothesize that the medial septum can serve as an in vivo model for the study of ethanol's actions on the CNS, especially in relationship to the righting reflex. (This work was supported by N.C.A.R.A. grant #8304).

87.13 PINEAL AND MELATONIN EFFECTS ON ETHANOL PREFERENCE IN THE SYRIAN HAMSTER. R.J. Salin-Pascual*, H.B. Moss*, and L.Tamarkin*, (SPON: D. Jimerson). Clinical Psychobiology Branch, NIMH and Lab. of Clinical Studies, DICBR, NIAAA, Bethesda, MD 20205

In a free choice experiment, Syrian hamsters will preferentially drink more 5% or 15% ethanol (ETOH) than water. It is unclear why these animals choose ETOH and it is also not known what area(s) of the brain regulates ethanol consumption in these animals. In a previous report surgical removal of the pineal gland was reported to raise ethanol consumption. The present study re-evaluates this observation and determines the effect of pineal hormone replacement by thrice daily melatonin injection on this behavior. Three groups of female hamsters (4 to 6 animals/cage) were exposed to 5% ETOH and water for 1 week, then these 3 groups were presented with 15% ETOH. A fourth group of animals (n=5) was maintained on the 5% ETOH solution. After 4 weeks of exposure to the ETOH/water free choice condition, the hamsters exposed to 5% ETOH and one group (n=4) of hamsters exposed to 15% ETOH were pinealectomized (Px). After a 4 week recovery during which the animals were maintained on the free choice condition, these animals and one (n=6) group of animals were injected thrice daily with 25 ug of melatonin per injection s.c. Daily injections occurred during the light hours (0900h, 1200h and 1500h) of a 14L:10D lighting schedule (lights on 0100h to 1500h). During the baseline period, the melatonin treatment period and the recovery period fluid consumption was monitored daily for each group. For each day an ETOH: water preference ratio was calculated, the data were summarized by week of treatment and significance was determined by analysis of variance.

ETHANOL PREFERENCE RATIOS (EPR)

	Intact	Px(15% ETOH)	Px(5%ETOH)	Intact (saline)
Basal	2.76	2.45	2.92	3.11
Week 1 melatonin	1.42	1.38	1.11	2.69
Week 2 melatonin	1.18	0.92	1.19	2.56
No treatment	1.76	3.76	2.63	2.43

Melatonin treatment resulted in a significant ($p<0.05$) reduction in ETOH consumption in intact and in both groups of Px animals. Pinealectomy itself did not alter the EPR compared to intact animals. Additionally, thrice daily injection did not significantly effect the EPR in intact animals. These data suggest that melatonin alters ethanol consumption which may provide us with some insight into the neurochemical basis of ethanol preference in this species.

87.14 EFFECTS OF ETHANOL ON MID-BRAIN DA AND NON-DA NEURONS. G.L. Gessa*, F. Muntoni*, V. Boi* and G. Mereu. Institutes of Pharmacology and Biology, University of Cagliari, Italy.

The behavioral stimulation induced in rodents and humans by moderate doses of ethanol is thought to be mediated by the activation of central dopamine (DA) transmission. Accordingly, the systemic administration of low doses (0.5-2.0 g/kg) of ethanol increases striatal DA release and DA synthesis, and as we have demonstrated, the firing rate of nigral A9-DA cells. We have also found that a low dose of ethanol depresses the activity of nigral non-DA cells in the pars reticulata (PR), which integrates the striato-nigral feed-back loop and exerts an inhibitory control on the neighboring A9-DA neurons. Thus we proposed that the activation of A9 neurons by ethanol may be the consequence of the preferential depression of such PR inhibitory inter-neurons.

Our question was whether a similar mechanism was also applicable to those DA neurons located within the ventral tegmental area (VTA) which project to limbic and cortical areas (A10 neurons). Indeed VTA-DA neurons have been implicated in the reinforcing properties of cocaine, amphetamine, heroin and brain self-stimulation. Therefore we tested the effect of i.v. ethanol on the activity of DA and non-DA neurons of VTA, comparing it to that observed for A9 and PR cells.

Extracellular single unit recordings of A9 and A10 neurons, as well as PR and VTA non-DA neurons, were performed in paralyzed-locally anesthetized rat preparations. Ethanol was administered intravenously (i.v.) in incremental doses as a 20% (w/v) solution in H_2O.

Ethanol stimulated both A9 and A10 DA neurons. However while the cumulative dose needed to increase by 50% the basal activity (ED_{50}) of A9-DA neurons was 863±72 mg/kg (N=22), the ED_{50} of A10 neurons was 162±25 mg/kg (N=16). By contrast the majority of non-DA cells of both areas were inhibited by i.v. ethanol. About 83% of PR neurons (N=35), whereas only 52% of the non-DA cells of the VTA (N=14), responded to the treatment with the remainder of the cells unaffected. The cumulative dose inhibiting 50% (ID_{50}) of activity in responsive cells was 483±31 mg/kg and 534±63 mg/kg for PR and VTA area, respectively.

These experiments confirm that i.v. ethanol increases the firing of A9 neurons and extend this observation to A10 neurons. The high sensitivity of the latter, more than 5 times that of A9 cells, might be correlated to the fact that meso-limbic and meso-cortical DA projections are proposed to be involved in the behavioral effects of various forms of reward, including those by drugs of abuse. Whether the mechanism of the stimulant action of ethanol on A10 neurons is mediated by an action of non-DA neurons of the same area is presently not clear.
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87.15 TIME COURSE OF ETHANOL EFFECTS ON IMMUNOREACTIVE VASOPRESSIN IN RAT PLASMA. D. L. Colbern, J. ten Haaf*, B. Tabakoff, and Tj.B. van Wimersma Greidanus. Dept. Physiol. Biophys., Univ. Ill. Chicago Med. Ctr., P.O. 6998, Chicago, IL 60680, and Rudolf Magnus Inst. Pharmac., Univ. of Utrecht, The Netherlands.

We previously reported that vasopressin (VP) levels are significantly elevated 5 min after an intraperitoneal (ip) injection of ethanol (2 g/kg, 15% v/v) and inhibited at 60 min. The present study more fully examined the short-term time course of VP release following an acute injection of ethanol. Plasma osmolality and blood ethanol concentration were also measured.

Male, Wistar rats (250-300g) were given food and water ad libitum and maintained on a 14:10, light:dark schedule (lights on 05:00 h). Rats were injected ip with a 15% v/v ethanol solution (2.0 g/kg, 95% ethanol in saline) 5, 15, 30, 60 or 90 min before decapitation. Control animals were given an equal volume of 0.9% saline (1.77 cc/100g). Injections were started at 14:30 h, and decapitations were completed by 17:00 h.

Plasma samples (1.5 ml) were extracted with Vycor glass powder, and VP content was determined by radioimmunoassay using an antiserum (W1E) which had less than 0.01 % crossreactivity with oxytocin. Blood ethanol concentration was determined by gas chromatography, and the osmolality of fresh plasma was measured with a Wescor freezing point osmometer.

Plasma VP levels in saline-treated rats remained unchanged over the 90 minute sampling period. Five min after ethanol injection, VP levels were increased (3.1 ± 0.7 pg/ml) compared to levels in saline-treated rats (1.2 ± 0.2). By 15 min, VP had decreased to control values. VP release was inhibited 30 and 60 min after ethanol injection (0.4 ± 0.1 and 0.3 ± 0.1 pg/ml, respectively), but levels returned to control values at 90 min (1.8 ± 0.9). Ethanol concentration was highest at 5 min (257.6 ± 11.9 mg%), declining thereafter (to 155.9 ± 4.5). Plasma osmolality was inversely correlated with blood ethanol concentration.

The effects of ethanol concentration and injection volume were also examined. Under the conditions described above, ethanol doses of 2.0 g/kg were administered as 10, 15 or 20% solutions. Comparison groups received equivalent volumes of saline (2.66, 1.77, or 1.33 cc/100g) or sham injections 5 or 60 min before decapitation. Again, for all ethanol concentrations, VP levels were increased at 5 min. Neither ethanol concentration nor injection volume differentially affected VP levels. Plasma osmolality and blood ethanol content were also unaffected by different concentrations of ethanol, although plasma from both saline- and ethanol-injected rats was diluted compared to those receiving sham-injections.

Vasopressin levels in response to ethanol follow a biphasic time course, and are not merely inhibited. Rapid, early release of VP may mediate some of the effects of acute ethanol injection.

87.16 INVOLVEMENT OF ENDOGENOUS ADENOSINE-RECEPTIVE SYSTEMS IN THE STIMULUS PROPERTIES AND BEHAVIORALLY DEPRESSANT EFFECTS OF ETHANOL. R.C. Michaelis, F.A. Holloway, and G.A. Hunter*. Dept. of Psychiatry and Behavioral Sciences, Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

It has been suggested that ethanol-induced sleep and motor incoordination are mediated in part by stimulation of adenosine-receptive systems in the CNS. This study examined the possibility that the discriminative stimulus properties and response rate-reducing effects of high doses of ethanol are mediated by an agonist action on adenosine-receptive neurons. Two groups of rats were trained to discriminate the stimulus properties of ethanol from those of saline. One group was trained with 1.0 g/kg ethanol throughout the entire study. In the other group, the training dose of ethanol was gradually increased as tolerance developed in order to maintain a significant response rate reduction. Results from both groups suggested that the discriminative stimulus properties of ethanol are not mediated through adenosine-receptive systems. No stimulus generalization was seen after administration of either of the adenosine analogs N6-cyclohexyladenosine (CHA) or 5' -N-ethylcarboxamide adenosine (NECA). In addition, the stimulus properties of ethanol were not antagonized by either caffeine or 8-phenyltheophylline. Finally, neither the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) nor the adenosine uptake inhibitor dipyridamole were able to enhance the stimulus properties of a low dose of ethanol. In contrast to the stimulus properties of ethanol, preliminary findings suggest a synergism between ethanol and adenosine agonists with respect to the ability of these drugs to reduce operant response rate.

87.17 MORPHOLOGICAL EVIDENCE OF ALCOHOL-INDUCED PRECOCIOUS NEURONAL AGEING. M.M.Paula-Barbosa, M.A.Tavares and M.M.Borges (SPON : R. ROSENBERG). Department of Anatomy, Oporto School of Medicine, Oporto, Portugal.

It is well known that young chronic alcoholics show deficits in cognitive functions that in several respects parallel those found in the aged. Besides, the morphological studies of the effects of chronic alcohol consumption on CNS made on necropsic material emphasise the similarities between brains of young chronic alcoholics when compared with the aged brains of non-alcoholics. From these observations it was advanced the concept that chronic alcohol consumption could induce a premature ageing of neurons. To substantiate this hypothesis a sound morphological basis is nevertheless required. In fact, the identification of neuronal senescence markers, is difficult to establish in necropsic material since apart from bad fixation it is impossible to exclude other factors present in most alcoholic patients, which, per se, may act on neuronal structure. To gain an insight into this problem we studied, using qualitative and quantitative methods, the cerebellar cortex of groups of 6 alcohol-treated rats for 1,3,6,12 and 18 months. Pair-fed animals were used as controls.

Accurate parameters for morphological senescence of neurons were sought. 1. Lipofuscin deposition - a significant increase in the amount of pigment formation was observed in experimental animals as early as 6 months of alcohol consumption, whereas in controls it became evident only in the older group. Purkinje and basket cells in the cerebellar cortex and hippocampus pyramids of both CA_1-CA_3 subdivisions were the most affected neurons. 2. Empoverishment of dendritic arborization - marked abnormalities in dendrites and their spines were depicted after 6 months of alcohol intake, increasing with the duration of the experiment. Dendritic trees areas appeared reduced in overall size, with less complex branching patterns and changes in its spatial orientation. The number of dendritic spines per unit length was conspicuously decreased. 3. Neuritique plaques - scattered single neurites were seen after 3 months of treatment. Neuritic plaques of the primi - tive type were found in the cerebellar cortex molecular layer after 6 months of alcohol intake. 4.Neuronal degeneration -the number of neurons per unit volume of cerebellar cortex (Nv) and their total number were found to be significantly reduced in alcohol treated animals. The most affected were granular cells.

The presence of accurate neuronal ageing indicators appearing together and simultaneously in alcohol-fed animals and its absence in their respective controls, make temtping to suggest that chronic alcohol intake might induce a precocious neuronal ageing.

88.1 DEPENDENCE OF MEPP FREQUENCY AT THE FROG NMJ ON TERMINAL LENGTH AND EXTERNAL Ca^{2+}. P. A. Pawson and A. D. Grinnell, Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, CA 90024, USA.

We are seeking physiological explanations for the wide variability in transmitter release properties of frog sartorius neuromuscular junctions (NMJs). These terminals regularly differ by 20-fold or more in quantal content or release per unit length. Previously we have presented evidence that stronger junctions have a greater Ca^{2+} influx per action potential than do weaker junctions (with lower quantal contents or release/unit length) (Pawson & Grinnell, Brain Res(1984)323:311). It is also well known that stronger junctions show higher spontaneous quantal release (i.e. mEPP frequency). We have therefore turned our attention to the spontaneous release phenomena with an eye towards a more complete characterization of physiological differences between junctions.

Identified junctions, where release can be correlated with terminal morphology, were used. We have found that spontaneous release at frog NMJs, similar to their mammalian counterparts, shows a positive dependence on the external Ca^{2+} concentration. Moreover, mEPP frequencies of stronger junctions show a greater dependence on external Ca^{2+}. This phenomena is most striking when all external Ca^{2+} is removed. Under these conditions, all junctions become much more similar in their resting mEPP frequencies. This suggests that stronger junctions have a greater resting Ca^{2+} influx.

We have recently found another possible explanation for some of the physiological differences in mEPP frequency. Although mEPP frequency is positively correlated with quantal content, we have found that often, among junctions of a similar quantal content class, there is a positive dependence of mEPP frequency upon terminal length. This suggests that under some conditions mEPP release and evoked release are not strictly coupled as a function of terminal length. Curiously, we have found that this dependence on terminal length appears to disappear in a Ca^{2+}-free/EGTA Ringer, even under conditions that greatly augment spontaneous release. We are presently investigating this dependence on terminal length, seeking to understand these observations.

(Supported by an MDA Fellowship to PAP and grants from the USPHS to ADG).

88.2 **CHANGES IN RELEASE AT THE FROG NEUROMUSCULAR JUNCTION UPON STRETCH.** M.J. Werle and A.A. Herrera. Neurobiology Section, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371.

Both nerve stimulus-evoked and spontaneous transmitter release at the frog neuromuscular junction are known to increase when the muscle is passively stretched (Turkanis, J. Physiol. 230:391, 1973). We have improved upon earlier studies of this phenomenon by examining the effects of stretch at identified junctions, where physiological changes and light microscopic structure can be correlated in detail. Sartorius muscles from adult Rana pipiens were used.

In a sample of 27 junctions, quantal content (m) in low Ca^{2+}/high Mg^{2+} Ringer increased an average of 2.7X when the muscles were stretched from 90% to 120% of in vivo length. After nerve terminal and cholinesterase staining, terminal lengths ranging from 156 to 914 μm were measured for 14 of these junctions. A significant positive correlation was found between nerve terminal length and the increase in m ($r=0.91$, $P<0.01$). In a separate sample of 27 identified junctions, a significant positive correlation was also found between nerve terminal length and the increase in miniature endplate potential frequency (MEPP f; $r=0.46$, $P<0.05$). Thus the enhancement of release by stretch appears to correlate with total nerve terminal length by an as yet unknown mechanism.

Earlier studies (ibid.) showed that tetrodotoxin (TTX) decreased but did not abolish the effects of stretch on MEPP f. To further test whether the presynaptic voltage-dependent Na^+ channel is involved in the stretch enhancement of MEPP release, we replaced Na^+ in the Ringer with dimethylamine, a cation that is impermeable through the voltage-dependent Na^+ channel but permeable through the acetylcholine channel (Hille, J. Gen. Physiol. 75:469,1980). Each of several identified junctions was then tested in the presence and absence of external Na^+ for the effects of stretch on MEPP f. There was no significant difference in the mean increase in MEPP f upon stretch in normal Na^+ (2.4X) or 0 Na^+ (2.3X). The replacement of Na^+ with dimethylamine produced a substantial decrease in resting MEPP f (73%) as well as slight decreases in membrane potential (10%) and MEPP amplitude (19%), but these changes were reversible upon the return of Na^+. Since the stretch effect is not eliminated by TTX or diminished by replacing Na^+ with an impermeant cation, we conclude that the voltage dependent Na^+ channel is not involved in the stretch enhancement of MEPP f. Further experiments are in progress. (Supported by NIH grant NS18186).

88.3 **DIFFERENCES IN SYNAPTIC EFFICACY AT FROG NEUROMUSCULAR JUNCTIONS.** A.A. Herrera, L.R. Banner* and P. Mason*. Neurobiology Section, Deparment of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371.

Studies of naturally occurring differences in the effectiveness of synaptic transmission at neuromuscular junctions (Herrera, et al., J. Neurocytol. 14, in press, 1985) have proven useful in determining the morphological and physiological basis of long-term changes in synaptic efficacy (Herrera et al., Neuroscience, in press, 1985; Herrera & Grinnell, J. Neurosci., 5, in press, 1985). In a continuing search for preparations in which synapses show large differences in efficacy, we have examined cutaneous pectoris (CP) and cutaneous dorsi (CD) muscles of Rana pipiens.

Both muscles are composed of twitch fibers with identical mean contraction times of 29 ms and similar mean fiber diameters (CD 38 μm, n=168; CP 42 μm, n=223). Reducing $[Ca^{2+}]$ in the Ringer from 1.8 to 1.0 mM causes nerve stimulus evoked twitch tension to fall by an average of 66% in the CD (n=21) while CP tension falls only by 11% (n=17), indicating substantially higher safety margins for neuromuscular transmission in CP muscles. We find no differences in muscle fiber action potential threshold or input impedance, suggesting that this difference in safety margin is due to presynaptic factors. Indeed, in Ringer containing 0.5 mM Ca^{2+} and 3 mM Mg^{2+}, CP junctions showed 4X higher EPP quantal content (CP mean 7.6, n=149; CD 1.8, n=90) and 5X higher MEPP frequency (CP 0.91 Hz, n=148; CD 0.18 Hz, n=82). Nerve terminals in the CP are slightly longer (CP mean 504 μm, n=223; CD 409 μm, n=168) but do not otherwise appear different in the light microscope. Since it seemed that this 23% difference in terminal length could not account for a difference in release of several hundred per cent, these data suggested to us that there are intrinsic differences in release per unit terminal length. Recordings made from single identified junctions in 0.5 mM Ca^{2+}/3 mM Mg^{2+} Ringer confirm this, showing that the CP has 2.5X higher quantal content per unit terminal length (CP 1.18 quanta/100 μm, n=26; CD 0.47 quanta/100 μm, n=21) and a 3.7X higher rate of MEPP release per unit terminal length (CP 1.88 Hz/1000 μm, n=26; CD 0.51 Hz/1000 μm, n=17). We are currently measuring evoked and spontaneous release in normal Ringer (1.8 mM Ca^{2+}) after blocking excitation-contraction coupling and muscle fiber action potential generation with formamide. Correlated morphological observations using freeze fracture electron microscopy suggest that these differences in transmitter release may be related to differences in some aspects of the ultrastructure of presynaptic active zones (Ko et al., Soc. Neurosci. Abstr., this volume). (Supported by NIH grant NS18186 and the Muscular Dystrophy Association).

88.4 **A COMPARISON OF ACTIVE ZONE ULTRASTRUCTURE IN TWO MUSCLES WITH MARKEDLY DIFFERENT SYNAPTIC EFFICACY.** Chien-Ping Ko, Jon W. Propst and Albert A. Herrera. Neurobiology Section, Dept. of Biological Sciences, University of Southern Calif., Los Angeles, Ca. 90089.

Thin section electron microscopy has shown that transmitter release efficacy is correlated with indirect estimates of active zone (AZ) length (Herrera et al., J. Neurocytol., 14: in press, 1985). We examined this question more directly using freeze-fracture electron microscopy. We chose to study AZ ultrastructure from nerve terminals in two muscles: the cutaneous pectoris (CP) and cutaneous dorsi (CD) of Rana pipiens. These muscles differ widely in synaptic safety factor and release per unit nerve terminal length, with the CP being stronger (Herrera et al., Soc. Neurosci. Abstr., this volume).

Average safety factors were determined as described previously (Grinnell and Herrera, J. Physiol., 307:301,1980). After pinning to 1.05x rest length, muscles were fixed in frog Ringer containing 3% glutaraldehyde. Endplate regions were dissected and prepared for freeze-fracture using standard techniques (Ko, J. Cell Biol., 98:1685,1984).

Morphometric analysis of nerve terminals showed differences between CP and CD. Data taken from 11 CP terminals with 342 AZ segments and 9 CD terminals with 388 AZ segments gave the following results. Although AZ spacing did not show any apparent difference between the two muscles, AZ length per 100 microns of nerve terminal length was slightly larger in CP. The length of individual AZ segments was greater in CP, as was the percentage of terminal width occupied by AZ. The number of AZ segments per junctional fold was higher in CD terminals. Preliminary results suggest that differences in synaptic efficacy between strong and weak junctions can be related to some aspects of AZ ultrastructure. (Supported by NIH and MDA grants.)

88.5 **THE CORRELATION BETWEEN SYNAPTIC EFFICACY AND ACTIVE ZONE ULTRASTRUCTURE STUDIED WITH FREEZE FRACTURE OF IDENTIFIED NEUROMUSCULAR JUNCTIONS.** J.W. Propst and C.-P. Ko. Neurobiology Section, Dept. of Biological Sciences, University of Southern Calif., Los Angeles, Ca. 90089.

Since quantal content per unit terminal length can vary widely in the same muscle, it has been difficult to correlate differences in synaptic strength with synaptic structures. We have developed an innovative technique to study the relationship between synaptic efficacy and active zone (AZ) ultrastructure by freeze fracture of physiologically identified neuromuscular junctions.

Cutaneous pectoris muscles from Rana pipiens were dissected and pinned to 1.15x rest length. Quantal content was measured in 0.35mM Ca^{++}/4mM Mg^{++} Ringer solution from individual muscle fibers. The fibers were marked by the injection of Lucifer Yellow. After fixation, endplates were visualized with a cholinesterase stain we modified to be compatible with later freeze fracture. The muscles were glycerinated and identified fibers teased out. Endplates were drawn using a camera lucida. Single fibers were frozen in Freon-22, stored in liquid nitrogen, and fractured in a complementary replica device.

The overall success of our method was quite high, with 20% of fibers fractured showing active zones. The most important result was an apparent correlation between quantal content per 100 microns of terminal length and the amount of active zone per 100 microns (r=0.68). The preliminary data from 5 experiments (m/100u, AZ/100u) is given below: (0.41,114u); (0.41,178u); (0.52,128u); (0.92,173u); and (1.62,193u). In addition, AZ length seemed to be related to AZ spacing and terminal width. Although there has been a recent attempt at single fiber freeze fracture (Pawson and Grinnell, Soc. Neurosci. Abstr., 10:918, 1984), to the best of our knowlege, the present study is the first report showing freeze fracture of physiologically identified endplates, and a relationship between synaptic efficacy and AZ length. Additional experiments are in progress to supplement the results described above, and to examine if other aspects of AZ ultrastructure are related to transmitter release. (Supported by NIH and MDA grants).

88.6 **FREEZE-FRACTURE STUDY OF THE EFFECTS OF PROTEOLYTIC ENZYMES ON ACTIVE ZONES IN THE FROG NEUROMUSCULAR JUNCTION.** R.R. Nystrom* and C.-P. Ko. (SPON: G. Augustine). Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

It has been shown that proteolytic enzymes digest the basement membrane and cause synaptic disjunction of nerve terminals and muscle endplates (Betz and Sakmann, J. Physiol. 230:673, 1973). Since the basal lamina (BL) is believed to play an important role in the differentiation of the active zone (AZ) (Sanes et al., J. Cell Biol. 78:176, 1978), it is of interest to study the effect of BL removal by proteolytic enzymes on the AZ ultrastructure.

Cutaneous pectoris muscles from Rana pipiens were treated with 0.1% collagenase and 0.01% protease according to the protocol of Betz and Sakmann. Muscles were then fixed and processed for freeze-fracture. In some cases the nerves were stimulated during slow fixation. Also, other enzyme-treated muscles were incubated with filipin in a fixative solution, in order to obtain information about membrane lipid heterogeneity (Nakajima and Bridgman, J. Cell Biol. 88:45, 1981).

Following the proteolytic enzyme treatment, disorganization of AZs was observed. Although some AZs retained the normal organization of two double rows of intramembrane particles, they were much shorter than normal. In addition, they were located and oriented randomly. Dimples, which might represent openings of synaptic vesicles, were seen even at these disrupted AZs. Filipin-sterol complexes were absent from disrupted AZs in enzyme-treated muscles, as is the case with normal non-disrupted AZs.

This study shows that treatment of frog neuromuscular junctions with proteolytic enzymes causes a disorganization of AZs and that these disorganized AZs are still functional. It is not known, however, whether this effect is due to BL removal or direct action of the enzymes. In addition, the results suggest that the absence of filipin-sterol complexes is closely associated with AZ particles. (Supported by MDA postdoctoral fellowship and NIH, MDA grants).

88.7 PHENOTYPE-SPECIFICITY OF THE MEMBRANE-ASSOCIATED FORMS OF THE CATECHOLAMINE SYNTHESIZING ENZYMES. O. Hwang*, C. Abate* and T.H. Joh. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

The catecholamine synthesizing enzymes tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) have been generally believed to be soluble cytosolic proteins. Although particulate forms had been reported, these were thought to be the aggregated forms of the soluble enzymes as the enzymes' high tendency to aggregate was known. Dopamine -hydroxylase (DBH), on the other hand, is known to exist in two forms: soluble and membrane-bound. We have recently reported (Docherty et al., Brain Res., in press) that anti-TH antibody causes the complement-mediated specific immunolysis of dopaminergic synaptosomes isolated from rat striatum, which suggests the presence of a TH-like protein in the membrane.

To investigate this further, we have carried out a series of proteolytic digestion of the membrane fraction of catecholamine-containing tissues, followed by immunoblot with antibodies to TH, DBH and PNMT. The results showed that in the dopaminergic synaptosomal plasmamembrane, there is a TH population which is protected from the proteolytic digestion by the presence of the membrane suggesting the possibility of a membrane-associated form. In bovine adrenal medulla, which is primarily noradrenergic and adrenergic, however, this proteolytically protected form of TH was not present. Instead, a protected form of PNMT, in addition to the membrane DBH, was found. These results have been confirmed by Triton X-114 extraction studies.

Thus, we found the proteolysis resistant, probably membrane-associated, forms of the catecholamine biosynthetic enzymes. These membrane-associated forms seem to be phenotype-specific: TH in dopaminergic cells, DBH in noradrenergic cells, and PNMT in adrenergic cells. We conclude that the coordinate expression of the soluble and the membrane-associated enzymes may determine the phenotype of the catecholamine-synthesizing cells.

88.8 THE RELEASE OF DOPAMINE AND SEROTONIN FROM TWO POPULATIONS OF SYNAPTOSOMES ISOLATED ON PERCOLL GRADIENTS. P.J. Robinson*, W. Lovenberg*, and P.R. Dunkley* (SPON: H. Pollard), NHLBI, National Institutes of Health, Bethesda, MD 20205, and The Neuroscience Group, Faculty of Medicine, University of Newcastle, N.S.W. Australia, 2308.

A rapid procedure has recently been described for the preparation of synaptosomes from rat brain utilizing Percoll gradient centrifugation (Rostas et al., Abs. Soc. Neurosci. 10, 917, 1984). Two distinct sub-populations of synaptosomes were isolated and their homogeneity and viability were assessed. In this study a modified procedure was employed to isolate the two fractions, to determine their content of dopamine (DA) and serotonin (5-HT), and to demonstrate the release of these neurotransmitters. Rat striatum was homogenized in 0.32 M sucrose/1 mM EDTA, then dithiothreitol was added (to 0.25 mM). After centrifugation for 10 min at 1000 g the supernatant (2 ml) was layered onto gradients of 2 ml steps of 3, 10, 15 and 23% Percoll (in sucrose/EDTA/dithiothreitol) and centrifuged at 32,500 g for 5 min. Synaptosomes collected from the 10-15% interface (fraction 6) and the 15-23% interface (fraction 8) were washed once in 0.32 M sucrose and twice in Krebs solution (containing 0.1 mM calcium). Synaptosomes were preincubated at 37°C for 60 min, followed by 60s incubation in the same buffer, or in depolarizing Krebs. Incubations were terminated by centrifugation and the pellet homogenized in 0.1 M perchloric acid and recentrifuged. DA and 5-HT were determined in the supernatant by HPLC.

Striatal fraction 8 synaptosomes contained a significantly greater amount of endogenous DA (43.6 ng/mg protein) than fraction 6 (29.3 ng/mg), and upon depolarization endogenous levels were significantly reduced to 84% (fraction 8) and 86% (fraction 6). In contrast, endogenous 5-HT levels were greater in fraction 6 (2.46 ng/mg) than fraction 8 synaptosomes (1.39 ng/mg), and upon depolarization levels were reduced to 77 and 72%, respectively. Most of the neurotransmitter released from the synaptosomes was recovered in the extrasynaptosomal buffer as 5-HT, or as DA plus DOPAC. The results demonstrate for the first time that the two synaptosomal populations isolated on Percoll density gradients are functionally capable of releasing endogenous neurotransmitter stores. The results also raise the possibility that a relative enrichment of dopaminergic synaptosomes in fraction 8 and of serotonergic synaptosomes in fraction 6 has been achieved. This supports the suggestion that these fractions may represent two populations of functionally distinct synaptosomes.

88.9 ACETYLCHOLINESTERASE IN CLONAL NICOTINIC SYNAPTIC TRANSMISSION. F.-C. T. Chang and M. Adler. Neurotoxicology Branch, Physiology Division, USAMRICD, APG, MD 21010-5425.

A cell culture model system was utilized to investigate the actions of acetylcholinesterase (AChE) inhibitors on nicotinic synaptic transmission. The presynaptic element consisted of neuroblastoma x glioma hybrid cell NG108-15, which synthesizes, stores and releases acetylcholine (ACh); the postsynaptic element was subserved by the clonal G8-1 myotube, which expresses nicotinic ACh receptors in high density but has low AChE activity. This biclonal synapse is devoid of junctional folds, connective tissues and Schwann cell processes. The primary cleft is approximately 20-50 nm and contains discontinuous basal lamina.

We have previously demonstrated some direct effects of the irreversible organophosphorous agent diisopropylfluorophosphate (DFP) on the ACh receptor-channel macromolecule. These consisted of a depression in the miniature synaptic potential (MSP) amplitude, a shortening in the open channel lifetime and an enhancement in the desensitization rate. However, washout of unreacted DFP did not lead to the typical increases in the synaptic potential decay phase that accompany inhibition of AChE at the motor endplate. In this study, we have investigated possible reasons for the failure of clonal synapses to show prolongation of the synaptic response after AChE inhibition. The absence of prolongation suggests that in the NG108-15/G8-1 synapse, AChE is not rate limiting for removing transmitter from the synaptic cleft. This may arise because AChE is not sufficiently concentrated or correctly localized in the synapse. There are two possible consequences: (1) Diffusion of ACh may be adequate to clear transmitter from the synaptic cleft. If so, the macroscopic miniature synaptic current (MSC) lifetime should approach the limit imposed by the ACh channel gating reaction, irrespective of AChE activity. (2) Alternatively, if diffusion is slow (as at the motor endplate), the MSC decay will be longer than the mean single channel lifetime under all conditions. These two possibilities were examined by comparing the single channel open time measured in patch-clamp experiments with whole cell voltage-clamp recordings of the MSC. Under control conditions, the mean single channel open time at 37°C and -80 mV was 1.82 ± 0.14 msec (n=4). The MSC decay time constant, measured at the same temperature and membrane potential, was found to exceed this value by a factor of 1.5 to 4.3. Neither the mean channel open time nor the MSC decay time constant showed any systematic changes after exposure and subsequent washout of 100-500 μM DFP. These results indicate that the failure of DFP treatment to prolong the MSP and MSC decay stems from the fact that it is already prolonged under control conditions.

88.10 MOLECULAR EFFECTS OF ROUS SARCOMA VIRUS TRANSFORMATION ON CULTURED CHICKEN MYOTUBES. D.T. Anthony* and L.L. Rubin. Rockefeller Univ., New York, NY 10021.

Chicken myotubes infected before fusion with a temperature sensitive mutant (tsNY68) of Rous sarcoma virus (RSV) do not cluster acetylcholine receptors (AChRs) either spontaneously or in response to exogenous clustering factors (Anthony, D.T. et al., PNAS, 81: 2265, 1984). This defect in AChR clustering is observed even at the nonpermissive temperature for viral transformation, but nonetheless is due to the presence of the transforming gene src of RSV, which codes for a membrane associated tyrosine protein kinase $pp60^{src}$. We have tried to identify changes in transformed muscle cells which make them unable to cluster AChRs and might, thereby, prove useful in specifying molecular events which normally contribute to the maintenance of high densities of AChRs at the neuromuscular junction. First, we investigated the possibility that residual $pp60^{src}$ kinase activity at the nonpermissive temperature is sufficient to phosphorylate an AChR cluster-associated molecule; in fact, it appears that AChRs themselves are phosphorylated. AChRs were purified from ^{32}P-orthophosphate labeled cell cultures by chromatography on cobratoxin-Sepharose followed by con A-sepharose. The α-subunit, identified in ^{35}S-methionine labeled cultures, was not phosphorylated. Two other subunits, molecular weights 46-50 kd and 55-60 kd, were phosphorylated on serine and tyrosine residues in infected cells. The function of AChR phosphorylation is currently being explored. We also looked for other differences between infected and noninfected cells and found that a 37 kd protein was greatly decreased in the infected cells. Using anti-chick gizzard tropomyosin antibodies (supplied by Dr. F. Matsumara, Cold Spring Harbor Labs), we identified the 37 kd component as one of several tropomyosin isoforms. Future studies will try to determine whether the 37 kd tropomyosin and its interaction with microfilaments has any role in the clustering of AChRs.

88.11 AMPLITUDE FLUCTUATION ANALYSIS OF GROUP Ia SINGLE-FIBER EPSPs IN CAT SPINAL MOTONEURONS. H.-R. Lüscher and H.P. Clamann. Dept. of Physiology, University of Zürich, Switzerland.

The complex morphology of the Ia-motoneuron synapse determines many aspects of its function. The terminal branches of Ia fibers contact a motoneuron with numerous boutons at different sites. Impulses in a Ia fiber do not activate all these boutons; instead, boutons respond to a variable fraction of the impulses ranging from 0 to 1. It is not justified to lump these diverse boutons with their unique behaviors into a single equivalent bouton and to apply classical quantal analysis to this central synapse.

Analysis of amplitude fluctuations of single fiber EPSPs may be used to gain insight into the junctional mechanisms at these synapses, but is limited by the noise of other EPSPs. It is possible to remove the masking effects of this noise and to calculate the amplitude distribution of the fluctuating EPSP by the process of maximum likelihood estimation (MLE). This requires two assumptions: 1) the mechanisms generating the noise are statistically independent of those producing the EPSP fluctuations, and 2) signal and noise add linearly. The variance σ^2 of the gaussian noise amplitude can be measured directly and the problem reduces to finding the mean (μ_k) and membership probability (p_k) of each component of a gaussian mixture distribution of amplitudes. A Monte-Carlo study evaluated the performance of the MLEs for μ_k and p_k of mixtures of computer-generated normal distributions. The estimator reliably found these parameters when $\min|\mu_k-\mu_{k+1}|>1.2\sigma^2$. No a priori assumptions about the statistical distribution of the EPSP amplitudes are made.

The amplitude of 1024 EPSPs produced by a single Ia-motoneuron contact system were measured and the probability density (PD) of the peak amplitude fluctuations was calculated together with the PD of the noise amplitude. MLEs for μ_k and p_k for each of k components of a finite mixture of normal distributions were used to determine the distribution of amplitudes of the fluctuating EPSPs. Most EPSP amplitudes fluctuated among discrete levels which were integer multiples of the increment between successive levels. This increment ranged from 86 μV to 160 μV (mean 121 μV, n=19 contact systems). The PD of the EPSP peak amplitude could not be described by Poisson or binomial statistics. These results suggest that the incremental EPSP reflects the all-or-none action of a single synaptic bouton and that the probability of transmission failure varies among the boutons making up a single Ia-motoneuron contact system. The results are consistent with the hypothesis of incomplete action potential propagation into the terminal arborization as a mechanism for EPSP fluctuations. (Supported by Swiss National Foundation Grant no. 3.308-0.82.)

88.12 QUANTAL ANALYSIS OF POSTSYNAPTIC CURRENT FLUCTUATIONS IN HIPPOCAMPUS. G. Barrionuevo, S. Bench* and T.H. Brown. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

The quantum hypothesis and the conventional analytical techniques derived therefrom are valuable for analyzing changes in synaptic microphysiology. Because the conventional methods of quantal analysis are based on release probability models whose assumptions may not hold and are difficult to assess (Brown et al., PNAS, 73:2913, 1976), we have been developing a new method that provides accurate information about the synaptic microphysiology even when these questionable assumptions are relaxed.

The new method, which is based on a cepstral-like algorithm, was tested in three independent circumstances. We first applied the method to computer-simulated synaptic release data that were generated by the convolution of a compound binomial release function with a Gaussian quantal density function and a Gaussian noise density function (Bench et al., Fed. Proc., 44:1388, 1985). Next, the method was applied to experimental data obtained from the crayfish neuromuscular junction (Keenan et al., Soc. Neurosci. Abstr., 11: in press). In both cases the new method accurately extracted the quantal release parameters.

Here we report preliminary results of a third test of the new method. Mossy-fiber postsynaptic current fluctuations were examined in CA3 neurons of the hippocampal slice (cf. Brown and Johnston, J. Neurophysiol., 50:487, 1983; Johnston and Brown, In: Brain Slices, 1984). The object of these experiments was to determine whether the new method could correctly detect predicted changes in the mean quantal content m and the mean quantal size q at this cortical synapse. The postsynaptic cell was voltage-clamped at two different holding potentials (-65 and -95 mV). At each potential a series of paired-pulses were delivered to the mossy-fiber axons and several hundred evoked synaptic currents were collected for each pair of stimulations at both holding potentials. Facilitation of the second response amplitude should increase m but not q. By contrast, voltage clamping at more negative holding potentials, which increases the synaptic driving force, should increase q but not m.

The predicted changes in m and q were confirmed. The results of the three separate tests of the new method suggests that this approach may be useful in determining the quantal basis of long-term synaptic potentiation in hippocampus and other structures in which conventional methods of quantal anlysis may be inappropriate. (Supported by AFOSR Contract 49620, NIH Grant NS18295, and a McKnight Foundation Scholars and Development Award).

88.13 AFFERENT INPUT TO NON-PYRAMIDAL NEURONS IN CA1 OF GUINEA PIG HIPPOCAMPUS: LESION-INDUCED TERMINAL AND TRANS-SYNAPTIC DEGENERATION. D.D. Kunkel* and P.A. Schwartzkroin (SPON: D. Sutton) Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195.

Dendrites of pyramidal and granule cells have been regarded as the postsynaptic targets of afferents to hippocampus. Recently, it has been reported that commissural axons also terminate on non-pyramidal cells of the hippocampus (Brain Res., 1983, 265:289-293). In the course of our studies of hippocampal interneurons, we have investigated the direct afferent input to these non-pyramidal neurons. In adult guinea pigs, the fimbria/fornix was transected or a unilateral hippocampal lesion was made. Animals were allowed to survive for 1 to 7 days post-lesion, were then sacrificed and the brains prepared for electron microscopy. EM analysis of CA1 strata radiatum and oriens showed significant terminal degeneration associated with pyramidal cell dendrites (spines) at all survival periods.

Non-pyramidal cell types in these regions were identified on the basis of morphological criteria established from combined morphological/electrophysiological studies. Following the lesion, degenerating terminals contacting, or just retracting from, the non-pyramidal neuron profiles were common. The form of terminal degeneration on non-pyramidal neurons progressed through a distinct electron-lucent phase. At early survival periods (1-3 days), vacuolated and swollen terminals were common; they often contained synaptic vesicles in densely packed arrays near the synaptic specialization. At later survival periods (4-7 days), terminals were swollen, vesicles depleted and mitochondria were distorted. The electron-dense form of degeneration was seen only during this latter period. Postsynaptic changes in the dendrites of non-pyramidal cells were also observed in some cases. Particularly at later survival periods (3-7 days), many of the postsynaptic non-pyramidal neurons appeared to be in an early stage of degeneration, with condensed electron-dense cytoplasm. Such postsynaptic alterations were not seen in the pyramidal cell dendrites or somata receiving degenerating afferent terminals. A sub-set of interneuron profiles also seemed unaffected.

It is unclear if the effects of the lesion lead to complete interneuron degeneration. Electrophysiological studies showed that abnormally large EPSPs were produced in lesioned guinea pigs, but that IPSP activity was not completely abolished. Trans-synaptic degenerative effects specific to a sensitive interneuron population could have significant consequences, not only following afferent lesions, but also as a result of abnormal activity in afferent pathways.

These studies were supported by NIH grants NS18895 and NS20482, and NSF grant BNS82009906.

88.14 EFFECTS OF PRE-INCUBATION ON CA3 PYRAMIDAL CELLS OF HIPPOCAMPUS IN VITRO. A.K. Davis*, D. Janigro*, and P.A. Schwartzkroin, Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195

Tissue pre-incubation is a procedure commonly used by investigators studying brain slices in vitro. Maintaining slices in normal, oxygenated medium, often at relatively cool temperatures, is an attractive method of holding large numbers of slices before subjecting individual slices to specific treatments. However, the effects of the pre-incubation procedure itself have not been carefully evaluated. Using the in vitro hippocampal slice technique, we have examined the effects of tissue pre-incubation on cellular properties of hippocampal CA3 pyramidal cells.

Pre-incubated slices were maintained in an oxygenated, static bath holding chamber, at 21 or 30°C, prior to transfer of the slices to an interface chamber (constant perfusion, 35°C.) for electrophysiological study. Characteristics of these cells were compared to those of cells in slices which were transferred directly to an interface chamber after slice cutting. Intracellular recordings from CA3 pyramidal cells revealed significant differences between the pre-incubated (P) and non-pre-incubated (NP) tissue. Pre-incubated tissue showed more negative resting membrane potentials (NP = -57 mV, P = -66 mV); increased input resistance (NP = 39 meghoms, P = 47 meghoms); larger synaptic potentials in response to a given stimulus strength (both EPSPs and IPSPs, with a particularly large effect on the late IPSP); lower threshold for stimulus-induced burst discharge; and larger hyperpolarizing afterpotentials. CA3 cells in pre-incubated slices were also more likely to show spontaneous synchronized burst discharge, as seen in simultaneous recording from pairs of pyramidal cells. The cells in the CA3 region of pre-incubated slices were relatively resistant to hypoxia-induced spreading depression (SD). Latencies to SD in NP slices averaged 3.15 minutes, while SDs in pre-incubated slices were often not elicited by the hypoxic challenge or seen only after exposures of 30 to 55 minutes in 12% O_2. CA1 pyramidal cells in pre-incubated slices showed little difference from their non-pre-incubated counterparts in most cell properties, including sensitivity to hypoxia.

A variety of factors could be involved in the observed differences between pre-incubated and non-pre-incubated tissue. Whatever the mechanisms, however, it is important to note that tissue pre-incubation can alter the character of CA3 activity. Such changes may complicate comparison of data between laboratories and between in vitro and in vivo preparations.

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88.15 EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON THE FINE STRUCTURE OF THE DENTATE FASCIA IN MICE. J.A. Markham, E. Fifková and A.J. Scheetz*. Dept. of Psych., Univ. of Colo., Boulder, CO 80309.

Long-sleep (LS), short-sleep (SS), and heterogeneous (HS) lines of mice that were bred for differential sensitivity to ethanol at the Institute of Behavioral Genetics, University of Colorado at Boulder, were used in these experiments. Thirty mice from each line were divided into three groups and fed with a standard laboratory diet, control isocaloric liquid diet, and isocaloric liquid diet with ethanol, respectively. The ethanol diet was administered according to the following scheme: for the first 7 days only the liquid control diet was given and was followed by diets with increasing concentrations of ethanol derived calories, 11.6%, 23.3%, and 35% for 2, 11, and 7 days, respectively. Consumption of the liquid diets was monitored twice a day and the body weight once a day. Although all strains tolerated well the ethanol diet, all animals on the 35% ethanol diet started to lose weight during the last two days. The loss represented on the average 6.5% of the body weight. Therefore, the experiment was terminated at this time. However this weight loss was not associated with a decreased consumption of the ethanol diet. Animals were perfused transcardially and prepared for electron microscopy. The liquid control diet had no effect on the weights of the HS and SS as compared with the laboratory diet. However, the LS gained weight significantly (13%) on the liquid control diet. Fifty percent of the material has been so far evaluated morphometrically: cross section area, perimeter, and density of dendritic spines and axon terminals have been measured. Given the distinct stratification of the input to the dentate molecular layer, the layer was divided accordingly into thirds, and the results were expressed as an average per third, per line, and per diet. There were no differences in the dimensions and density of dendritic spines across the lines. The ethanol treatment did not have any consistent impact on these parameters when comparison was made with the laboratory diet. However, with the liquid control diet, variations in dimensions of spines were noted. The density of axon terminals did not change across the lines as a function of the various diets. There was also no consistent effect on axon terminals of the HS with the ethanol diet. However, axon terminals of the SS were consistently smaller with the ethanol treatment in all thirds of the dentate molecular layer. On the other hand, the LS terminals were larger in the proximal and distal thirds of the dentate molecular layer. These results are in agreement with the differential sensitivity of the LS and SS to ethanol administration.

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88.16 EFFECT OF CHRONIC ETHANOL EXPOSURE ON THE FINE STRUCTURE OF THE STRATUM ORIENS OF CA_1 HIPPOCAMPAL FIELD. A.J. Scheetz*, J.A. Markham and E. Fifková. Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

Lines of mice with differential sensitivity to ethanol (10 long-sleep, LS; 10 short-sleep, SS; 10 heterogeneous, HS) obtained from the Institute of Behavioral Genetics (University of Colorado at Boulder) were exposed to a chronic ethanol diet for 20 days during which the percentage of ethanol derived calories was raised from 11.6% to 35%. Prior to the start of the ethanol diet administration, mice were exposed for a week to the liquid isocaloric diet without ethanol. With such a pretreatment mice tolerated the ethanol diet for a longer period of time than they would without the pretreatment. The experiment was terminated when some of the ethanol exposed mice started to lose weight for two days. Mice which were fed with the laboratory diet (10 per line) and with control liquid diet (10 per line) were used as controls. Mice were perfused transcardially with a mixture of aldehyde and prepared for electron microscopy. In order to minimize the possible impact of the aldehyde fixation and tissue preparation on the fine structure of the hippocampus, animals from each line were maintained in groups of three and were fed with the laboratory control diet, control liquid diet, and the liquid ethanol diet, respectively. These groups were perfused and prepared for electron microscopy at the same time. Ultrathin sections were prepared from the stratum oriens of the CA_1 hippocampal field, which contains the basilar dendrites of the CA_1 pyramids and commissural afferents. Cross section area and perimeter of dendritic spines and axon terminals were measured. Preliminary data of these measurements have shown that the average cross section area of spines was the smallest in the HS and the largest in the SS. On the other hand, axon terminals were the smallest ones in the SS and the largest in the HS. With ethanol exposure, axon terminals in the HS and LS became smaller while in the SS they were enlarged. The effect of ethanol on dimensions of dendritic spines is being studied.

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88.17 THREE DIMENSIONAL STRUCTURE OF DENDRITIC SPINES IN THE RAT HIPPOCAMPUS (CA1) AND CEREBELLUM. K.M. Harris, J. Trogadis*, and J.K. Stevens. Neuroscience Dept., Children's Hospital, Boston, MA 02115 and Playfair Neuroscience, Univ. of Toronto, Toronto Ontario M5T 2S8.

Several studies have shown that dendritic spine morphology and density varies during development, with location in the CNS, and with synaptic activity. Theoretical models of spine function have suggested that changes in spine shape might provide a mechanism for modulating synaptic efficacy. Quantitative analyses of parameters that might account for differences in spine size and shape should begin to provide a rationale for this variability.

Long-evans hooded rats were perfused with 2% paraformaldehyde and 2.5% gluteraldehyde in .1 M sodium cacodylate buffer at pH 7.35 and 37°C. Hippocampus and cerebellum were removed, sliced at 400 um, postfixed with 1% OsO_4 or K-Fe reduced OsO_4 and some were stained en bloc with uranyl acetate and embedded in epon. Silver serial sections were mounted on formvar coated slot grids and stained with lead citrate. Four dendritic segments, 4-6um long with 51 spines from the middle of stratum radiatum were reconstructed using a computer system (Stevens and Trogadis, Adv. Cell. Neuro., 1985). For comparison, a cerebellar dendritic segment, 4 um long with 27 spines was reconstructed.

Spine volumes ranged from 0.016 to 0.235 um^3 in CA1 and from 0.07 to 0.15 um^3 in cerebellum. Smooth endoplasmic reticulum (SER) occupied about 15% of CA1 and cerebellar spine volume, with larger spines containing more SER (r=.80). CA1 spine surface areas were 0.22-2.14 um^2 and synapses 0.0056 - 0.213 um^2 occupied about 8% of this surface. Cerebellar spine surface areas were .33 - 1.66 um^2 and synapses ranging from 0.035-.265um^2 occupied about 10% of the spine surface. In area CA1, spine lengths, head and neck diameters and neck lengths showed no significant relationships with one another, and long spines were either thin or fat, and thin spines were either short or long suggesting that spine shapes are part of a continuum. Spine shape and size was more uniform in cerebellum.

Sixteen axonal varicosities presynaptic to CA1 spines and 15 varicosities presynapic to cerebellar spines were reconstructed. In CA1, 15 of these varicosities made only the 1 spine synapse. One CA1 varicosity also formed a second synaptic junction on a spine of a neighboring dendrite. In cerebellum, 6 varicosities formed 2 synaptic junctions with 2 spines on the same dendritic segment, and 1 varicosity formed a second asymmetric synapse on the shaft of a neighboring dendrite. In CA1, the volume of these varicosities ranged from .033-.232 um^3 and was correlated with spine volume (r=.72) and synaptic area (r=.67).

These preliminary results suggest that spine size is related to SER content, synaptic area and size of the presynaptic varicosity.

SPECIAL LECTURE

89 SPECIAL LECTURE. NEUROCHEMICAL SPECIALIZATIONS IN THE BASAL GANGLIA: THE OLD MOTOR SYSTEM IN A NEW LIGHT. A.M. Graybiel, MIT.

The fiber pathways of the basal ganglia include through-conduction lines and side loops associated with the striatum, pallidum and substantia nigra. By way of the pallidum and substantia nigra, the basal ganglia can influence not only parts of the premotor and supplementary motor cortex, and brainstem sensorimotor regions such as the superior colliculus, but also regions of prefrontal cortex and other structures associated with the limbic system. A remarkable chemical anatomy has been discovered in relation to the basal ganglia, so that many of the fiber pathways associated with the basal ganglia can now be viewed as making up neurochemically specialized subsystems of the extrapyramidal motor system. Much of this neurochemical specificity becomes established at the main input side of the system, the striatum, by means of a compartmental organization that affects both its neurotransmitter systems and its input-output relations.

Key elements of this compartmental organization are the striosomes ("striatal bodies"), first recognized as macroscopically visible zones of low acetylcholinesterase (AChE) activity in sections through the striatum. In all mammals studied (including human) these AChE-poor striosomes form parts of three-dimensional labyrinths. Nearly all of the neurotransmitter-related compounds known to exist in the striatum are now known to be arranged in patterns reflecting the striosomal mosaics. This is true for a number of neuropeptides and for ligand binding sites thought to serve as neuropeptide receptors, and holds for markers of the classical GABA and acetylcholine transmitter systems of the striatum as well. There is also strong evidence that at least part of the dopamine-containing innervation of the striatum observes striosomal ordering: during ontogeny the early-developing "dopamine island" fibers innervate regions that become striosomes at maturity, and subsets of nigral fibers innervating striosomes can be labeled in adults.

This neurochemical patterning is of increasing interest because of evidence that striosomal compartmentalization reflects a plan of organization impressed on nearly all afferents and efferents of the striatum and on the arrangement of striatal interneurons as well. Fibers from the cortex, thalamus and amygdala either "fill" or avoid striosomes depending on their sites or origin, and neurons of the striosomes and of the extrastriosomal matrix have different efferent connections. It is rapidly becoming possible, therefore, to relate the specialized neurochemical properties of the striosomes and extrastriosomal matrix with different chemically coded efferent pathways, and to relate these to particular afferent and interneuronal modulation. The functional implications of these findings are potentially great, for they suggest that individual subsystems in the basal ganglia may be open to specific intervention in the clinical situation and to finely individuated control under normal physiological conditions.

91 SYMPOSIUM. MOLECULAR SYNAPTOGENESIS: TRANSITION FROM GROWTH CONE TO SYNAPSE. M.C. Fishman, Harvard, MGH, HHMI (Chairperson); C.S. Goodman, Stanford University; P.H. Patterson, California Institute of Technology; R.T. Ambron, Columbia University; M. Nirenberg, NIH.

The growth cone of the presynaptic neuron evidences directed outgrowth, and selective cell contact and fasciculation. It eventually ceases probing and evolves into a mature synapse upon appropriate target neurons. M.C.F. will provide an introductory overview of cellular and molecular mechanisms by which a presynaptic cell may find appropriate targets. He will indicate evidence for changes in gene expression and protein transport in the presynaptic cell during the transition from growth cone to synapse. C.S.G. will discuss the cellular and molecular analysis of cell recognition by neuronal growth cones in grasshopper and Drosophila embryos where experimental manipulations and monoclonal antibodies have suggested the differential expression of many different surface recognition molecules. The genes for these molecules are being isolated. P.H.P. will discuss the identification of neurite outgrowth promoting factors, such as the extracellular matrix proteoglycan, and will emphasize the interplay of neuronal proteases and their inhibitors with this proteoglycan. R.T.A. will discuss the use of large identified Aplysia neurons in culture for combined electrophysiological, biochemical, and morphological studies of the cell cell interactions that accompany selective synaptogenesis, and discuss efforts to isolate proteins of the growth cones of single neurons. M.N. will discuss his observations that elevation of cAMP levels of neuroblastoma or hybrid cells shifts relatively undifferentiated cells to a differentiated state and increases synaptogenesis. Cloned cDNA probes have been obtained for species of mRNA that are positively or negatively regulated by dibutyryl cAMP in these cells.

92 SYMPOSIUM. COORDINATION OF RESPIRATORY AND CARDIOVASCULAR HOMEOSTASIS. J.L. Feldman, Northwestern University (Chairman); D.L. Eckberg, Medical College of Virginia; G.L. Gebber, Michigan State University; C. B. Saper, University of Chicago; K.M. Spyer*, Royal Free Hospital, U.K..

Homeostatic regulation in mammals requires gas exchange between air and blood in the lung and appropriate delivery of this blood to and from the body tissues. Thus, oxidative metabolism is sustained and acid-base balance maintained. This requires precise nervous system control and coordination of ventilation and blood flow. In the past few years, it has become increasingly apparent that there are many common anatomical, physiological and pharmacological features of respiratory and cardiovascular control systems. A central substrate for the coordination of these functions is suggested by the observations: i) respiratory premotor and motor neurons are markedly influenced by cardiovascular variables, and; ii) preganglionic autonomic neurons, e.g. cardioinhibitory vagal motoneurons, are actively inhibited by central inspiratory activity. This symposium is an opportunity to fuse the results of several complementary approaches to cardiopulmonary regulation. The central theme concerns homeostasis and movement, which represent the major actions of the brain; we will address issues of rhythmogenesis and pattern generation and discuss mechanisms for brainstem and spinal cord integration and the role of cortical structures in modulating these functions. Data will be presented from man, cat and rodent. Dwain Ekberg will discuss the respiratory modulation of human autonomic outflow based on experimental observations on humans. Single unit sympathetic nerve activity and sophisticated analysis of heart rate provide the basis for assessment of autonomic cardiovascular outflow. This talk will set the groundwork for the subsequent talks, which address detailed observations in reduced mammalian preparations. Jack Feldman will review nervous system control of respiratory movements with emphasis on the basic neural properties underlying respiratory rhythm generation and sensorimotor integration. Recent evidence inplicating these mechanisms in central modulation of cardiovascular control will be discussed. Gerry Gebber will review the generation of sympathetic nerve discharge. He will discuss the physiology of both intrinsic brainstem and bulbospinal neurons controlling basal sympathetic nerve discharge. Recent results on the brain stem locus of respiratory-sympathetic interactions will be presented. Mike Spyer will discuss central mechanisms coordinating respiratory and cardiovascular functions. He will review the physiology and pharmacology of brainstem mechanisms controlling heart rate and blood pressure. Recent results on the respiratory inputs to cardioinhibitory vagal motoneurons and preganglionic sympathetic neurons will provide a basis for describing the integration of respiratory and cardiovascular control. Cliff Saper will describe the common features of the central nervous system anatomy underlying cardiovascular and respiratory function and will discuss the separation, overlap and pharmacology of brainstem structures important in cardiovascular and respiratory control. This data will lead to a discussion of the structural basis for cardiopulmonary coordination. He will include recent physiological data supporting convergence of respiratory and cardiovascular inputs onto parabrachial nucleus neurons.

OPIOID RECEPTORS

93.1 OPIOID RECEPTOR SUBTYPES IN DROSOPHILA MELANOGASTER. C. Santoro,* L.M. Hall and R.S. Zukin, (SPON: N. Sharpless) Departments of Biochemistry and Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

Multiple classes of opioid receptors have been defined by many approaches, but the molecular basis of this diversity is not yet clear. The question arises as to whether the different receptor types represent different gene products or are posttranslational modifications of a single gene product. The fruit fly Drosophila melanogaster provides a particularly suitable model system in which to address this issue because of the ease with which it can be manipulated genetically. Opioid receptor types present in membrane extracts prepared from heads of the wild-type Canton-S Drosophila melanogaster have been characterized. The μ selective opioid ligand [^{3}H]dihydromorphine (DHM) binds to an apparent single class of high affinity sites with an affinity constant of 125 nM and a maximal number of binding sites of 1590 fmol/mg protein. Binding of [^{3}H]DHM was also analyzed by a Hill plot; the analysis showed a Hill coefficient of 1.0. The binding is saturable and is inactivated by heat treatment suggesting a proteinaceous component. This binding can be displaced by several μ selective ligands but is unaffected by 10^{-5} M D-Ala^2-D-Leu^5-enkephalin (DADLE, a δ selective ligand). In addition, we have been unable to detect any specific [^{3}H]DADLE binding in these extracts suggesting there are no δ receptors in this preparation. We have also looked at K receptor binding. The prototypic K ligand [^{3}H] ethylketocyclazocine (EKC), in the presence or absence of the μ and δ blockers (DAGO and DADLE), binds to an apparent single class of receptors with an affinity constant of 90 nM and a B_{max} of 750 fmol/mg protein. Preliminary data also suggest the presence of σ receptors. Thus we have defined at least two opioid receptor subtypes in Drosophila. In feeding experiments, when wild-type Drosophila are grown throughout development on medium containing either morphine sulfate or etorphine, development is substantially delayed relative to drug-free controls. At the highest concentrations of morphine sulfate used, only 0 to 10% developed to the adult stage. These experiments provide a pharmacological screening strategy for the isolation of single gene mutations affecting this receptor system. Such mutants can potentially provide the means to manipulate genetically each distinct class of receptors independently of the others. This would allow the determination of the physiological and behavioral significance of each class of receptors.

93.2 CHARACTERIZATION AND VISUALIZATION OF BRAIN KAPPA OPIATE RECEPTORS: A COMPARATIVE STUDY OF RAT AND GUINEA PIG. M. Eghbali*, A. Tempel, S. Henriksen, and R. S. Zukin Departments of Biochemistry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461 and Department of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

A comparative study of the biochemical properties and anatomical distribution of the K receptor in guinea pig and rat brain was carried out. K receptors were labelled using the prototype K ligand [^{3}H]ethylketocyclazocine ([^{3}H]EKC) in the presence of the D-Ala^2,N-Me-Phe^4,Gly-ol^5-enkephalin (DAGO) and D-Ala^2, D-Leu^5-enkephalin (DADLE), which block the binding of the radioligand to μ and δ receptors. Scatchard analysis of [^{3}H]EKC binding to rat brain membranes (in the absence of blockers) revealed a curvilinear plot which could be resolved into 2 components; K_d1 = 0.73 ± 0.14 nM, B_{max} 1 = 94 ± 19 fmol/mg; K_d2 = 8.7 ± 0.6 nM, $B_{max}2$ = 252 ± 1 fmol/mg. In the presence of DAGO and DADLE, Scatchard analysis gave a straight line; receptor affinity and density were similar to those of the lower affinity component obtained in the absence of blockers. Longer incubation times did not significantly alter these results. In the case of guinea pig brain, the inclusion of μ and δ blockers again afforded a linear Scatchard plot (K_d = 7.3 nM, B_{max} = 118 fmol/mg at 60 min). Increasing the incubation time to 150 min in this case resulted in an increase in K_d and decrease in B_{max}. Possible mechanisms underlying this change in K_d are 1) a time-dependent conformational change in the K receptors, 2) a coupling of the receptors to a second protein such as N_i, or 3) aggregation of receptor proteins. In order to test the first hypothesis, N-ethylmaleimide (NEM) inactivation of K receptors was carried out. K receptors in guinea pig brain homogenate incubated 60 min exhibited a markedly different rate of N-ethylmaleimide inactivation than did K receptors incubated 150 min. These findings suggest that guinea pig brain K receptors undergo a time-dependent conformational change. Visualization of [^{3}H]EKC binding to rat and guinea pig brain slices by autoradiography in the presence of DAGO and DADLE revealed similar distribution patterns of K sites in the midbrain and hindbrain, but striking differences in the thalamic and hypothalamic areas. These data suggest that, although there is a similar density of K receptors in rat and guinea pig brain, the neuroanatomical distributions of the receptors differ in the two species.

93.3

WITHDRAWN

93.4 ELECTROENCEPHALOGRAPHIC CHARACTERIZATION OF MU AND DELTA OPIOID RECEPTOR ACTIVITY IN RATS. F.C. Tortella, L. Robles*, H.I. Mosberg* and J.W. Holaday, Neuropharmacology Br., Dept of Med. Neurosciences, Walter Reed Army Inst. of Research, Washington, DC 20307 and Dept. of Med. Chem., Univ. of Michigan, Ann Arbor, MI 48109.

The availability of highly selective ligands for the μ and δ opioid receptor types has allowed a critical evaluation of the receptor mechanisms responsible for enkephalin-induced electroencephalographic (EEG) responses in rats. For example, we reported that the conformationally constrained δ selective enkephalin analogs D-Pen^2-D-Pen^5 enkephalin (DPDPE) and D-Pen^2-L-Pen^5 enkephalin (DPLPE) do not produce EEG seizure activity in rats (Tortella et al., Neuropeptides 5:233, 1984). Furthermore, the highly selective μ receptor ligand Try-D-Ala-Gly-NMe-Phe-Gly-ol (DAGO) was recently reported to be 9x more potent than the mixed mu- and delta-directed ligand D-Ala^2-D-Leu^5 enkephalin (DADL) as an activator of EEG seizures in rats (Tortella et al., Fed. Proc. 44: 1343, 1985). Consonant with these findings, enkephalin-induced non-convulsive ictal activity appears to be solely mediated at μ opioid receptors. In the present study, DAGO- and DPDPE-induced EEG activity were compared using computer derived spectral analysis, and the opioid antagonists β-funaltrexamine (β-fNA, selective μ), naloxone (μ and δ), and ICI 174,864 (ICI, selective δ) were used to confirm agonist opioid receptor specificity.

Male S.D. rats (225-275 g) were prepared with bipolar cortical electrodes, right lateral i.c.v. cannulae and, when appropriate, external jugular vein catheters. Injections of DAGO (0.25-4.4 nmol, i.c.v.) resulted in two distinct EEG responses: 1). initial nonconvulsive ictal afterdischarges characterized by marked increases in EEG spectral activity in the 1-2 Hz and 12-30 Hz bands, 2). and a sustained period of high voltage slow-wave activity (HVSA) with a peak frequency of 2.0-2.5 Hz. These EEG effects of DAGO were antagonized by naloxone (0.01-1.0 mg/kg, s.c.) and β-fNA (9.8 μg, i.c.v.), but not by ICI (3.0 mg/kg, i.v.). In contrast, DPDPE (35-140 nmol, i.c.v.) failed to cause seizure activity in any of the rats tested. However, the administration of DPDPE produced a complex EEG response characterized by an increase in spectral activity in the 0-10 Hz band: most noteably HVSA with a peak frequency of 4.5-5.5 Hz, and intermittent theta driving (6.5-7.5 Hz) associated with intense behavioral arousal. Similar EEG profiles were observed with DPLPE. The DPDPE-induced HVSA was partially antagonized by high (10 mg/kg)(δ ?), but not low (1.0 mg/kg)(μ ?), doses of naloxone. Furthermore, pretreatment with ICI (3.0 mg/kg, i.v.) clearly antagonized the DPDPE-induced HVSA, causing a 5 fold shift to the right in the EEG power dose-response curve. Studies with β-fNA and DPDPE are in progress. DPDPE-induced theta activity was not altered by any of these antagonists.

Given the respective receptor selectivities for the various agonists and antagonists used, we propose that at least four distinct EEG responses are produced by enkephalins: a μ receptor mediated ictal activity, a μ receptor mediated HVSA (2.0=2.5 Hz), a δ receptor activated HVSA (4.5-5.5 Hz), and a non-opioid theta driving.

We thank M. J. Rance and R. Cotton of ICI Pharm. for ICI 174,864.

93.5 COMPARISON OF CROSS-LINKED β-ENDORPHIN LABELED OPIATE RECEPTORS FROM RAT, LEECH, AND TETRAHYMENA. B. Zipser†*, M.R. Ruff†, W. Higgins* and C.B. Pert†. †Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, Maryland 20205, and *University of Maryland, College Park, Maryland.

Cross-linking is a relatively recent biochemical strategy for covalently affixing reversible ligands to their recognition molecules for subsequent electrophoretic analysis. [^{125}I]Tyr^{27}-β-endorphin (prepared as originally described by Smythe and co-workers) binds stereospecifically to rat brain membranes. While some studies have suggested that the β-endorphin receptor is a unique "epsilon" opiate receptor, a larger body of evidence suggest that β-endorphin has high affinity for most if not all of the opiate receptors types and subtypes. Cross-linking opiate receptors from different tissue sources can potentially reveal much information about the molecular basis of apparent opiate receptor heterogeneity. Cross-linking, however, only fixes 1% of the bound trace and SDS-PAGE while exquisitely sensitive, can fail to reveal substantial inter-molecular differences. Cross-linking was performed with the homo bi-functional reagent Disuccinimidyl Suberate (DSS). The iodinated cross-linking products of tetrahymena, leech CNS, and rat brain membranes (both type 1 and type 2 conditions) appeared indistinguishable on SDS-PAGE gel with major cross-linking products at 58K and 100-110K. The strong cross-linked bands produced by incubation in the presence of the inactive opiate ((+)-naloxone) was completely abolished by the same (10^{-6}M) concentration of its active isomer (-)-naloxone. Although we have thus far failed to distinguish between opiate receptors from a mammal, an invertebrate, and a unicellular organism, we continue to explore various conditions of binding, and electrophoresis, (e.g., reduced and unreduced) to examine possible receptor differences, both intra and inter species. Electrophoresis of proteolytic digests of cross linked bands will be performed as a particularly sensitive method for distinguishing heterogeneity. Thus far, our cross-linking experiment suggest that the recognition molecule (the opiate receptor) which binds all opiate alkaloids and peptides is stable across evolution. As proposed, apparent physiological receptor heterogeneity (Bowen, W.D., et al., Proc. Natl. Acad. Sci. USA, 78:4818, 1981) is due to coupling to other membrane components.

93.6 APPARENT DOWNREGULATION OF THE μ NON-COMPETITIVE δ BINDING SITE IN RAT BRAINS FOLLOWING REPEATED ELECTRO-CONVULSIVE SHOCK J.A. Danks*[1], F.C. Tortella [2], J.W. Holaday [2], V. Bykov*[1], A.E. Jacobson*[3], K.C. Rice*[3] and R.R. Rothman[1] [1]Lab. of Preclin. Pharmacol., NIMH, St. Elizabeth's Hosp., Wash., DC 20032; [2]Neuropharm. Branch, Dept. Med. Neurosci., Walter Reed Army Inst. Res., Wash., DC 20307 and [3]Lab. of Chemistry, NIADDK, Bethesda, MD 20205

It has been shown that repeated electroconvulsive shock (R-ECS) in rats results in a potent activation of endogenous opioid systems (Holaday et al, Mod. Prob. Pharmacopsych. 17,142,1981). However, discrepant data on the effects of R-ECS on binding parameters have been reported. The present studies were conducted to reevaluate the effects of R-ECS on opiate receptor binding parameters using selective site-directed agents.

Male S-D rats (275-300 g) were given repeated supramaximal transauricular ECS (50mA, 60Hz, 2sec) or sham ECS (S-ECS) daily for nine days. One day following the last ECS or S-ECS, brains were removed and frozen on dry ice. Brains were thawed, and lysed P2 membrane preparations were made. The lysed P2 preparations were incubated with 0.4M NaCl for one hour at 25°C to remove endogenous opioid ligands. Membranes were then treated with the site-directed opiate alkylating agents, BIT (to eliminate μ-non-competitive sites) or FIT (to eliminate μ-competitive sites) at a concentration of 1 μM (Rice et al, Science 220,314,1983). Membrane pellets were frozen at -80°C until assayed. To determine the Bmax and KD values, binding surfaces were constructed using ^{3}H-D-Ala^2-D-Leu^5 enkephalin (^{3}H-DADL) as the ligand in assay conditions as previously described (Rothman et al, Neuropeptides 4,257,1984). Each experimental group consisted of 54 data points from three separate membrane preparations. Results indicate that R-ECS significantly decreased the μ-non-competitive binding sites (* $p<0.05$):

	KD ± SD (nM)	Bmax ± SD (fmoles/mg prot.)	% of control
μ-competitive remain (BIT treated):			
S-ECS control	1.6 ± 0.1	174 ± 7	100
R-ECS	1.6 ± 0.1	157 ± 8	90
μ-non-competitive remain (FIT treated):			
S-ECS control	8.6 ± 1.0	183 ± 25	100
R-ECS	8.4 ± 1.5	133 ± 36*	72*

It was previously shown that R-ECS increases the apparent number of ^{3}H-DADL binding sites (Holaday et al, Life Sci. 31,2359,1982). The present studies, conducted using a different assay condition along with selective site-directed alkylating agents, provide preliminary evidence of the opposite effect. Specifically, R-ECS decreases (downregulates) the μ-non-competitive δ binding sites by approximately 28%. Further studies are being conducted to confirm and extend these observations on the effects of R-ECS on opiate binding parameters.

93.7 CHARACTERIZATION OF SOLUBLE β_h-ENDORPHIN-LABELED RECEPTORS FROM POST-MORTEM HUMAN STRIATUM. Daiga M. Helmeste and Choh Hao Li*. Laboratory of Molecular Endocrinology, University of California, San Francisco, CA 94143.

Tritiated human β-endorphin (^{3}H-β_h-EP) labeled receptors were solubilized from human striatal membranes using the zwitterionic detergent, CHAPS. Using a polyethyleneglycol precipitation method, we demonstrate that the solubilized receptor binds ^{3}H-β_h-EP in a displaceable manner.

	IC_{50} (nM)	
	membrane	soluble
β_h-EP-(1-31)	1.6	2.3
β_h-EP-(1-5)	22	> 1 μM
β_h-EP-(6-31)	no displacement at 1 μM	
Morphine	24	-
Naloxone	17	-
U50-488H	2112	-

While β_h-EP-(1-31) retained high affinity in the solubilized preparation, β_h-EP-(1-5) (met-enkephalin) lost its ability to compete for binding with high affinity. This phenomenon was also seen in the solubilized rat preparation.

Size differences (Stokes radius) could be demonstrated between human and rat, as estimated by Sepharose 6B molecular exclusion chromatography of prelabeled receptor preparations.

	Stokes radius (Å)
Human Caudate	80 ± 3
Human Putamen	72 ± 3
Rat Whole Brain	64 ± 1

Investigations of differences in other molecular parameters are in progress and their implications for a model of the opiate receptor will be discussed.

[DMH is supported by the Canadian MRC. Human CNS from Canadian Brain Tissue Bank, Toronto, Canada and National Neurological Research Bank, Los Angeles, CA.; work supported in part by NIDA (DA-03434)].

93.8 ENDOGENOUS LIGAND FOR THE SIGMA OPIOID RECEPTOR ("SIGMAPHIN") IN THE BRAIN: PRELIMINARY EVIDENCE. T.-P. Su* and A.D. Weissman.* (SPON: W.B. Pickworth) NIDA Addiction Res. Ctr., Baltimore, MD 21224.

The sigma opioid receptor was postulated by Martin, W.R. et al. (J. Pharmacol. Exp. Ther., 197:517, 1976) to mediate psychotomimetic effects of certain opiates and other drugs. Those effects included dysphoria, hallucinations and delirium. Sigma opioid receptors later were identified in guinea-pig brain homogenates using [^{3}H]SKF-10047 (Su, T.-P., J. Pharmacol. Exp. Ther., 223:284, 1982). Ligand selectivities of the sigma opioid receptor indicated that dextro-isomers of certain opioids were more potent than their counterpart levo-isomers and that several antipsychotics such as haloperidol and chlorpromazine were among the most potent ligands in the sigma receptor binding assay (Su, 1982). It was also indicated that sigma receptors were not dopamine receptors (Su, 1982). These findings on the sigma opioid receptors were later confirmed by several laboratories using [^{3}H]EKC (Tam, S.W., Proc. Natl. Acad. Sci., 80:6703, 1983), [^{3}H]3-PPP (Largent, B.L., et al. Proc. Natl. Acad. Sci., 81:4983, 1984) and [^{3}H]d-SKF (Martin, W.R., et al. J. Pharmacol. Exp. Ther. 231:539, 1984). Recently, in vivo data also showed that in pigeons, the increase in responding to food presentation induced by a sigma ligand d-SKF, could be antagonized by haloperidol (Katz, J.L., et al. J. Pharmacol Exp. Ther. 232:452, 1985). Taken together, these findings suggest that the sigma opioid receptor may repesent a psychotomimetic pathway in the brain. This study examined the possibility of existence of an endogenous ligand for sigma opioid receptors.

Guinea-pig brain was homogenized in 12 ml of ice-cold 0.1 N acetic acid using a Polytron. The suspension was further homogenized with a Dounce homogenizer and then stirred at 4°C for 2 hr. The suspension was then centrifuged at 12,000 rpm for 30 min. The supernatant was applied to a Sephadex G-50 column (1.5 x 90 cm). After lyophilization, each fraction from the column was assayed for inhibition of [^{3}H]SKF binding to sigma opioid receptor sites.

Three major peaks inhibiting [^{3}H]SKF binding to sigma opioid receptors were obtained. Peak I had an apparent mol. wt. of about 10,000 daltons. Peak II was broad, containing a larger number of fractions with major inhibitory activity at about 3,000 daltons. Peak III was the most potent and had an apparent mol. wt. of about 300 daltons. The experiment was repeated four times. Each experiment produced a similar inhibitory profile.

These results suggest that endogenous ligands for psychotomimetic sigma opioid receptors may exist in the brain. Results of this study also indicate that endogenous sigma opioid receptor ligands ("SIGMAPHIN") in the brain may be heterogenous.

93.9 OPIOID BINDING SITES INDUCED BY ETHANOL IN NG108-15 CELLS ARE FUNCTIONAL DELTA-OPIOID RECEPTORS. M.E. Charness and L.A. Querimit*, Department of Neurology and The Ernest Gallo Clinic and Research Center, University of California San Francisco, CA. 94110

Long-term ethanol exposure increases the expression of delta-opioid binding sites in cultured neural cells (Science 222: 1246-1248). We investigated whether these binding sites exhibit several pharmacologic properties of the delta-opioid receptor: Na^+ modulation of agonist binding, down-regulation of binding sites by agonists, and inhibition of cAMP production.

NG108-15 cells were cultured in the presence or absence of 200mM ethanol for 48 hours to increase delta-opioid binding sites by 85%. Inhibition of [^{3}H]d-[Ala^2]d-[Leu^5]enkephalin (DADL) binding by Na^+ was unaltered in the ethanol-treated cells. Moreover, 24 hours exposure to 10^{-8} M etorphine caused similar down-regulation of binding sites in ethanol-treated (46% ± 1.1) and control (61 ± 8.9%) cells.

The opioid receptor of NG108-15 inhibits cAMP production through its interaction with the inhibitory GTP binding protein complex, N_i. In this cell line, maximal opioid inhibition of cAMP occurs with fractional occupancy of receptor binding sites. Thus, if the binding sites induced by ethanol are functional receptors, then opiates should inhibit cAMP production more potently than in control cells, but to the same maximal level.

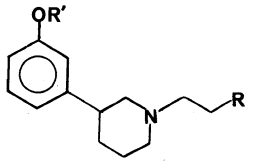
Cyclic AMP production in intact washed cells was determined by RIA. Basal levels were significantly higher in ethanol-treated cells (control 9.4 ± 1.8; ETOH 24.5 ± 1.6 pmol/mg/min), and 10^{-4} M phenylisopropyl adenosine raised cAMP to higher levels in the ethanol-treated cells (control 93.7 ± 8.1; ETOH 131 ± 6.6 pmol/mg/min). In control cells, etorphine inhibited stimulated-cAMP production by a maximum of 73 ± 4.9% with half-maximal inhibition (K_i) occurring at 2.7×10^{-9} M. In ethanol-treated cells, etorphine inhibited stimulated-cAMP production by 89 ± 2.1%, with a K_i of 7.7×10^{-10} M. Thus, ethanol induces the expression of functional opioid receptors, rendering these cells 3.5-fold more sensitive to opiates. The concomitant increase in opiate efficacy suggests that ethanol also alters receptor-effector coupling.

93.10 INHIBITION OF OPIOID BINDING TO RAT NEURAL MEMBRANES BY FAB FRAGMENTS FROM A MONOCLONAL ANTIBODY DIRECTED AGAINST THE OPIOID RECEPTOR. Jean M. Bidlack and Peter C. Mabie. Center for Brain Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

A monoclonal antibody, OR-689.2.4, capable of inhibiting opioid binding to rat neural membranes has been obtained (Bidlack and Denton, Neuropeptides, 5: 225-228, 1984). This monoclonal IgM was generated by immunizing a BALB/c mouse with a partially purified opioid receptor preparation. A procedure has been developed for obtaining Fab fragments from this mouse monoclonal IgM. The Fab fragments were able to inhibit opioid binding to membranes to a much greater degree than the whole IgM. The binding of the μ ligand, [^{3}H][D-Ala^2, MePhe^4, Gly(ol)^5]- enkephalin (DAGO) and the δ ligands, [^{3}H][D-Ser^2, Leu^5, Thr^6]- enkephalin (DSLET) and [^{3}H][D-Pen^2, D-Pen^5]- enkephalin (DPDPE), to neural membranes was inhibited noncompetitively by the Fab fragments. To measure κ binding sites, [^{3}H]bremazocine in the presence of μ and δ blockers was used. The Fab fragments were not able to significantly suppress binding to κ sites. The antibody competitively inhibited up to 95% of the binding of [^{125}I-Tyr^{27}] β_h-endorphin to membranes. Heat-denatured antibody or control mouse IgM did not inhibit the binding of [^{125}I-Tyr^{27}]β-endorphin. To ascertain the relative affinity of the antibody for the different types of opioid binding sites, varying concentrations of antibody and the opioid peptides, DAGO, DSLET, β_h-endorphin 1-31, β_h-endorphin 1-27, β_h-endorphin 6-31, were tested for their combined ability to inhibit the binding of 0.4 nM [^{125}I-Tyr^{27}]β-endorphin to membranes. The combination of antibody with opioid peptides did not produce an additive effect but instead, produced an effect suggestive of common or interacting sites. β_h-endorphin 6-31 did not block the inhibition of binding by the antibody. The other peptides were able to block the antibody's inhibition of [^{125}I-Tyr^{27}]β-endorphin binding. The relative affinities were β_h-endorphin 1-31 = β_h-endorphin 1-27 > DAGO > DSLET. The antibody is directed against a 35,000 dalton protein. These studies suggest that the μ, δ and β-endorphin binding sites share a common or interacting site, while the κ opioid receptor does not share this site. (Supported by NIDA grant DA03742.)

93.11 Synthesis and Characterization of New Ligands for Brain Sigma (σ)-opiate Receptors. Frank J. Arnold, Randall B. Murphy, Miroslav Trampota and David I. Schuster: Department of Chemistry, New York University, 4 Washington Place, New York, NY 10003 USA

As part of a study to prepare irreversible ligands for brain sigma opiate receptors, we have synthesized twelve analogs of the putative neuroleptic drug 3-PPP having the following structure:



Several of these compounds are highly potent in a recently described binding assay to rat brain homogenates (Langent, Gundlachand,Snyder, PNAS 81, 4983-4987, 1984). This assay quantifies sigma-opiate like activity using [^{3}H]-Haloperidol, in the presence of 25 nM spiperone to block D_2 receptor binding. The most active compounds are those in which R is phenyl(I), benzoyl (II), Ph-CH(OH)-(III), Ph-C(n-C_5H_{11})(OH)-(IV) or p-N_3-phenyl(V). In this series the values of Kd observed against 2nM [^{3}H]-Haloperidol ranged from 22 nM (for III) to 50 nM (for V). The substitution of R^1 = Me for R^1 = H does not dramatically effect biological activity. For most compounds the Hill slopes were essentially unitary. In general for this series an increase in hydrophobicity appears to be correlated with increased sigma-opiate activity. Substitutions of p-NH_2, p-NO_2 and p-I on the phenyl ring appear to decrease activity somewhat. None of these compounds have significant potency on the rat striatal D_2 receptor, as quantified by the binding of [^{3}H]-spiroperidol.

Due to the relatively selective action and high affinity of these compounds for the putative rat brain sigma-opiate receptor, we suggest that they may prove useful as ligands in this system.

93.12 THE SUBCELLULAR COMPARTMENTATION OF OPIATE RECEPTORS REFLECTS THEIR BIOSYNTHETIC AND FUNCTIONAL STATES. C. Klein*, R. Levy* and R. Simantov. Dept. of Genetics, Weizmann Institute of Science, Rehovot 76100, Israel.

The early analysis of the subcellular distribution of opiate receptors in the brain (Pert et al. Brain Res. 70:184, 1974; Simantov et al. Brain Res. 107:650, 1976) indicated that binding sites for opiates are present in several intracellular structures. Yet, most attention was focused over the years on the membrane-bound receptors and, until recently, little was known about the others. The recent studies conducted by Coscia and associates (J. Biol. Chem., 256:10117, 1981; Brain Res., 250:101, 1982) effectively revealed the properties of brain microsomal receptors. In these studies we have used the clone M8, isolated from NG108-15 neuroblastoma-glioma hybrids, to characterize the intracellular opiate receptors. The cell organelles were separated on a Percoll gradient and four enzyme markers were used to define the fractions containing the plasma membrane, microsomal, lysosomal and Golgi elements. Analysis of receptor density in these fractions from untreated cells shows that in addition to the membranes, all the three intracellular compartments contained opiate binding sites. The enzyme markers indicated that appearance of receptors in these elements does not result from contamination with plasma membranes. Moreover, the study showed that receptors in the lysosomal fraction were resistant to low pH. Yet, the affinity of ^{3}H-DADL to lysosomal and plasma membrane receptors (of untreated cells) was similar. Leu-enkephalin induced time-dependent loss of receptors in all the four compartments but a kinetics analysis showed that the rate of receptor loss in these structures was not identical.The percentage of receptors appearing in the lysosomal fraction that could still bind ^{3}H-DADL was increased upon treating cells with leu-enkephalin, and the extent of this increase was correlated with the extent of receptor loss from the membranes. This supports the notion that the peptide promotes internalization of receptors which are still functional in some lysosomes. Yet, some difference in this respect between two lysosomal fractions was observed. As an additional approach to follow the intracellular fate of the receptors, cells were labeled with ^{3}H-diprenorphine, chased with various opiates, and the distribution of ^{3}H-diprenorphine-receptor in the cells was monitored with a Percoll gradient. Opiate agonists and antagonists altered the distribution of the labeled receptors between the plasma membrane, lysosomal and Golgi elements. Anti-lysosomal agents and primary amines were used to further clarify the opiate mode of action. The study sheds light on the involvement of the Golgi apparatus and the lysosomes in the metabolism of opiate receptors.

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CATECHOLAMINES: RECEPTORS I

94.1 **INTERACTION OF β-ADRENERGIC RECEPTORS WITH THE INHIBITORY GUANINE NUCLEOTIDE-BINDING PROTEIN.** Stewart N. Abramson, Robert G. L. Shorr*, T. Kendall Harden and Perry B. Molinoff. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, Smith Kline and French Labs, Philadelphia, PA 19101, and Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27514.

β-Adrenergic receptors on membranes prepared from wild-type S49 lymphoma cells and the adenylate cyclase-deficient mutant (cyc$^-$) of S49 lymphoma cells have been shown to bind the agonist (^{3}H)hydroxybenzylisoproterenol (^{3}H-HBI) with high affinity and with characteristics consistent with the formation of a ternary complex composed of agonist, β-adrenergic receptor, and a guanine nucleotide-binding protein (Abramson and Molinoff, Neurosci. Abs. 84.7, 1984). To further characterize this process receptors labeled with either the agonist (^{3}H)HBI or the antagonist (^{125}I)iodopindolol (^{125}I-IPIN) were solubilized from wild-type and cyc$^-$ S49 cell membranes with digitonin. The ligand-bound receptor complexes were then passed over HPLC gel exclusion columns. Receptors from wild-type S49 cells labeled with (^{125}I)IPIN were included in the columns as were receptors labeled with (^{3}H)HBI. However, based on their retention times, receptors that were labeled with (^{3}H)HBI had a larger apparent molecular size than receptors that were labeled with (^{125}I)IPIN. Similar results were obtained for receptors from cyc$^-$ S49 cells. These observations are consistent with the hypothesis that receptors from both wild-type and cyc$^-$ cells can interact with another membrane protein, presumably a guanine nucleotide-binding protein, when occupied by an agonist. Wild-type S49 cells contain both the stimulatory (N_s) and the inhibitory (N_i) guanine nucleotide-binding proteins of adenylate cyclase. Cyc$^-$ S49 cells contain a functional N_i protein, however, they do not contain a functional N_s protein. To determine whether the guanine nucleotide-binding protein associated with the β-adrenergic receptor in membranes prepared from cyc$^-$ cells is N_i, cyc$^-$ S49 cells were treated with pertussis toxin to inactivate N_i. Treatment of cells with pertussis toxin inhibited by 95% the subsequent ability of the toxin to label N_i with (^{32}P)-NAD in membranes prepared from treated cells. Treatment of cells with pertussis toxin also reduced the K_{act} values of GTP S and Gpp(NH)p for inhibition of forskolin-stimulated adenylate cyclase activity in membranes prepared from treated cells. Although treatment of cyc$^-$ cells with pertussis toxin did not affect the binding of (^{125}I)IPIN to membranes prepared from these cells, the binding of (^{3}H)HBI was decreased 60-70% in membranes prepared from treated calls. Inhibition of the binding of (^{3}H)HBI to β-adrenergic receptors by treatment of cells with pertussis toxin is consistent with the hypothesis that agonist-bound β-adrenergic receptors interact with N_i. Such an interaction may be physiologically important in regulating the activity of adenylate cyclase or in processes such as desensitization. (Supported in part by USPHS NS 18479 and a Predoctoral Fellowship from the PMA Foundation.)

94.2 **Regulation of β-Adrenergic Receptors on Cultured Cells.** K.A. Neve and P.B. Molinoff. Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

The turnover of β-adrenergic receptors on cultured cells was determined by measuring the rate of recovery of the binding of ^{125}I-iodopindolol after irreversible blockade of β-adrenergic receptors. The effect on β-adrenergic receptors of N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), a pseudobase that irreversibly inactivates α-adrenergic and dopaminergic receptors, was investigated. Scatchard analysis of the binding of ^{125}I-iodopindolol to membranes prepared from L6 myoblasts resulted in linear plots with K_d and B_{max} values of approximately 45 pmolar and 500 fmoles/mg protein, respectively. Nonspecific binding, defined in the presence of 50 μM (-)-isoproterenol, was less than 10% of total binding at a concentration of the radioligand that was close to its K_d value. Addition of EEDQ to the growth medium of L6 myoblasts resulted in a time- and concentration-dependent reduction in the binding of ^{125}I-iodopindolol. Treatment with EEDQ did not alter the K_d value for the binding of ^{125}I-iodopindolol, but caused a decrease in B_{max} that was not reversed by extensive washing of the membranes. At a concentration of 1 μmolar, EEDQ had no effect on the density of β-adrenergic receptors after up to two hours. When the concentraction of EEDQ was 10 μmolar, there was a 35% decrease in the density of receptors after one hour, and a 50% decrease after treatment for two hours. Treating cells with 100 μmolar EEDQ for one hour caused a greater than 90% reduction in the density of β-adrenergic receptors measured on membranes prepared from the cells. When EEDQ-containing medium was replace by fresh medium, the density of receptors on L6 cells treated with 100 μmolar EEDQ for one hour recovered to within 25% of the density on untreated cells within 24 hours. The degradation rate constant calculated from the rate of recovery was 0.053/hour, and the rate of reappearance of receptors was 33 fmoles/mg protein/hour. The recovery of the binding of ^{125}I-iodopindolol was blocked by treatment with cycloheximide (5 μg/ml), and probably represents the synthesis of new receptors. One of the problems involved in determining the turnover rates of receptors in this manner is the possibility that irreversible blockade alters the synthesis or degradation of receptors. To determine if blockade of the receptors by EEDQ will itself alter the metabolism of receptors, the rate of recovery of binding after treatment with EEDQ was determined in the presence of the antagonist sotalol. Blockade of the receptors by sotalol did not alter the turnover rate of β-adrenergic receptors, indicating that the rate constants measured after treatment with EEDQ probably reflect resting rates of metabolism of β-adrenergic receptors. EEDQ is a useful reagent for studies of this nature because it is inexpensive and easily obtained. In addition, when membranes are prepared for assays of radioligand binding, little washing of membranes is required to reduce the residual amount of EEDQ in membranes below concentrations that affect the binding of ^{125}I-iodopindolol (Supported by the USPHS NS 18479 and MH 14654.)

94.3 DETECTION AND CHARACTERIZATION OF β-ADRENERGIC RECEPTORS IN COATED VESICLES OF BOVINE BRAIN. D.M. Chuang*, O. Carter*, J. W. Spain*, D.B. Bennett*, B.L. Roth*, M.B. Laskowski and C.J. Coscia, (SPON: L. de Medinaceli). LPP, NIMH, St. Elizabeths Hosp., Washington, D.C. 20032 and St. Louis Univ. St. Louis, MO 63104

Evidence has accumulated suggesting that β-adrenergic receptors (BAR) are internalized in frog erythrocytes and several cultured cell lines. However, it is uncertain as to whether internalization of BAR occurs in the CNS. Since clathrin-coated vesicles (CVs) have been implicated in both endocytotic and intracellular transport of a variety of receptors, we have isolated these organelles and examined them for the presence of BAR binding and adenylate cyclase (AC) activities. CVs were prepared from bovine forebrain as described by Pfeffer and Kelly. Microsomal pellets were subjected to linear D_2O/Ficoll gradients and the CV-enriched (60-80%) fraction applied to a controlled-pore glass bead column to achieve further purification. The two major bands of protein eluting from the column were monitored by electron microscopy and SDS-PAGE. Peak II contained almost exclusively CVs, whereas Peak I, which appeared in the void volume, contained larger smooth vesicles and few CVs. BAR binding to Peaks I and II was assessed with ^{125}I-cyanopindolol (CYP) as ligand in Sepharose 4B column assays. ^{125}I-CYP was found to bind specifically to sites in both Peaks I and II with a Bmax of 28±4 and 32±3 fmol/mg protein, respectively. Binding of ^{125}I-CYP to both fractions was displaced by various BAR agents in a stereospecific manner. The addition of 50 μM $Gpp(NH)_p$ did not affect displacement of CYP binding to Peak II sites by (-)isoproterenol, whereas a significant "right shift" was noted when Peak I or a synaptic plasma membrane preparation (SPM) from bovine hippocampus was used. ^{3}H-CGP 12177, a hydrophilic BAR ligand, specifically bound to SPM and to a lesser extent to Peak I, but failed to label the BAR present in Peak II, suggesting that receptors present in CVs were cryptic in nature. AC activity could also be detected in both Peaks I and II (spec. act. = 21±0.6 and 24±0.5 pmol cAMP/mg/min, respectively). These activities were unaffected by GTP or isoproterenol+GTP. In contrast, a moderate stimulation of the cyclase activity present in SPM was induced by these agents. Rechromatography of Peak II on the glass bead column revealed that appreciable amounts of protein, CYP binding and AC activity were recovered in Peak I; this change in chromatographic migration was facilitated by pre-exposure of CVs to 0.5 M Tris, a condition known to cause at least partial dissociation of clathrin from these vesicles. This suggests that at least some of the protein, as well as AC and CYP binding activities in Peak I were derived from CVs, possibly due to loss of clathrin. In conclusion, BAR and AC present in brain CV preparations might be molecular entities undergoing endocytotic or intracellular transport.

94.4 COMPARATIVE ANATOMY OF BETA-ADRENERGIC RECEPTORS IN THE RAT AND GUINEA PIG: IN VITRO AUTORADIOGRAPHY WITH [^{125}I]-CYANOPINDOLOL. R.M. Booze, E.A. Crisostomo, and J.N. Davis. VA Medical Center and Depts. of Med. (Neurology) and Pharmacology, Duke University Medical Center, Durham, NC 27705.

Species diffrences have been reported in the distribution of B-adrenergic receptors in mammalian nervous systems. B-adrenergic receptors are present in the brains of some mammals, such as the rat, cow, and human, but are sparse or absent in the brains of swine and guinea pigs. We used in vitro receptor autoradiography to study the species differences in B-adrenergic receptor distribution between rats and guinea pigs.

In vitro receptor autoradiography was performed with [^{125}I]-cyanopindolol (CYP), a specific B-adrenergic receptor antagonist. The selective B-adrenergic antagonists ICI-89,406 and ICI-118,551 wre used to localize the B2- and B1-adrenergic receptor subtypes, respectively. The brains from three adult male rats and guinea pigs were cryostat-sectioned (16 um) in either the coronal, horizontal, or sagittal planes. Adjacent sections were incubated with either [^{125}I]-CYP and the appropriate ICI drug or with dl-propranolol for estimation of non-specific binding. The radiolabeled sections were apposed to film for 30 hours and densitometric measurements performed on the resulting autoradiographs.

Striking qualitative and quantitative species differences were evident in the localization of B-adrenergic receptor subtypes. In guinea pigs, B_2-adrenergic receptors were limited to hippocampal area CA_1 and the cerebellar molecular layer. In rats, the substantia nigra, superior colliculus, cortical layer IV, olfactory tubercle, and cerebellar molecular layer were heavily labeled for B_2 receptors. B_1 receptors were found in cortical layers I and V, hippocampus, substantia nigra, superior colliculus, amygdala, basal forebrain, striatum, and lateral septum of both species, although guinea pigs had lower receptor densities relative to rats. Furthermore, the thalamus was not labeled for B-adrenergic receptors in guinea pigs, but discrete labeling corresponding to specific thalamic nuclei was observed in rats.

The use of in vitro receptor autoradiography has provided the first detailed anatomical description of B-adrenergic receptors in guinea pigs. The results indicate that highly specific qualitative and quantitative variations exist in the neuroanatomical distribution of B-adrenergic receptors in rats and guinea pigs. Thus, caution should be used in generalizing the relationships of neurotransmitters and receptors from studies of a single species.

(Supported by the VA and NS06233)

94.5 EVIDENCE FOR SUBTYPES OF ALPHA-1 ADRENERGIC RECEPTORS LABELLED BY [^{3}H]PRAZOSIN. A. Leslie Morrow and Ian Creese. Dept. of Neuroscience, U.C.S.D. School of Medicine, La Jolla, CA 92093.

[^{3}H]Prazosin (Praz) is considered to be a selective high affinity ligand for the study of central and peripheral alpha-1 adrenergic receptors. [^{3}H]Praz binding is characterized by monophasic saturation in all systems studied. However, phentolamine and WB4101 competitions with [^{3}H]Praz are shallow in rat cortex, exhibiting Hill Coefficients of .62 (1). Praz, indoramine and dihydroergocriptine competitions with [^{3}H]Praz are steep, monophasic and model best to a single binding site, while phentolamine and WB4101 curves model to two binding sites in equal proportion. There is no additivity of the inhibition by phentolamine and WB-4101 at concentrations which saturate their high affinity component of [^{3}H]Praz binding, indicating that WB4101 and phentolamine have highest affinity for the same half of [^{3}H]Praz binding. Antagonist affinities are 0.69, 0.62 and 2.44nM for Praz, WB4101 and phentolamine at the high affinity component, designated alpha-1A. The lower affinity component, designated alpha-1B, has affinities of 0.69, 24.0 and 55.0nM respectively.

[^{3}H]WB4101 labels two binding sites in rat cortex, with picomolar and nanomolar affinity respectively. The nanomolar binding site represent labelling of an S-1 serotonin receptor (2). The picomolar site has characteristics of an alpha-1 receptor binding site. Praz, WB4101 and phentolamine affinities for this [^{3}H]WB41-01 binding site correlate with their affinities for the alpha-1A component of [^{3}H]Praz binding. [^{3}H]WB4101 labels the alpha-1A component of [^{3}H]Praz binding with 0.15nM affinity and Bmax equal to one half of the total [^{3}H]Praz Bmax (5.96 ± .32 vs. 11.03 ± .30 pmoles/g tissue).

Agonist competitions with [^{3}H]Praz are shallow with Hill slopes less than 1.0 and with a rank order of affinity of epinephrine > norepinephrine > phenylephrine. EPI and NE affinities at the two subtypes are the same and the rank order of agonist affinities is preserved when binding to the alpha-1A component is blocked. The ratio of the potency of phentolamine vs. prazosin is between 2 - 10 at the alpha-1A component and between 50 - 100 at the alpha-1B component. Using this ratio as a pharmacological distinguishing criterion, tissues containing only one subtype may be identified. We discuss these data in relation to antagonist potencies of alpha-1 receptor mediated responses which correlate with alpha-1A or alpha-1B binding sites.

(1) Morrow, et al., (1985) Eur. J. Pharmacol. 109:285.
(2) Norman, et al., (1984) Eur. J. Pharmacol. 106:461.

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94.6 THE EFFECTS OF ALPHA-2 ADRENERGIC AGONIST PREINCUBATION ON RECEPTOR BINDING AND cAMP GENERATION IN THE HUMAN COLON CARCINOMA CELL LINE HT29. Susan B. Jones*, John T. Turner and David B. Bylund. Spon: William S. Stark. Dept. of Pharmacology, University of Missouri, School of Medicine, Columbia, MO 65212.

The term desensitization is used to describe the phenomenon of reduced cellular responsiveness to an agonist following a previous exposure to that agonist. For the alpha-2 receptor desensitization can be experimentally defined by a reduction in the inhibition of the basal or forskolin stimulated adenylate cyclase activity of cyclic AMP accumulation. Downregulation of receptor number often accompanies desensitization.

To date, few desensitization studies of alpha-2 receptors have been reported. In the human platelet, it was found that preexposure of human platelets to 100 μM epinephrine caused a decrease in [^{3}H]yohimbine binding, but that this decrease was caused by competition at [^{3}H]yohimbine sites by epinephrine taken up by platelets (Karliner, Mol. Pharm. 21:36, 1982). Exposure to agonist did not alter the maximum alpha-2 receptor response in NG 108-15 cells, but caused a two-fold reduction in apparent potency of (-)epinephrine in inhibiting cyclase, indicating that NG 108-15 cells have "spare" receptors (U'Prichard, Ann. N.Y. Acad. Sci. 430, 1984). Previously we have done similar alpha-2 adrenergic receptor desensitization experiments with isolated human properitoneal adipocytes. Exposure to epinephrine reduced the B_{max} of [^{3}H]p-aminoclonidine by 43% but not [^{3}H]yohimbine B_{max}. The ability of 10 μM epinephrine (plus propranolol) to lower cAMP levels was not affected.

We have examined the effects of alpha-2 agonist preincubation on receptor binding and cAMP generation in the human colon carcinoma cell line HT29. We found no decrease in receptor number with a 60 min preexposure of HT29 cells to 10 μM UK-14,304 with either [^{3}H]UK-14,304 or [^{3}H]yohimbine binding. There was a decrease in affinity with [^{3}H]UK-14,304 binding indicating possible loss of the high affinity binding site. [^{3}H]yohimbine binding affinity was not affected. In cyclic AMP whole cell assays, preincubation with a low concentration of UK-14,304 caused a marked increase in forskolin stimulated cAMP. The molecular interactions at the alpha-2 adrenergic receptor are complex events in which several mechanisms may be involved.

94.7 CHARACTERIZATION OF ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES IN HUMAN BRAIN: PRAZOSIN INHIBITION OF [^{3}H]YOHIMBINE BINDING. David B. Bylund, and Anton C. Petrash*. Dept. of Pharmacology, University of Missouri, School of Medicine, Columbia, MO 65212.

Pharmacologic differences in the characteristics of alpha-2 adrenergic receptor binding between rodent and non-rodent species has led to the speculation that subtypes of the alpha-2 receptor may exist.

The first pharmacologic difference is that [^{3}H]yohimbine is more potent in non-rodent species with K_D values of 2nM or lower compared to rodent species with values in the range of 5 to 10 nM. The second difference is that while oxymetazoline tends to be more potent in non-rodent than in rodent species, prazosin tends to be more potent in rodent than in non-rodent species. In rat brain, we found that prazosin inhibited [^{3}H]yohimbine binding with Hill slopes less than 1.0. These data were consistent with equal densities of low (approx. 300nM) and high (approx. 7 nM) affinity binding sites. We have tentatively called these A and B subtypes, respectively (Bylund, D.B., Pharmacol Biochem Behav 22(5), 1985 (in press)). The alpha-2 receptors of the human platelet for example are all of the "A" subtype.

In order to further characterize these putative subtypes, inhibition binding studies were conducted in our laboratory using human brain tissue from three regions: cerebral cortex, cerebellum, and caudate nucleus. The K_i values for prazosin inhibition of [^{3}H]yohimbine binding were determined and the results analyzed using standard computer techniques. In the cerebral cortex and cerebellum, the prazosin inhibition curves generally modeled as a single site. However, in the caudate we were able to resolve the prazosin inhibition data into high (B subtype) affinity and low (A subtype) affinity sites, with K_i values of about 5 nM and about 300 nM respectively. Approximately 60% of the sites were of the B subtype and 40% were of the A subtype.

In saturation binding studies in the same tissues, [^{3}H]prazosin binding had a K_D of 0.04 nM, indicating that the affinity of prazosin for alpha-1 receptors is about 250X the affinity of the alpha-2B site. In addition, the K_D for [^{3}H]yohimbine binding in the caudate is about 0.5 nM, or about 10X more potent than prazosin at the alpha-2B site. Thus, the alpha-2B site does not appear to be an alpha-1 receptor.

94.8 QUANTITATIVE ASSESSMENT OF RAT CORTEX α_2-ADRENOCEPTOR RADIOLIGAND BINDING AND REGULATION. J.M. Stolk* and D.C. U'Prichard; Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD 21228 and Nova Pharmaceutical Corporation, Baltimore, MD 21224.

Considerable evidence supports the heterogeneous interaction of agonists and some antagonists at α_2-adrenoceptors (α_2AR). Allosteric regulation of low and high affinity states for agonists have been postulated from data on modulation of equilibria between states by guanine nucleotides and divalent cations. During studies to characterize α_2AR binding site regulation in rat cortex, we observed an apparent lack of conservation between agonist affinity state binding sites and pursued the issue further.

We systematically evaluated the binding of agonists (p-aminoclonidine, UK-14304) and antagonists (Idazoxan [RX]; rauwolscine [Rau]) to washed rat cortical membranes under defined conditions using a modified Latin-Square design. Displacement of labelled ligands (0.3-0.6 nM final conc.) was measured using common membrane suspensions in the presence or absence of Mg^2 (0.3 mM final conc.) or GppNHp (10^5 M final conc.); data from each multiple ligand study were analyzed together using the non-linear curve-fitting program, LIGAND (Munson and Rodbard). As anticipated, high affinity agonist binding was facilitated in Tris buffer (50% of total sites displayed high affinity for PAC and UK), whereas Rau binding to the site with low affinity for agonists was facilitated in Na/K-phosphate buffer (10% of total sites displayed high affinity for PAC and UK); RX bound with equal affinity to both states of the α_2AR in rat cortex. Contrary to predictions by the allosteric model of α_2AR regulation, the total number of binding sites in Tris buffer was significantly lower in the presence of GppNHp than in the presence of Mg^{2+} (134 ± 11 vs. 190 ± 17 fmol/mg), and the site with high affinity for agonists was selectively affected (62 ± 13 vs. 124 ± 13 fmol/mg). The binding site density changes were accompanied by significant alterations in K_D values for both agonist and antagonist ligands at the high affinity site for agonists (for example, K_D values for p-aminoclonidine and Rau were 2.14 ± 0.41 and 48.8 ± 18 nM, repectively, in the presence of GppNHp, and 1.07 ± 0.15 and 209 ± 28 in the presence of Mg^{2+}). We currently are evaluating changes in total binding site density in independent studies with RX (which binds with equal affinity to both states of the α_2AR) to verify the results observed in the multiple ligand analyses.

We tentatively conclude that the present model for allosteric regulation of α_2AR may be insufficient to account for the quantitative data obtained in the present study. (Supported in part by USPHS Research Grant MH32842 and RSDA MH00018)

94.9 RECEPTOR RESERVE AT CENTRAL DOPAMINE (DA) AND ALPHA$_2$ AUTORECEPTORS. E. Meller*, E. Matyjek*, C.H. Adler, K. Bohmaker*, A.J. Friedhoff, and M. Goldstein. Department of Psychiatry, New York University Medical Center, New York, NY 10016.

The extent of receptor reserve at central DA and alpha$_2$ autoreceptors was studied using the irreversible antagonist N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; Meller et.al., JPET, in press; Adler et. al., Fed. Proc. 44, 1833,1985). The in vivo GBL method was used for assessing receptor reserve at striatal DA autoreceptors. Rats were treated with vehicle or EEDQ (6 mg/kg, i.p.) and 24 hr later dose-response curves for apomorphine (APO;0.1-10 mg/kg, i.p.) reversal of GBL-induced L-dopa accumulation were generated. The dose-response curve for APO was shifted 4.3 fold to the right in EEDQ-treated animals relative to controls (ED_{50} (control) = 0.19 mg/kg; ED_{50} (EEDQ) = 0.83 mg/kg); however, at this dose of EEDQ the maximum response was not reduced. Utilizing the methods of Furchgott (Ann. NY Acad. Sci. 144:882-889, 1967) the fraction (q) of receptors remaining after EEDQ treatment was found to be 0.31 (i.e. 31%). Thus a large receptor reserve (≥ 70%) appears to exist at terminal DA autoreceptors in the rat striatum. Since the presence of a large receptor reserve (relative to its absence) has the effect of reducing the ED_{50} of an agonist, the reported autoreceptor selectivity of several DA agonists (EMD 23, 448, 3-PPP, CGS 14948A, ciladopa) may be related to differences in receptor reserve at pre- and postsynaptic DA D_2 receptors. EEDQ was also used to study receptor reserve in vitro at rat central alpha$_2$ autoreceptors mediating inhibition of NE release. Cumulative dose-response curves for the inhibition of K^+ (20mM)-stimulated ^{3}H-NE release by the full alpha$_2$ agonist UK-14,304 were obtained in rat occipitoparietal cortex slices of control and EEDQ-treated (0.8 mg/kg, i.p., 4 hr) animals (Frankhuyzen and Mulder, 1982; Adler et. al., Fed. Proc. 44, 1833, 1985). EEDQ did not affect either ^{3}H-NE uptake or its spontaneous release; however, K^+-evoked release was increased slightly (10-20%). In slices of cortex from EEDQ-treated rats, maximal inhibition of ^{3}H-NE release by UK-14,034 (100μM) was reduced by 34% relative to controls; the EC_{50} was shifted 7-fold to the right (control: 0.024μM; EEDQ-treated: 0.17μM). The following parameters were calculated: q = 0.15 ± 0.03 and K_A (dissociation constant) = 1.41 + 0.8μM (n=7). A plot of receptor occupancy vs. response resulted in a hyperbolic curve indicative of the presence of spare receptors; 50% response was obtained at 1.6% receptor occupancy. These studies demonstrate the utility of EEDQ as a tool to investigate the presence of receptor reserve at central alpha and DA receptors and to provide new insights into CNS receptor function. Supported by grants MH 02717, NINCDS 06801, MH 08616, and MH 35976.

94.10 DOPAMINE-D2 RECEPTORS AND LIGHT COUPLE TO CYCLIC GMP-PHOSPHODIESTERASE, PHOSPHOLIPASE A2 AND PHOSPHOLIPASE C IN ROD OUTER SEGMENT MEMBRANES. C. L. Jelsema, Y. Ishihara, M. R. Brann*(SPON: S. P. Fracek) Lab of Cell Biology, NIMH, Bethesda, MD 20205.

Recent reports of a non-cyclase role of G_i in the activation of phospholipase A_2 and C in neutrophils and mast cells raised the possibility that transducin, the G_i-like GTP-binding protein of rod outer segment (ROS) membranes, might similarly activate phospholipases either in conjunction with its role in the light activation of cGMP phosphodiesterase (cGMP PDE) activity, or in distinction from it. The existence of dopamine-D2 receptors(R) and their coupling to a pertussis toxin, cholera toxin-sensitive G protein has been recently demonstrated in the ROS membranes (see abs. M. R. Brann and C. L. Jelsema), suggesting the possibility that transducin may also couple D2 Rs to their effector system. Since D2 Rs appear to couple to G_i in the pituitary, their role in phospholipase and cGMP PDE activation was investigated along with the effect of light on these enzyme activities in ROS membranes.

In dark-adapted ROS membranes, D2 R activation lead to increased cGMP-PDE activity in a manner analogous to light activation, suggesting that D2 Rs similarly couple to transducin. The amount of increase in cGMP PDE activity in response to 10 uM apomorphine(APO) was about a third that of light-induced activation. The specificity of the APO effect for the D2 R was evidenced by the capacity for (-)sulpiride and (+)butaclamol to block the response. Both light and D2 R activation of cGMP PDE were accompanied by increases in phospholipase A_2 and C activity in dark-adapted membranes, supportive of a role for transducin in phospholipase A_2 and C activation. APO increased the phospholipase A_2 activity of dark-adapted ROS membranes nearly four-fold, whereas light induced a 12-fold increase. With phospholipase C activity, APO induced a 50% increase, while light lead to a 3-fold increase in activity. The effects of APO on both phospholipases, similar to effects of APO on cGMP PDE activity, were D2-R-specific as both (-)sulpiride and (+)butaclamol reverse the effect of APO in dark-adapted ROS membranes. These results suggest that D2 Rs are coupled to transducin in the ROS membranes, consistent with a more general role for transducin in signal transduction. In addition, our results support a role for transducin in phospholipase activation.

94.11 "D_1-LIKE" DOPAMINE RECEPTORS: RECOGNITION SITES WITH SELECTIVITY FOR SCH23390 THAT ARE NOT LINKED TO ADENYLATE CYCLASE

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It has been generally accepted that dopamine receptors are divisible into two major classes: D_1 linked to stimulation of cAMP synthesis; and D_2 which is not so linked (Kebabian and Calne, 1979). Until recently, the D_2 class was believed to be responsible for most of the important functional changes mediated by dopamine receptors, including psychopharmacological, behavioral, and neurochemical effects. However, in 1981 it was reported that SCH23390 was a biochemically selective D_1 antagonist, inhibiting adenylate cyclase with a K_i three orders of magnitude lower than the K_i for competing with D_2 radioligands. Recent studies have demonstrated that SCH23390 also has extremely potent behavioral and psychopharmacological effects, suggesting that previously unknown functional roles exist for D_1 receptors.

In 1984, we reported that the amygdala has no detectable dopamine-sensitive adenylate cyclase activity (Soc. Neurosci. Abstr. 10:881, 1984). According to accepted criteria, this requires there to be no D_1 receptors in this region. Contrary to this notion, we have found significant quantities of high affinity specific binding sites for SCH23390. In addition, results from the same tissue samples demonstrate that the ratio of binding of [^{3}H]-SCH23390 and [^{3}H]-spiperone is similar in striatum versus amygdala, whereas only the former region has detectable dopamine-sensitive adenylate cyclase activity.

These data clearly demonstrate that commonly used definition of D_1 receptors must be revised to include sites not linked to adenylate cyclase. The results demonstrate multiplicity of D_1 receptors at the functional level, whether or not significant multiplicity exists in the affinity of these sites for [^{3}H]-SCH23390.

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94.12 IN VIVO EEDQ SPECIFICITY FOR D-1 DOPAMINE RECEPTOR BLOCKADE: LACK OF EFFECT ON Ns OR THE CATALYTIC SUBUNIT OF ADENYLATE CYCLASE.

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Peripheral administration of EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) acts to potently and irreversibly block binding to striatal D-1 dopamine receptors (Hamblin, M.W. and Creese, I., Life Sciences, 32:2247, 1983). The specificity of in vivo EEDQ modification of receptors versus Ns or the catalytic subunit was investigated to assess the feasibility of using EEDQ to investigate the stoichiometry of receptor/effector interactions. Rats were injected with EEDQ (1-10mg/kg i.p.) and sacrificed 4 hours after treatment. Striatal homogenates from each animal were individually assayed for D-1 dopamine receptor-stimulated adenylate cyclase and D-1 dopamine receptor binding using the novel selective D-1 antagonist, [^{3}H]SCH 23390. [^{3}H]SCH 23390 binding exhibits a pharmacologic profile similar to the non-selective D-1 dopamine receptor radioligand [^{3}H]flupentixol with the advantage of yielding greater percent specific binding (90%).

We examined the characteristics of D-1 dopamine receptor-stimulated adenylate cyclase and also assayed for possible EEDQ induced modification(s) of the guanine nucleotide sensitive and catalytic moieties by assessing both guanine nucleotide and forskolin-stimulated cAMP production. No difference in basal adenylate cyclase activity was observed between EEDQ-treated and control animals. Comparable stimulation over basal activity was also observed in the presence of 0.1mM GTP (186%), 0.1mM Gpp(NH)p (789%) and 10mM NaF (633%). Likewise, 1uM forskolin (2049%), and 1uM forskolin +0.1mM GTP (3710%) stimulated treated and untreated tissue equally. However, EEDQ treatment markedly reduced D-1 receptor-mediated stimulation of cAMP production. Thus, EEDQ appears to be receptor specific in this system, leaving catalytic and guanine nucleotide sensitive subunits functionally intact. Both an EEDQ dose-dependent reduction in D-1 dopamine-stimulated adenylate cyclase activity and [^{3}H]SCH 23390 Bmax with no change in K_D was observed. This EEDQ-induced reduction in D-1 dopamine-stimulated adenylate cyclase and [^{3}H]SCH 23390 binding could be prevented by pretreatment with SCH 23390 (0.5mg/kg i.p.). Interestingly, the loss in [^{3}H]SCH 23390 binding did not correlate directly with the reduction in adenylate cyclase activity (e.g. at 4mg/kg EEDQ, 80% of receptor binding was lost while D-1 stimulated adenylate cyclase was reduced by only 64%). These data suggest that peripherally administered EEDQ may be used to selectively modify D-1 dopamine receptor binding sites, without modifying effector moieties. In addition, they may indicate that the stoichiometry of receptor/effector interaction may not be 1:1.

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ACTION POTENTIALS AND ION CHANNELS II

95.1 ION CHANNELS IN ASCIDIAN OOCYTES: SPECIES VARIATION AND SPATIAL DISTRIBUTION.

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Properties of macroscopic currents in two species of tunicate oocytes (Boltenia villosa & Ciona intestinalis) have been studied using two-electrode and whole cell patch voltage-clamp techniques. We have successfully obtained two-microelectrode impalements with resting potentials of -77mV to -88mV in both species of oocytes. Even small leakages (<100pA) from impalement of the ca. 150μm diameter oocytes resulted in loss of membrane potential to -20mV to -10mV because of the I-V characteristic of the inwardly rectifying K current, which comprises most of the resting conductance of the cell. Long-lasting action potentials were obtained from both oocytes in response to short, depolarizing stimuli.

Under voltage-clamp, Boltenia showed a rapid Na current (peak, 1-7nA at -30mV) and a slow, small Ca current (peak, 35-100pA at 0mV). The currents superficially resemble those reported by Takahashi et al. in Halocynthia oocytes, but with the major difference that in Boltenia, Ca current inactivation appears to be Ca-dependent, rather than voltage-dependent as in Halocynthia. The contribution of this calcium component to the action potential is variable since at the lower Na current densities the action potential often fails to reach threshold (-10 to 0mV) for regenerative Ca response. Ciona, in contrast, shows two inward currents (peak ca. 700pA for both, at -30mV & +10mV, respectively) both of which are carried by Ca ions. Both of these Ciona Ca currents are considerably slower than the Boltenia Na current, and consistently produce action potentials with large positive overshoots.

To investigate the spatial distributions of these channels, we have surgically bisected oocytes and studied the resulting animal or vegetal half-eggs ("merogones"). Two-microelectrode impalements of merogones have yielded high, stable resting potentials indicating minimal cell damage in the surgical procedure. We have also applied the "half-cell" variation of the patch clamp to the merogones. Preliminary results on the Na current using both techniques indicate no major differences in current density between the animal and vegetal halves.

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95.2 ACTION POTENTIALS PERSIST AT RESTRICTIVE TEMPERATURES IN TEMPERATURE SENSITIVE PARALYTIC MUTANTS OF ADULT DROSOPHILA.

J. C. Nelson* and D. H. Baird (SPON: R. J. Wyman), Dept. of Bio., Yale U., New Haven, CT 06511.

Action Potentials (APs) are not blocked in the Giant Fiber (GF) pathway of adult Drosophila when using temperature sensitive (ts) paralytic mutations above the temperature at which paralysis occurs. This is surprising because two mutations, nap and para, were reported to reversibly block APs in larval preparations (Wu, et. al., PNAS 75:4047, 1978; Wu and Ganetzky, Nature 286:814, 1980). We studied three ts-paralytic mutants postulated to affect sodium channel function and/or AP propagation - nap^{ts-1}, $para^{ts-2}$, and $seizure^{ts-1}$ (Jackson, et. al., Nature 308:189, 1984; Wu and Ganetzky, 1980). $shibire^{ts-1}$ was used as a control.

The GF pathway was stimulated by two electrodes in the brain. The GFs run from the brain to the thoracic neuropile where each makes two electrical synapses: one with the Tergotrochanteral motoneuron (TTMN) which drives the jump muscle (TTM); and the other with the Perhiperally Synapsing Interneuron (PSI), which chemically synapses with the motoneurons (DLMNs) to the wing depressor muscle (DLM). Electrical recordings were taken from the DLM and the TTM, while behavioral paralysis was monitored visually. Responses persist in the GF pathway up to 45°C in wildtype (wt) flies. The response requires sodium channels: they can be eliminated by perfusing flies with 20 nM TTX, which blocks neuron APs, but not muscle responses.

Some ts-paralytics affect the pathway as expected. shibire shows behavioral paralysis, loss of synaptic transmission (Salkoff and Kelly, Nature 273:156, 1978), and blockage of the GF pathway all at 30°C. As expected the GF pathway is blocked at chemical synapses. Thus, behavioral paralysis corresponds with physiological paralysis. However, while para flies are behaviorally paralysed and spontaneous neuronal activity stops at 30°C, the GF pathway responds up to 45°C. Similarly, in a large majority of nap flies, the GF pathway responds up to 45°C. The other nap flies have a variety of defective responses. Even at 22°C, the latency from brain stimulation to TTM response is longer than wt. Around 35°C the GF pathway may fail, but it will return if the stimulus voltage is increased slightly - higher temperatures require higher voltages. Sometimes above 35°C, part of the pathway (DLM) remains, but part (TTM) fails and does not return with higher voltage. We find no physiological abnormality in seizure around 40°C, but the paralytic temperature is so close to wt that defects may not be experimentally detectable. Deficiencies uncovering nap and seizure did not lower the behavioral paralysis temperature, or make the physiology more abnormal.

APs are not blocked in the GF pathway in para, seizure, and most nap flies. shibire reliably blocks the GF pathway at known synaptic sites. (support: NIH - GM 75027-08; NS-07314)

95.3 DIFFERENTIAL SENSITIVITY TO TTX OF Na-POTENTIALS IN NOCICEPTIVE NEURONS FROM FOUR SPECIES OF LEECHES. J. Johansen and A.L. Kleinhaus Dept of Neurology, Yale U. Sch. Med. New Haven, Ct. 06510.

We have recently shown that the nociceptive neurons (N cells) in the leech can be segregated into a medial type (Nm) and a lateral type (Nl) according to their physiological and pharmacological properties. We now report that the Na-dependent action potential (AP) of the two cell types have differential sensitivities to TTX and that the AP's of the N cell homologues from different leech species also vary considerably in their response to TTX.

The normal AP of the N cells was exclusively Na-dependent; its AP varied logarithmically with $\{Na\}_o$ was unaffected by the presence of Mn and absence of Ca, and could not be elicited in Na-free Ringer. The TTX block of the maximal rate of depolarization (Vmax) of the Na-dependent AP of the Nm cell in Macrobdella followed a reverse Langmuir curve for bimolecular reaction with an ED50 of 9 uM. Almost complete blockage was obtained at 50 uM. In contrast, TTX inhibition of Vmax in the Nl cell reached saturation at a level of only 55% reduction in Vmax even when doses in excess of 200 uM TTX were applied. The simplest interpretation of this data is that there are two types of Na-channels in the Nl cell. This is further supported by the fact that the dose-response data could be well fitted by a reverse Langmuir curve assuming that the Nl cell possesses two populations of Na-conductances: one insensitive to TTX and one (like the Nm cell) with an ED50 to TTX of 9 uM, with the two populations present in a 0.45 to 0.55 ratio respectively. Similar results were obtained for the two pairs of N cells in Haemopis. In the N cells of Hirudo 50 uM TTX blocked Vmax by only 25% with no difference between Nm and Nl. In the phylogenetically more distant glossiphoniid leech Haementeria which possesses only one N cell homologue, the AP was completely blocked by 50 nM TTX with an approximate ED50 of 10 nM.

These results provide evidence that the Nl cell of Macrobdella has two types of Na-channels that can be segregated by their different sensitivity to TTX. The Nm cell only possesses one type with an ED50 in the low uM range. In contrast, the Nm cell of Haementeria is a 1000 times more sensitive with an ED50 in the low nM range as is the case for most mammalian Na-channels. This diversity in sensitivity to TTX may reflect structural differences in Na-channels of leech N cells at the molecular level both between species and within a single cell type. Furthermore, the variation in pharmacological sensitivity of identified homologous neurons in different leech species may provide insight into leech phylogeny and evolution of ion-conductances.

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95.4 DISTRIBUTION OF SODIUM CHANNELS IN DENERVATED SKELETAL MUSCLE. J. H. Caldwell and R. L. Milton*. Dept. of Molecular and Cellular Biology, National Jewish Hospital and Dept. of Physiology, Univ. of Colorado Medical School, Denver, CO, 80206.

Voltage-gated sodium channels are not uniformly or randomly distributed along the length of a normal skeletal muscle fiber. Loose patch voltage clamp recording has been used to show that sodium channels are 5-10 fold more concentrated near the nerve terminal than in extrajunctional membrane (Beam, Caldwell & Campbell. Nature 313:588, 1985; Roberts and Almers, Biophys. J. 47:189a, 1985). This increased density occurs only within 100-200 μm of the endplate.

Two other regions of the muscle fiber have concentrations of sodium channels that are strikingly different from extrajunctional membrane; (1) membrane underneath the nerve terminal has a high density of Na channels and (2) membrane near the tendon has a low density of Na channels with most of the decrease (~10 fold) occurring within 250 μm of the tendon (Caldwell, Campbell & Beam, unpublished).

We have studied the effect of denervation upon the distribution of Na channels in both snake and rat muscle. Measurements of sodium current with the loose patch clamp were made from endplate, perijunctional, extrajunctional, and near-tendon membrane. The nonuniform distribution found in innervated muscle persists in both rat flexor digitorum brevis (denervated two weeks) and the snake external abdominal oblique (denervated four weeks). Current density (referred to the area of the pipette orifice) remained 100-125 mA/cm^2 at both snake and rat endplate membrane. The perijunctional density in rat muscle increased ~20% (from 25 mA/cm^2 to 31 mA/cm^2). The abrupt decrease in current density near the tendon also remained.

It is not known when or how this nonuniform distribution is formed during development, but it is likely to be imposed upon the muscle by the nerve. The present experiments show that the nerve is not required for the maintenance of the sodium channel distribution.

95.5 THE SODIUM CURRENT OF DISSOCIATED BULLFROG SYMPATHETIC NEURONS. Stephen W. Jones, Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794.

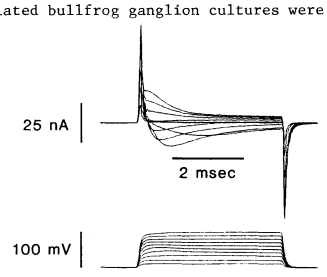
Neurons of vertebrates clearly have voltage-sensitive sodium currents closely analogous to that of the squid giant axon, but technical problems have limited detailed characterization of somatic sodium currents. Neurons dissociated from bullfrog paravertebral sympathetic ganglia by the method of Kuffler & Sejnowski (J. Physiol. 341:257, 1983) are suitable for patch electrode recording techniques. The dissociated cells are essentially spherical, and lack an axon.

Sodium currents were recorded in the whole-cell configuration with cesium-containing pipets in nominally calcium-free Ringer's, at room temperature, from neurons dissociated 1-5 days previously and stored at 4°C. A single electrode voltage clamp amplifier (Axoclamp 2, 30-50 kHz switching frequency) was used with .5-2 MΩ electrodes; capacity transients generally settled in 0.2-0.5 msec and clamp error at peak current was typically 4 mV. Large depolarizing commands (to +50 to +100 mV) sometimes also evoked outward currents activating over several msec, which were largely separated from the more rapid sodium currents.

The sodium current in dissociated bullfrog sympathetic neurons turns on and off rapidly (see figure). The voltage sensitivities of peak activation and of steady-state inactivation are near 7 mV per e-fold. Half maximal peak conductance occurs near +5 mV, with half maximal steady-state inactivation near -35 mV. Recovery from inactivation is rapid at -80 mV (half maximal in 1-3 msec). The reversal potential for the current changes appropriately for changes in external sodium concentration. 1 μM tetrodotoxin reversibly blocks most of the current.

These properties are generally consistent with those of sodium currents in other preparations.

Supported by NIH grants NS 20751 to S. W. J. and NS 18579 to Dr. Paul R. Adams. The dissociated bullfrog ganglion cultures were prepared by Claire Adams.



Sodium currents evoked by depolarizing steps from -40 mV. Each trace is the average of 4 records; capacity transients and leak not subtracted.

95.6 SOLUBILIZATION AND PURIFICATION OF THE VOLTAGE-SENSITIVE SODIUM CHANNEL FROM *DROSOPHILA MELANOGASTER*. K. Hubbard*, S.D. Wilson*, and L.M. Hall. Dept. of Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

At least five different single gene mutations in Drosophila melanogaster appear to affect the structure and/or regulation of the voltage-sensitive sodium channel involved in propagation of action potentials in neurons. All of these mutants have the phenotype of temperature- or stress-induced paralysis. Some have been implicated as affecting sodium channel activity or regulation on the basis of mutant-induced effects on ^{3}H-saxitoxin binding (Jackson et al., Nature, 308: 198, 1984) while others have been implicated on the basis of their interactions with mutants which affect ^{3}H-saxitoxin binding and/or propagation of action potentials (Ganetzky, Genetics, 108: 897, 1984). It is of interest to determine which of these mutants define structural genes for the sodium channel and which define gene products which affect channel regulation or environment. In this regard, it would be helpful to know how many structural proteins comprise the sodium channel in Drosophila. Purification studies of sodium channels from vertebrate sources suggest the channel consists of 1 to 3 subunits depending on the source of material (Agnew, Ann. Rev. Physiol., 46: 517, 1984). As a first step in the purification of sodium channels from Drosophila, we have developed a procedure for the stable solubilization of the channel. While several nonionic and zwitterionic detergents can be used for solubilization, the Drosophila sodium channel, like that of other species, appears to be sensitive to its detergent/lipid environment and binding activity is often unstable following solubilization. One zwitterionic detergent, 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate (CHAPS), allowed stable solubilization of ~75% of the total saxitoxin-binding activity from membrane preparations. Stability of the solubilized sodium channels was improved further by optimizing the lipid:detergent ratio using phosphatidylcholine. When the solubilized saxitoxin-binding component was chromatographed on a Sepharose 6B column and its elution profile compared with molecular weight markers including catalase (MW 248,000), ferritin (MW 450,000), and β-galactosidase (MW 540,000), the Drosophila sodium channel: detergent complex was found to be a large macromolecular complex similar to that described for vertebrate sodium channels. Solubilization followed by Sepharose chromatography represents a useful first step for the purification of the saxitoxin receptor from normal and mutant Drosophila strains. (Supported by a grant from the Muscular Dystrophy Association and by NIH grant NS16204. LMH is an Irma T. Hirschl-Monique Weill-Caulier Career Scientist Awardee).

95.7 SEPARATION AND CHARACTERIZATION OF THE POLYPEPTIDE SUBUNITS OF THE RAT BRAIN SODIUM CHANNEL. Donald J. Messner* and William A. Catterall. (SPON:M. Leibovitz). Department of Pharmacology, University of Washington, Seattle, WA 98195.

The voltage-sensitive Na channel from rat brain consists of 3 polypeptide subunits that have been designated α, β1, and β2. Their molecular weights as determined by SDS-polyacrylamide gel electrophoresis or gel filtration chromatography in SDS are 260,000 for α, 36,000 for β1, and 33,000 for β2. β2 is attached to α by disulfide bonds, while β1 is attached to the αβ2 complex non-covalently. All three subunits contain complex carbohydrate chains as shown by specific binding of wheat germ agglutinin. Chemical or enzymatic deglycosylation of the subunits gives polypeptide chains with apparent molecular weights of 220,000 for α, 23,000 for β1, and 21,000 for β2. Partial proteolytic maps of the SDS-denatured β1 and β2 subunits indicate they are non-identical polypeptides.

The β1 subunit dissociates from the detergent-solubilized Na complex upon incubation in high salt. Treatment with 2M $MgCl_2$ causes complete loss of the β1 subunit and of [^{3}H]saxitoxin (STX) binding activity. At intermmediate concentrations of $MgCl_2$, the loss of β1 and the loss of [^{3}H]STX binding activity are closely correlated. Tetrodotoxin (TTX) quantitatively stabilizes the solubilized complex against both the loss of β1 and the loss of [^{3}H]STX binding activity, indicating a cause-and-effect relationship between dissociation and loss of activity.

Treatment of the solubilized Na channel with dithiothreitol causes release of the β2 subunit. Dissociation of β2 is not blocked by TTX. Preliminary experiments suggest there is no correlation between the amount of β2 in the Na channel complex and the ability of the preparation to bind [^{3}H]STX. We conclude from these studies that the presence of β1, but apparently not of β2, is required for the integrity of the [^{3}H]STX binding site of the solubilized and purified rat brain Na channel.

95.8 BIOSYNTHESIS OF THE VOLTAGE-SENSITIVE SODIUM CHANNEL FROM RAT BRAIN. J.W. Schmidt* and W.A. Catterall. Department of Pharmacology, University of Washington, Seattle, WA 98195.

The voltage-sensitive sodium channel from rat brain has three glycoprotein subunits called α, β1, and β2. The α and β2 subunits are linked by disulfide bonds. We have begun to study how the channel is assembled and compartmentalized by using the technique of immunoprecipitation of subunits from metabolically labeled rat brain cells. Primary cultures of brain cells isolated from 15 day embryos and maintained in vitro for 21 days were pulse labeled with [^{35}S]-methionine for 0.5 hrs and chased for 0 to 4 hrs. Cell membranes were solubilized, glycoproteins isolated by affinity chromatography on wheat germ agglutinin (WGA)-Sepharose, α subunit immunoprecipitated with antiserum that was raised against purified sodium channel, proteins resolved by SDS-PAGE, and visualized by autoradiography. α subunits with complex carbohydrate chains capable of binding to WGA appear with $t_{1/2}$ of 45 minutes. Glycoproteins that failed to adsorb to WGA were affinity purified on lentil lectin-Sepharose. Immunoprecipitates of this glycoprotein pool show a single autoradiographic band that migrates slightly more rapidly than mature α and is rapidly ($t_{1/2}$=45 min) converted to a form that will bind to WGA. These experiments identify newly synthesized α subunits and show that they are first made with high mannose carbohydrate side chains which are processed to complex chains.

To determine when subunit assembly occurs, cells were pulse labeled for 2 hrs and chased for 0 to 24 hrs. Soon after attainment of capacity to bind to WGA, 1/3 of the newly synthesized α subunits are disulfide-linked to β2 subunits ($t_{1/2}$=90 min). We have previously shown that, in brain cells of this developmental stage, only 1/3 of the α subunits are disulfide-linked to β2 and located on the cell surface. Most α subunits are not linked to β2 subunits and exist in an intracellular pool. Cell surface channels were selectively isolated by incubating intact cells with antiserum at 4°C after pulse/chase labeling. Only the newly assembled α-β2 complex is located at the cell surface. Free α subunits are found only in the intracellular compartment. At least 50% of newly synthesized α subunits are degraded within the first 12 hours. In contrast, both the cell surface α-β2 and intracellular free α pool that remains after 12 hr are metabolically stable, with apparent half-life >5 days. These results indicate that, when α subunits reach their mature glycosylation state, they can be linked to β2 subunits and transported to the cell surface. Early in development, most α subunits do not immediately form disulfide bonds to β2 and remain as an intracellular pool of inactive subunits. The metabolic stability of this pool suggests that it may be readily available for formation of functional cell surface channels.

95.9 SINGLE K^+ CHANNELS FROM RAT BRAIN INCORPORATED INTO LIPID BILAYERS ON PATCH-CLAMP PIPETTES. J.Farley and B.Rudy. Prog. in Neurosci., Princeton Univ., Princeton, NJ 08544 and Dept. of Physiol. Biophysics, New York Univ. Med. Ctr., NY,NY 10016.

The diverse functional properties of K^+ channels in many neurons, their role in neuromodulation, and their apparent cell-specific control by calcium and cyclic nucleotides raise interesting questions as to their role in the regulation of neuronal excitability. We have used planar bilayers on patch-clamp electrodes[1] and a special flux assay[2] to detect and characterize K^+ channels in rat brain synaptosomal membranes. This flux assay allows the selective study of specific ion channels present in only a small fraction of a heterogeneous population of vessicles.

Artificial membranes (2-40 GΩ) were formed on the tips of fire-polished patch clamp electrodes from a 70:30 molar ratio of bovine brain lipids (PE:PC). Incorporation of ionic channels proceeded within 2-6 min in 109 of 117 experiments, following the addition of membrane vessicles to a 100 mM KCl^-/500 μ MCaCl solution.

To date, we have identified and characterized two different K^+ channels and a Cl^- channel, on the basis of their selectivity, unitary conductance(γ), gating kinetics, and sensitivity to calcium and TEA ions. The most commonly observed channel (seen in 88 experiments) displays: 1) a γ = 220 pS in a symmetrical 100 mM KCl solution, 2) cation selectivity (Pk:Pna ∿ 100:1), 3) voltage-dependent block by TEA (<10 mM) applied to the intracellular (bath) face of the channel, 4) modest voltage-sensitivity: at 200 μ M Ca^{2+} concentrations, the channel is often open at -80 mV, and 5) sensitivity to "intracellular" Ca^{2+}: the channel is reversibly activated and inactivated by EGTA-buffering of the calcium concentration. High(>200 μM) concentrations of Ca^{2+} partially inhibit this channel, which can be overcome by greater depolarizations. This channel appears to be similar to the "BK" channels described in many tissues[3-6]. The second channel, distinguished clearly in 63 experiments, displayed: 1) a γ =140 pS, 2) cation selectivity, 3) complex gating characteristics with at least two closed states, 4) resistance to intracellular TEA (10mM) and 4-AP (1mM) block, 5) strong voltage-dependency, and 6) greater sensitivity to intracellular Ca^{2+} than the large conductance channel.

Other classes of K^+ channels, Ca^{2+}- insensitive and with smaller conductances, have also been seen. A fourth Cl^- channel has also been studied. It displays: 1) γ =50 pS, 2) little voltage- or Ca^{2+}-dependency, and is not blocked by TEA or 4-AP.

1. Coronado & Latorre, Biophys. J., 43: 1983. 2. Garty et al., J. Biol. Chem., 258: 1983. 3. Marty, A. Nature, 291: 1981. 4. Barret et al., J. Physiol., 331: 1982. 5. Adams et al., Nature, 296: 1982. 6. Latorre, R. et al., PNAS, 79: 1982.

95.10 A NEW INTERPRETATION OF THE EFFECTS OF TETRAETHYLAMMONIUM IONS (TEA^+) ON SQUID GIANT AXONS. J.R. Clay* (SPON: W.J. Adelman, Jr.). Lab. of Biophysics, NINCDS, NIH at the Marine Biological Lab., Woods Hole, MA 02543.

Internal application of TEA^+ in squid axons produces a marked prolongation of action potential duration (Tasaki, I. and S. Hagiwara, J. Gen. Physiol.,40:859, 1957) and a significant reduction of outward going potassium ion current (Armstrong, C.M. and L. Binstock, J. Gen. Physiol.,48:859, 1965). These results suggest that the effect of TEA^+ on the action potential is caused by potassium channel blockade. The mechanism of blockade proposed by Armstrong, C.M. (J. Gen. Physiol.,50:491, 1966) is based on the hypothesis that a potassium channel must open before TEA^+ blockade can occur. The primary evidence for this model is the time course of potassium ion current in the presence of TEA^+ (and TTX) following a membrane depolarizing step. The current initially rises with a time course similar to that of the control followed by a slow decline of outward current, which is reminiscent of sodium channel inactivation. This result is consistent with a link between channel activation and TEA^+ blockade, although the correlation could be fortuitous since membrane depolarization is required for both processes to occur. The results in this study concerning the effects of moderately large TEA^+ concentrations (10-20 mM) on inward-going tail currents with elevated levels of external potassium ion concentration indicate that TEA^+ blockade is, in fact, independent of channel gating. The tail exhibits a slight, initial increase in the inward direction lasting about 0.2 msec (Armstrong and Binstock, 1965) followed by a return to baseline with a time constant which is identical to that of the control. This result suggests that TEA^+ blockade is, by definition, independent of channel gating. The Armstrong (1966) model predicts a tail current time constant in the presence of 10-20 mM TEA^+ which is significantly greater than the control An alternative model for TEA^+ effects is proposed based on Eyring rate theory.

Computer simulations of the action potential demonstrate that TEA^+ blockade of potassium current produces surprisingly little prolongation of action potential duration. TEA^+ also effects the sodium current by increasing the steady-state, non-inactivating component of sodium conductance for potentials positive to 0 mM (Yeh, J.Z. and T. Narahashi, J. Gen. Physiol., 69:293, 1977). Simulations with this effect alone also produce relatively little increase in action potential duration. However, the simulated results are in close agreement with the experimentally observed action potentials, when the effects of TEA^+ on both the sodium and the potassium currents are incorporated into the computer model.

95.11 PSYCHOTOMIMETIC "SIGMA OPIATES" AND PHENCYCLIDINES SELECTIVELY BLOCK THE SAME K CHANNELS IN PRESYNAPTIC NERVE TERMINALS. D.K. Bartschat*, R. G. Sorensen* and M. P. Blaustein. (SPON: E.K. Krueger) Univ. of Maryland, Sch. of Med., Baltimore, MD 21201.

Rat brain synaptosomes have at least four different types of K channels, as shown by ^{86}Rb and ^{42}K efflux studies (J. Physiol. 361:419, 1985). One type of channel, a voltage-regulated, non-inactivating K channel ("S_V"), is selectively blocked by the psychotomimetic agent, PCP, and behaviorally active analogues (Soc. Neurosci. Abstr. 10:863, 1984): the rank order of potency for block of these K channels closely parallels the behavioral potency of these drugs and their relative ability to displace ^3H-PCP from a high affinity binding site on synaptic plasma membranes. Of interest are several stereoisomer pairs of "sigma opiates", one isomer of which produces PCP-like behavioral effects and displaces bound ^3H-PCP more potently than the other. In the presence of 10 μM naloxone, which blocks classical opiate effects, dexoxadrol blocks S_V at 1000-fold lower concentrations than does its stereoisomer, levoxadrol; (+) SKF 10,047 (N-allyl-normetazocine or NANM) is 10-fold more potent than (-) SKF 10,047; and (-) cyclazocine is 2-3-fold more potent than (+) cyclazocine. This stereoselectivity for block of S_V closely parallels the effects of these isomers in behavioral and binding experiments (Table I). Furthermore, block of S_V by the phencyclidines and "sigma opiates" occurs at nano- to micromolar concentrations. This high affinity block of presynaptic K channels should prolong the action potential duration and increase voltage-gated Ca entry, thereby producing excessive neurotransmitter release, as is observed in PCP intoxication. These results are consistent with the view that the behavioral effects of PCP and "sigma opiates" may be related to blockade of some presynaptic K channels, and that the PCP/"sigma opiate" receptor is associated with a specific class of K channels. Supported by NIH grant NS-16106 & NRSA NS-07611.

Table I

Drug Pair	Relative Potency for: Block of S_V	Displacement of Bound ^3H-PCP	PCP-like Behavior
Dexoxadrol: Levoxadrol	~1000:1	418:1	>1000:1[a]
(+) NANM: (-) NANM	~10:1	6.4:1	~3:1[b]
(-) Cyclazocine: (+) Cyclazocine	2-3:1	1.8:1	~5:1[c]

a. J. Pharm. Exp. Ther. 228: 147, 1984.
b. Eur. J. Pharm. 84:225, 1982.
c. J. Pharm. Exp. Ther. 222:146, 1982.

95.12 THE RAT BRAIN PHENCYCLIDINE RECEPTOR CONSISTS OF TWO POLYPEPTIDES (M_R = 95 kD and 80 kD) THAT ARE SPECIFICALLY LABELLED BY ^3H-AZIDO-PHENCYCLIDINE. R.G. Sorensen* and M.P. Blaustein, Dept. of Physiol., Univ. of Maryland, Sch. of Med., Baltimore, MD 21201.

Phencyclidine (PCP) and behaviorally-active analogues bind with high affinity to synaptic membranes (Biophys. J. 47:384a, 1985) and selectively block voltage-regulated, non-inactivating K channels ("S_V") in rat brain presynaptic terminals (Bartschat & Blaustein, Soc. Neurosci. Abstr. 10:863, 1984). Thus, elucidation of the molecular composition of this PCP receptor may provide direct information about the subunit structure of certain rat brain K channels. To identify the brain PCP receptor, we prepared a tritiated photoaffinity analogue of PCP, m-azido- [^3H]-phencyclidine (^3H-Az-PCP); this photolabile ligand binds with high affinity ($K_{0.5}$ ≈ 0.9 μM) to the PCP receptor (Soc. Neurosci. Abstr. 10:863, 1984).

Synaptic membranes were incubated for 30 min at 37°C in 5 mM sodium phosphate, pH 7.0, with 0.5 - 2 μM ^3H-Az-PCP, without or with a 500 to 2000-fold excess of unlabelled PCP or other ligands (see below). The samples were then irradiated with 254 nm UV light for 5 min to photolyze the Az-PCP. The membranes were solubilized in SDS dissociation buffer and subjected to SDS-PAGE. The resulting gels were impregnated with "Fluoro-Hance" (RPI), dried, and fluorograms were prepared. Several polypeptides incorporated the label. However, in the presence of excess unlabelled PCP, incorporation of the label was markedly reduced in only two polypeptides, M_R 80 kD and 95 kD, respectively. The M_R = 95 kD peptide was labelled more heavily, and may therefore include the primary PCP binding site. Covalent labelling of these two polypeptides was also specifically blocked by other PCP analogues such as TCP (the thienyl analogue of PCP), by some K channel blockers (4-aminopyridine and tetrabutylammonium), and stereoselectively by certain behaviorally-active ("PCP-like") "sigma" opiates (dexoxadrol >> levoxadrol). The latter results parallel the ability of these ligands to displace ^3H-PCP from rat brain membranes and to block "S_V". The acetylcholine receptor (AChR) of Torpedo electric organ is also a PCP "receptor". However, this nicotinic AChR, with about 10-fold lower affinity for PCP than the brain "receptor" and with specific ^3H-Az-PCP labelling to subunits of M_R <66 kD (Haring et al., J. Neurosci. 4:627, 1984), differs from that of the rat brain PCP "receptor". Our data are consistent with the view that the rat brain PCP receptor is the voltage-regulated, non-inactivating K channel, and that this channel consists of at least two subunits of M_R = 80 kD and 95 kD, respectively. Supported by grant NS-16106 and NRSA NS-07611.

SENSORY SYSTEMS: SUBCORTICAL VISUAL PATHWAYS II

96.1 RESPONSES OF PRIMATE LATERAL GENICULATE NEURONS TO COLOR CONTRAST INDEPENDENT OF BRIGHTNESS CONTRAST. P. Gouras and H.U. Evers*. Dept. Ophthalmology, Columbia University, New York, N.Y. 10032

An edge formed by spectral (wavelength) contrast across which brightness (effective light energy) contrast can be independently controlled is used as a stimulus in order to determine what forms of pure color can be distinguished by neurons in the lateral geniculate nucleus (LGN) of anesthetized (Nembutal 10 mg/kg/hr) cynomolgus monkeys. The edge is projected onto a tangent screen focused on the monkey's retinas through a 1-2mm pupil and moved at 10 deg/sec or slower across the center of the receptive field of each neuron. Responses to the same stimulus are averaged and analyzed on a computer display. The precise alignment of each response provides a good monitor of any eye movement. The parvocellular layers contain mostly tonically responding neurons with small receptive field centers; but some very phasically responding cells are also found. Tonic on-center cells tend to be in the upper and off-centers in the lower layers. Most of the tonic cells show antagonistic interactions between two different cone mechanisms which we believe to be the middle (M) and long (L) wavelength sensitive cones. We have not yet encountered a cell subserving S cones. The phasic cells do not show cone opponent interactions but respond similarly to all wavelengths. In the magnocellular layers only phasic cells are found, also without overt cone opponent interactions. All cells studied so far cannot distinguish white from any other color (red, yellow, green or blue). It is therefore impossible for visual cortex to distinguish white from other colors given this input. Many of the tonic on- and off-center cells can distinguish reddish orange from greenish blue and they do this better when brightness contrast across the edge is minimized; presumably this brings their cone opponent inputs to a more optimum balance. For example, the so called "concealed" cone opponent cells tend to become overt cone opponent cells as brightness contrast is minimized. Phasic cells cannot distinguish any part of the spectrum from any other but they have high sensitivity for brightness contrast and movement. Their response to brightness contrast is minimal at approximately the same range that we see equal brightness contrast across the edge. These tonic cells are not so; some see yellow brighter than white, others see white brighter than yellow and at a different brightness scale than we. This arrangement must guarantee that a white/yellow edge can always be detected as a brightness gradient. For all these cells blue is darker than the other colors and a blue/black edge is an extremely ineffective stimulus. In order to tell white from yellow or blue from green as a color difference another input is required. We believe that this input must be transmitted by a small fraction of cells which receive an input from S cones. We have detected such cells in the retina where they represent about 5% of all optic nerve fibers. We expect to detect them in the LGN.

96.2 THE PROCESSING OF CONTRAST INFORMATION IN THE MAMMALIAN LATERAL GENICULATE NUCLEUS. E. Kaplan* and R.M. Shapley* (SPON: F. Brink)
The Rockefeller University, New York, NY 10021.

Contrast is one of the most important aspects of a visual stimulus, and it is thus important to understand the way in which it is encoded and processed by various stages of the visual system.

We used simultaneous recordings of synaptic (S) potentials and action potentials from lateral geniculate nucleus (LGN) neurons in cats and monkeys in order to study the way in which the LGN processes contrast information. The S potentials faithfully reflect the activity of the retinal ganglion cells which drive the LGN units. The stimuli were spots, annuli or gratings, presented on the face of a CRT screen and modulated or drifted at various temporal frequencies. Their contrast ranged from 2% to 80%.

Our preliminary results show that the contrast-response functions of most LGN units saturate before their inputs do. The amplitude ratio of the LGN response/retinal ganglion cell response decreases and the phase difference increases as contrast increases. This result appears to hold for a range of temporal frequencies, patterns and cell types. At this stage, neither the mechanism nor the functional significance of this compression of contrast information at the thalamus is clear.

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96.3 GABA IMMUNOREACTIVITY IN MONKEY LATERAL GENICULATE NUCLEUS (LGN) AT LIGHT AND ELECTRON MICROSCOPIC LEVELS. P. Pasik, T. Pasik, J. Hamori* and G. R. Holstein. Depts. Neurol. & Anat., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029, and 1st Dept. Anat., Semmelweis Univ. Sch. Med., 1450 Budapest, Hungary.

The recognition of presumably GABA-ergic elements in the LGN has been based on indirect methods that permit the visualization of neurons which uptake tritiated GABA (by autoradiography), or the identification of sites for GABA synthesis (by GAD immunocytochemistry) or catabolism (by GABA-T histochemistry). We have used the direct labeling of GABA-containing elements with an antiserum, raised against GABA-BSA conjugate, of proven specificity (Hodgson et al., J. Histochem. Cytochem., 1985), and further treatment with Sternberger's PAP technique. The procedure was made on preembedded 50 µm Vibratome sections and resin-embedded 1 µm sections from 3 M. mulatta and 1 M. fascicularis. No labeling was observed in control tissue. Light microscopy showed immunostained neuronal bodies in the pars dorsalis (LGNd). Most of them were smaller than unlabeled neurons and belonged to a bipolar type with poorly arborized long dendrites. The proportion of immunoreactive cells was higher in magnocellular than parvocellular laminae. Somata were also consistently labeled in the pars ventralis (LGNv) or pre-geniculate nucleus. The reaction product appeared in both cytoplasm and karyoplasm, the nucleolus always being negative. No stained glial elements were ever observed.

Ultrastructurally, GABA immunoreactivity was found in perikarya similar to those described for interneurons in our earlier work, exhibiting an occasional cluster of synaptic vesicles. Heavy label was present in dendrites as well as in vesicle-containing profiles which were postsynaptic to unlabeled terminals of retinal origin, and presynaptic to either unstained dendrites of principal cells, or to other immunoreactive dendrites. These elements, considered to be interneuron presynaptic dendrites, frequently formed triadic synaptic arrangements. In addition, there were other immunostained profiles in the LGNd neuropil of exclusively presynaptic character, with considerably larger mitochondria and lighter labeled synaptic vesicles.

These findings provide direct identification of GABA-ergic neurons in both the LGNd and LGNv, and strongly suggest that they are of inhibitory nature. That such cells are the LGNd interneurons (I-cells) is supported by: (1) similar differential proportion in the two laminar types as found for I-cells (Hamori et al., Exp. Brain Res., 1983); (2) same synaptology of labeled profiles as those of earlier defined I-cell presynaptic dendrites (Hamori et al., Brain Res., 1974); (3) frequent synapses between labeled profiles as with the previously described I-cell to I-cell junctions (Pasik et al., Exp. Brain Res., 1976). The second type of labeled bouton probably represents terminals of afferent axons from the thalamic reticular nucleus and/or the LGNv.

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96.4 GABA ANTISERUM REACTIVITY IN THE LATERAL GENICULATE NUCLEUS OF THE TREE SHREW (TUPAIA BELANGERI): A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS. R.N. Holdefer, T.T. Norton and R.R. Mize. Dept. of Physiological Optics, Sch. of Optometry, Univ. of Ala. at Birmingham, AL 35294 and Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163.

Recent reports of GAD immunoreactivity and ^{3}H-GABA uptake in the lateral geniculate nucleus have suggested that GABAergic neurons participate in the inhibitory circuitry of the LGN. In order to examine this issue more directly, we utilized an antiserum specific for GABA. Tree shrews were perfused with a phosphate buffered 4% paraformaldehyde, 0.2 - 0.5% glutaraldehyde fixative. 50 µm sections were reacted with GABA antiserum (Immuno Nuclear) and stained using the avidin-biotin (ABC) technique. Selected sections were prepared for electron microscopy.

At the LM level, dense, labelled neuropil was found throughout the LGN but was quite sparse within the interlaminar zones. Darkly-labelled cells were distributed homogeneously in all 6 layers of the LGN, in both the monocular and binocular segments. In many labelled cells, 1 - 4 proximal dendrites could be seen. These dendrites occasionally crossed laminar boundaries. The proportion of labelled cells was approximately 25 - 30% in semithin sections counterstained with toulidine blue. The mean area (± s.e.) of the labelled cells was 99.8 ± 5.0 µm^2 in contrast with the mean area of 186.5 ± 7.0 µm^2 for the unlabelled cells in the same section.

At the EM level the labelled cells were characterized by a relatively thin rim of cytoplasm surrounding a prominent nucleus. The nucleus usually was labelled more darkly than surrounding cytoplasm and was deeply invaginated. We found no clearly labelled glia. Other labelled profiles included myelinated axons, large dendrites and several types of vesicle-containing profiles. Many examples were found of labelled F2 profiles that were postsynaptic to retinal terminals within a glomerulus and that contained pleomorphic vesicles. The presence of numerous labelled axons in the LGN suggests that part of the labelled neuropil may come from extrinsic sources. Indeed, darkly-labelled cells were found in the thalamic reticular nucleus.

In conclusion, the homogeneous distribution of labelled cells across all 6 LGN layers suggests that similar, GABA-mediated inhibitory processes occur throughout the LGN. Our EM data support the conclusion that, as in other species (cat, galago, and macaque), GABAergic neurons in tree shrew participate in F2 synaptic interactions and may mediate feed-forward inhibitory processes in the LGN.

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96.5 TWO TYPES OF GABA-CONTAINING INTERNEURONS IN THE A-LAMINAE OF THE CAT LGN: A DOUBLE-LABEL HRP AND GABA-IMMUNOCYTOCHEMICAL STUDY. J. Zempel* and V.M. Montero. (SPON: C.N.Woolsey). Dept. of Neurophysiology, Waisman Center, Univ. of Wisconsin, Madison, WI 53705.

The A-laminae of normal and HRP-retrogradely labeled LGN of the cat were examined after immunocytochemistry with a GABA antiserum with three main objectives: a) to see whether the GABA antiserum labels the same type of cells that were previously identified with GAD antisera (Fitzpatrick et al. '84; Montero and Singer '85); b) to examine whether GABA+ cells can be retrogradely labeled from the visual cortex after massive injections of HRP in cortical areas 17 and 18; and c) to analyze quantitatively the size distribution of GABA+ cells in the medio-lateral extent of LGN.

The results showed that GABA+ cells were similar in several respects (distribution, size, proportion) to the population of cells identified with GAD antibodies, reinforcing the notion of the GABAergic nature of these cells. In vibratome (50 µm) or plastic-embedded semithin (1 µm) sections doubly reacted with HRP and GABA antiserum, the relay cells were characterized by black HRP Co-DAB grains in the cytoplasm and absence of the brown anti-GABA reaction product in the nucleus. In contrast, GABA+ cells showed the brown anti-GABA reaction product in the cytoplasm and nucleus, and absence of black HRP grains in the cytoplasm. As control, sections of the cortex surrounding an HRP injection site showed many double-labeled HRP-GABA+ cells. In the semithin sections (treated with post-embedding GABA immunocytochemistry) geniculate neurons were labeled either retrogradely with HRP or with anti-GABA reaction products, the GABA+ cells being about 23% of the total population. The results of the double labeled material suggest the interneuronal nature of GABA+ cells.

Cell body area measurements showed that the population of GABA+ cells in the A-laminae is composed of a large proportion (about 79%) of small cells (50-220 µm^2) and a smaller proportion (about 21%) of medium size cells (220-440 µm^2), corresponding to the beta and alpha GAD+ cells, respectively, previously described by Montero and Singer '85. The proportion of medium size (alpha) cells, however, increases from medial to lateral parts of LGN, resembling a similar medio-lateral increase of Y and type I cells in the A-laminae (Hoffmann et al. '72; LeVay and Ferster '77). These results suggest that the beta and alpha GABAergic interneurons are related to the X and Y relay cells, respectively, and are consistent with the notion of separate feedforward inhibitory pathways to X and Y relay cells in cat LGN (Lindström and Wróbel '84), via X and Y intrinsic interneurons (Dubin and Cleland '77).

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96.6 LOCALIZATION OF GABA IN TYPE 3 CELLS OF CAT LGN AND DEMONSTRATION OF THEIR SOURCE TO F2 TERMINALS: A STUDY USING A NEW DOUBLE-LABEL GOLGI-EM GABA-IMMUNOCYTOCHEMICAL METHOD. V.M. Montero. Dept. of Neurophysiology, Waisman Center, Univ. of Wisconsin, Madison, WI 53705.

Presumptive GABAergic cells in cat LGN have been identified by 3H-GABA uptake and immunoreactivity to GAD and GABA antisera by several workers. The pattern of dendritic arborization of these cells is, however, unknown. Furthermore, there is no direct evidence linking GABA processes in cat LGN (F1, F2 terminals) to intrageniculate GABA cells. To answer these questions, typical Guillery's types 1, 2 and 3 geniculate cells were selected in Golgi gold-toned sections embedded in plastic for subsequent processing with GABA immunocytochemistry. After drawing and photography of the cells, alternate semithin (1 µm) and thin sections were collected serially of dendritic and perikaryal regions. Post-embedding immunocytochemistry using a GABA antiserum was done in the semithin sections containing the perikarya. Taking advantage of the fact that the GABA antiserum labels not only the cytoplasm but also the nucleus of neurons (which is, fortunately, spared by the Golgi gold-toned impregnation), it was possible to differentiate double-labeled Golgi-GABA cells from cells that were only Golgi impregnated. Not surprisingly, only the perikarya of type 3 cells were found to contain GABA immunoreactivity. The GABA+ type 3 cells identified in this material were characterized by few slender, scarcely branching dendrites bearing many, or few, long stalked appendages and by extensive filiform, axon-like processes with appendages, taking origin from different regions of dendrites. Several of these Golgi gold-toned appendages were examined at the EM and identified as typical F2 processes; i.e., they contain pleomorphic synaptic vesicles and are postsynaptic to retinal terminals and presynaptic to dendrites.

In conclusion, the results provide supporting evidence for the GABAergic nature of type 3 cells in cat LGN, and for their giving origin to F2 terminals. In addition, since GAD+ and GABA+ cells in cat LGN are not retrogradely labeled with HRP from the visual cortex (Fitzpatrick et al. '84; Zempel and Montero '85, accompanying abstract) the results support the notion of the interneuronal nature of type 3 cells.

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96.7 PATTERNS OF DISTRIBUTION OF X ON AND OFF AND Y ON AND OFF CELLS IN THE A LAYERS OF THE DORSAL LATERAL GENICULATE NUCLEUS (LGNd) OF THE CAT. Douglas B. Bowling and Elzbieta Wieniawa-Narkiewicz, Depts. of Med. Physiology & Anatomy and the Lions Sight Centre, University of Calgary, Calgary, AB., Canada T2N 4N1.

Ganglion cells in the cat retina and cells in the LGNd can be classified based on whether they sum the effects of light linearly (X) or non-linearly (Y) within their receptive fields and on the sign of contrast to which they respond (On-center or Off-center). While these distinctions are usually clear, the functional roles and interactions of the different cells are not well understood. A likely place to look for clues to the functional relationship between the cells is in the A layers of the LGNd. Here the axons of the different kinds of ganglion cells converge and terminate with the potential for interaction or for specific patterns of segregation.

To examine the relationship in the LGNd between X and Y and On and Off cells in more detail we made tangential electrode penetrations through the A layers. We were able to measure the positions (depths) of cells more accurately than previously possible because the electrodes ran parallel to the layers.

We recorded from 239 cells in layer A and 177 cells in layer A_1. It is clear from the sample that cells of different physiological types have specific and well-ordered arrangements across layer A but are only weakly organized in their distributions across layer A_1. In layer A the Y cells are heavily concentrated near the dorsal and ventral borders with Y off cells being predominant ventrally. X cells are distributed more uniformly across the layer but are arranged in an On/Off/On tri-laminar pattern. X on cells outnumber X off cells by nearly 2 to 1 in dorsal and ventral thirds of the layer and this ratio is reversed in the central third of the layer. In layer A_1 there is a small but clear shift in the proportion of X on and Y off cells across the layer with X on cells being slightly more common near the dorsal border and X off cells more comon near the ventral border. Too few Y cells have been sampled in A_1 to determine their distributions there.

These results may reflect differential interactions between X on, X off, Y on and Y off cells at different depths in the layers as well as differential patterns of projection from geniculate to cortex.

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96.8 RELAY CELLS AND INTERNEURONS IN THE CAT'S LATERAL GENICULATE NUCLEUS WITH EXCITATORY INPUT FROM MORE THAN ONE RETINAL X-CELL. D. N. Mastronarde* (SPON: M. W. Dubin). Dept. of MCD Biology, Univ. of Colorado, Boulder, CO 80309.

This study continues the description of how the retinal input to the cat's lateral geniculate nucleus (LGN) diverges to form different types of cells with differing properties. Experiments involved recording from a single LGN cell, assessing its receptive field properties, and searching in the retina for ganglion cells that provided input to the LGN cell, as judged from cross-correlations. Elsewhere, I described two distinct types of relay cells that receive essentially only one excitatory retinal X-input: X_S-cells, whose responses to visual stimuli replicate the basic form of the response of a single retinal X-cell; and X_L-cells, whose responses lag behind those of retinal X-cells (Invest. Ophthalmol. Vis. Sci. Suppl. 24:265). Here I report on three kinds of cells that receive significant excitatory input from more than one retinal X-cell: multiple input X-relay cells (X_M-cells) with no Y-input, multiple X-input relay cells with Y-input (X/Y-cells), and X-interneurons. Results from the retinal searches indicated that these cells with multiple X-input form one or more cell types, all distinct from the single-input X-cells. Compared to X_S-cells, X_M and X/Y-cells had, on average, larger receptive field centers, better responses to a large moving or flickering stimulus, somewhat weaker suppressive (inhibitory) fields, shorter conduction times to visual cortex (0.45 to 1.15 ms, mean 0.7 ms, vs. 0.7 to 2.1 ms, mean 1.2 ms, for X_S-cells), and a higher probability of responding to stimulation of the optic chiasm (usually near 100%, vs. less than 50% for X_S-cells). The X/Y-cells differed from X_M-cells primarily in having significantly lower spatial resolution. The period of the finest grating resolved by the cells was: X_S = 0.38 ± 0.03; X_M = 0.59 ± 0.04; X/Y = 0.81 ± 0.08; Y = 1.15 ± 0.10 (degrees, mean ± SEM). This suggests that the functional role of X_M and X/Y-cells may be to fill in the large, 1.5-octave gap in spatial resolution between X_S and Y-cells with half-octave steps.

Some X-cells could not be antidromically activated from visual cortex, even though antidromic stimulation was routinely successful in activating even X_L-cells that had high thresholds and long latencies (2.5-5 ms). The X-interneurons had receptive field properties most closely resembling those of X_M-relay cells; however, they showed much weaker suppressive field effects and usually had higher maintained firing rates than X-relay cells. Searches for retinal inputs to eight cells all yielded evidence for input from more than one retinal X-cell but no evidence for Y-input. Thus, the X-interneurons identified by lack of antidromic activation also have a set of common properties that differ from those characteristic of the various types of X-relay cells.

96.9 FUNCTIONAL SUBGROUPS AMONG X-CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. A.L. Humphrey and R.E. Weller, Department of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

The X-cells in the cat lateral geniculate nucleus have been considered a single functional class. However, Mastronarde (ARVO Abstr. 24:265, 1983) demonstrated heterogeneity among X-cells in their retinal inputs and patterns of discharge to flashed spots of light and suggested that there may be two X subclasses, termed "normal" and "lagged". We have confirmed this heterogeneity and found additional correlates to it. We recorded from physiologically identified X-cells in laminae A and A1 of the LGN. Of 161 cells, 63% showed similar responses to a small flashing spot centered on the receptive field: 1) a brisk response onset at a latency ≤60 msec.; 2) achievement of 90% of peak response in ≤80 msec.; 3) no detectable inhibition; and 4) a rapid return to background firing at stimulus offset. This response pattern matches that of Mastronarde's "normal" X-cells. We tentatively refer to these as X_N-cells. Most of the remaining X-cells (27%) had a different response pattern to flashing spots: 1) following stimulus onset, an early period of reduced firing, probably due to inhibition; 2) a delayed onset of firing, having a latency ≥50 msec.; 3) achievement of 90% of peak response at ≥90 msec; and 4) in most of the cells, a transient increase in firing at stimulus offset. This pattern matches that of Mastronarde's "lagged" X-cells. We tentatively refer to them as X_L-cells. The remaining X-cells (10%) were intermediate in their latencies and discharge patterns, in agreement with Mastronarde's observations.

Despite some overlap, the X_N- and X_L-cells differ in other features as well. The geniculocortical conduction latencies for X_N-cells are smaller than those of the X_L-cells (<1.9 vs. >2.1 msec., respectively). Although both groups discharge at similar latencies to stimulation of the optic chiasm, the probability of firing is generally higher for X_N-cells (median=.5, range=.08-1.0) than for X_L-cells (median=.03, range=0-.07). Finally, our additional studies using drifting sine gratings reveal differences in the optimal temporal frequencies of the X-cell groups. Of X_N-cells, 91% prefer frequencies >2 Hz, while 71% of X_L-cells prefer frequencies ≤2 Hz. The lower optimal temporal frequencies and chiasm firing probabilities among X_L-cells may reflect strong, stimulus induced inhibition impinging upon them.

In conclusion, our data confirm the existence of physiological heterogeneity among X-cells and the presence of two major subgroups (X_N and X_L). However, due to overlap of the subgroups, and the existence of intermediate cells, it is not yet clear whether the subgroups comprise distinct classes or opposite ends of a continuum. (Supported by USPHS grants EY04091, EY03038 and EY05674.)

96.10 STRUCTURAL CORRELATES OF FUNCTIONAL SUBGROUPS AMONG X-CELLS IN THE CAT LGN. R.E. Weller and A.L. Humphrey, Department of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Functional heterogeneity exists among X-cells in laminae A and A1 of the cat lateral geniculate nucleus. Two major subgroups, termed X_N and X_L, have been defined which differ in terms of their latencies and patterns of discharge to visual stimuli and their axon conduction velocities (see preceding abstract). The goal of these experiments was to determine the correspondence between the two functional subgroups and the heterogeneous morphological cell types previously associated with the X-cell population (Friedlander et al., J. Neurophysiol. 46:80, 1981).

Single X-cells were recorded in the A-laminae with micropipettes filled with horseradish peroxidase. Each cell's functional subgroup was identified from its response to a small spot stimulus flashed on and off in its receptive field. Cells were classified as X_N if: 1) their response onset was <60 msec.; and 2) they attained 90% of maximal response at <80 msec. Cells were classified as X_L if: 1) they showed an early inhibition following spot onset; 2) their response onset occurred at >60 msec. and 3) was 90% maximum at >90 msec.; and 4) a transient discharge occurred at stimulus offset. Each identified cell was labeled with HRP to visualize its morphology.

We found consistent morphological differences between 7 X_L-cells and 5 X_N-cells recovered. All X_L-cells exhibit class 2 or class 2-3 morphologies (Guillery, J. Comp. Neurol. 128:21, 1966). Their small to medium sized somata (200-300μm^2) give rise to 3-7 primary dendrites, which issue narrow, sinuous dendrites distally. The most consistent feature is large clusters of grapelike appendages located at or near dendritic branch points. Depending on the cell, the distal dendrites may be smooth or beaded and they variably possess hairlike appendages or a few complex stalked appendages. The extrageniculate axons are the smallest observed (<1.0μm).

Three of the X_N-cells have medium sized somata (300-405μm^2) and exhibit class 1 morphology: 7-9 primary dendrites, straight, smooth distal dendrites having only a few grapelike appendages, and forming a radially symmetric dendritic tree. Their axons are >1.0μm in diameter. Until now, class 1 morphology has been associated exclusively with Y-cells, but X_N-cells may also show it. Two other X_N-cells exhibit class 3 morphology: very small (110-150μm^2) somata, fine, highly sinuous dendrites with numerous complex stalked appendages, and no extrageniculate axon.

These results suggest that the functional subgroups X_N and X_L have different morphological features. To confirm this, we are injecting additional X_N- and X_L-cells as well as cells with intermediate physiological properties. (Supported by USPHS grants EY04091, EY03038, and EY05674.)

96.11 EARLY DIFFERENTIAL EFFECTS OF VISUAL DEAFFERENTATION ON X AND Y CELLS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. W. Guido, W.L. Salinger, & C.E. Schroeder. Dept. of Psychology, Univ. North Carolina-Greensboro, NC 27412.

Previous studies have shown that signal transmission through retino-geniculate synapses terminates 3-4 days after visual deafferentation. Because LGN X and Y cells receive their inputs from axons that are functionally and morphologically distinct, we re-examined the early consequences of visual deafferentation to determine whether the breakdown of signal transmission proceeds at different rates for each of these cell types. Extracellular single unit recordings were made from sedated, unparalyzed cats, both before and immediately after one eye was removed. The proportion of X and Y cells in both normally innervated and deafferented layers was determined during repeated penetrations through the A-laminae of the LGN. Cells in layers with intact retinal inputs were classified as X or Y on the basis of their receptive field properties and axonal conduction velocity. Receptive field classification was made possible by surgical immobilization of the intact eye. Cells in the deafferented layers were found by monitoring responses to visual stimulation of the intact eye, or to electrical stimulation of the optic chiasm. Deafferented LGN cells were classified as X or Y solely on the basis of axonal conduction velocity. Within 5 hours and throughout the time tested (30 hrs), visual deafferentation resulted in a reduction in the number of cells recorded per electrode penetration as well as a decrease in cell threshold to electrical stimulation of the optic chiasm. Five to 10 hours after visual deafferentation, a progressive decline in the relative encounter rate of X cells began. By 20-30 hours it was evident that this decline was due primarily to a reduction in the proportion of X cells receiving input from retinal axon stumps with slow conduction velocities (slow X: 10-17 m/s; fast X: 18-25 m/s). By 30 hours, retino-geniculate transmission had collapsed to such an extent that one rarely recorded more than a single cell per penetration in the deafferented layers. Cells recorded in layers with intact retinal inputs remained stable with respect to these measures. Thus, prior to the complete collapse of retino-geniculate transmission, there appears to be an early, selective breakdown of signal transmission through the X cell pathway. The fact that this breakdown occurs earliest for a sub-population of X cells that receives its main input from slowly conducting afferents suggests that specific morphological features of the remaining axon stump (e.g., diameter, or axoplasmic volume) may play a critical role in determining the temporal course of degeneration in synaptic transmission. The use of a sedated unparalyzed preparation and choice of dependent measure may account for our detection of these early degenerative effects.

VESTIBULAR SYSTEM I

97.1 VERTICAL CANAL AFFERENT ACTIVITY AND ITS RELATIONSHIP TO CONTINUOUS NYSTAGMUS DURING PITCH WHILE ROTATING. T. Raphan, W. Waespe*, B. Cohen. Dept. of CIS, Brooklyn College and Depts. of Neurology, Mt. Sinai Sch. Med., New York, Univ. of Zurich, Switz.

Pitching the head back and forth while rotating about a vertical axis induces continuous horizontal nystagmus by activation the velocity storage integrator (Raphan et al. 1983). Canal and otolith afferents were recorded during sinusoidal pitching of the head while rotating to determine how their activation is related to specific parameters of slow phase eye velocity and how they might activate the velocity storage mechanism. Neurons were tested with rotational velocities up to 180°/sec and pitch amplitudes up to 40° about a plane coincident with the plane of the horizontal canal. Horizontal canal afferents responded with a jump in frequency at the start of rotation and decayed to their spontaneous levels as rotation continued. During sinusoidal pitching activity was modulated about the spontaneous level at twice the pitching frequency. The depth of modulation was proportional to the pitch angle and the peak excitation occurred with the lateral canal close to the horizontal plane over a pitching frequency of .05 to .2 Hz. Similar activity was observed during roll while rotating when continuous nystagmus was not prominant. This supports the contention that horizontal canal afferents do not contribute to the generation of continuous nystagmus during pitch while rotating. Otolith afferents were modulated according to pitch position depending on their polarization vectors and their activity was independent of the velocity of rotation about the horizontal axis. This shows that Coriolis forces during pitching while rotating are not significant in modulating the activity of otolith afferents and that excitation of the otoliths alone is not responsible for the continuous nystagmus. Vertical canal afferents were excited by pure pitching with depths of modulation being proportional to pitching velocity. Peak excitation occurred in phase with peak pitching velocity and 90° out of phase with otolith excitation. However, during pitching while rotating about a vertical axis there was a phase shift of the peak firing frequency so that it tended to be in phase or 180° out of phase with otolith afferent activity. This phase shift increased with rotational velocity for a pitching frequency of .05 to .2 Hz, consistent with the range over which continuous horizontal nystagmus is generated. The results suggest that during pitching while rotating the central vestibular system utilizes phase information from vertical semicircular canal afferents and the otoliths to generate a signal related to head velocity that excites the velocity storage integrator. Direct projections from afferents of horizontal and vertical canals and otoliths contribute to the modulation in horizontal slow phase eye velocity about the steady state value. Supported by EY-04148 and Swiss National Foundation.

97.2 COMBINED ANATOMICAL AND PHYSIOLOGICAL STUDY OF SEMICIRCULAR CANAL ORIENTATION IN THE RHESUS MONKEY. H. Reisine, J.I. Simpson, D. Rudinger* and V. Henn. Dept. of Neurology, University Hospital Zurich, 8091 Zurich, Switzerland and Dept. of Physiol. and Biophysics, NYU Medical Center, NY, NY, 10016.

Experiments were performed to examine the relationship between the morphology of the bony portion of the semicircular canals (SCCs) and the neural coding of head velocity in the spike train activity of single axons in the vestibular nerve. Twelve SCCs and nerves were examined in two juvenile rhesus monkeys. For nerve recordings animals were alert and rotated sinusoidally (± 25 deg; 0.333 Hz) on a three-axis turntable about either an earth horizontal or the vertical axis. Animal position with respect to the rotational axis could be oriented as desired. Modulation of unit activity varied with the animal position; a single axis relative to the head was determined about which rotation effected the largest response. The unit vector for this axis was specified in a stereotaxic system and was termed the neuronal sensitivity vector. An average sensitivity vector was calculated for each nerve from 2 to 6 neuronal sensitivity vectors. The angles between the neuronal sensitivity vectors for the same nerve ranged from 0.3 to 13.4 deg. Angles between average sensitivity vectors for the three SCC nerves from the same labyrinth ranged from 90.3 to 103.4 deg. The angles between average sensitivity vectors for individual SCCs of push-pull pairs varied between 167.4 and 177.7 deg.

Following perfusion, labyrinths were injected with a plastic casting resin, which when hardened, was exposed by removing the overlying bone. From coordinate measurements of 8 to 13 points along the circumference of each SCC cast the best fit plane was determined using principal component analysis. Angles between vectors normal to the planes of different SCCs from the same labyrinth ranged from 84.1 to 102.2 deg. The angle between normal vectors for individual SCCs of push-pull pairs ranged from 162.8 to 177.1 deg.

The angle between the average sensitivity vector and the normal vector for each SCC averaged 6.3 deg (range: 1.5 to 11.7 deg). This angle was smallest for lateral SCCs (1.5 to 6.0 deg), largest for anterior SCCs (6.4 to 11.7 deg) and intermediate for posterior SCCs (3.0 to 9.3 deg). This study provides the first physiological and anatomical measurements of SCC orientation made in the same animals. Therefore, the differences in these measurements probably reflect the degree with which physiological values can be predicted from anatomical measurements of the SCCs. Despite the differences, for both types of measurements angles between SCCs of the same labyrinth differed from 90 deg by, in some cases, more than 10 deg and angles between individual SCCs of push-pull pairs differed from 180 deg by as much as 17 deg. Supported by Swiss Foundation for Scientific Res.(3.718.80), EMDO Foundation, NASA (NAG2-336).

97.3 PATIENTS WITH PERILYMPH FISTULAS GATE VESTIBULAR INPUTS TO POSTURE, DEPENDING ON THE CONDITIONS OF SUPPORT. F.O. Black, D. Lilly, and L.M. Nashner, Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, Portland, Oregon 97209.

If patients with perilymph fistulas, i.e. small holes in the round and/or oval windows, are subjected to changes in external ear canal pressure, stimulation of the vestibular end organs results. In this study we have applied external canal pressure under altered support surface conditions. We show that, in most patients standing with eyes closed, vestibular inputs to posture are gated "off" when the support surface is fixed. As motion of the surface increases, however, a threshold is reached at which vestibular inputs are suddenly gated "on".

Patients with clinical signs of perilymph fistulas were exposed to sinusoidally modulated pressures (400 mm water, 0.1-0.5 Hz) to the external canal of one ear at a time while standing with eyes closed. Conditions were termed "normal" when the support surface was fixed and "sway-referenced" when the support surface rotated in proportion to the sway rotations of the body measured at the center of gravity. Under sway-referenced conditions, the gain of support surface to body sway rotation could be varied in increments from 0 (surface fixed) to 0.5 (surface rotates half as much as the body). Postural movements were measured by recording leg muscle EMG's, forces exerted by each foot on the surface, and the AP sway motions of the body center of gravity.

To determine the extent of postural movements in the absence of pressure stimulation, movements were recorded for 20 sec intervals under fixed and then sway-referenced conditions. Then, with the surface fixed, the pressure stimulus was imposed on one ear at a time (asymptomatic or least symptomatic ear first) for 20 sec. If postural movements following the waveform of the stimulus did not occur, the stimulus was re-imposed under sway-referenced conditions with the support surface gain incrementally increased during subsequent 20 sec trials. Finally, the process was repeated for the other ear.

Of 133 patients tested to date, 36 demonstrated postural movements correlated with pressure in the symptomatic ear but not the normal ear, 4 demonstrated responses to pressure bilaterally, and 94 were unresponsive to pressure. Correlated movements occurred in 13 patients under fixed support surface conditions. In the remaining 27, movements emerged only under sway-referenced conditions; in 5 patients at 0.12 gain, in 15 at 0.25 gain, and in 7 at 0.5 gain. Emergence of correlated movements under sway-referenced conditions appeared to be a gating phenomenon rather than the result of postural instability. The onset of pressure induced movements was frequently abrupt and occurred when the patient was stable.

This work sponsored, in part, by NIH Grants No. 19222 (Dr. Black) and 12661 (Dr. Nashner).

97.4 OTOLITH CONTRIBUTION TO ADAPTIVE CHANGE IN VOR DIRECTION. J. Baker, R. Harrison, N. Isu*, C. Wickland*, & B. Peterson. Dept. Physiol., Northwestern University Medical Sch., Chicago, IL 60611.

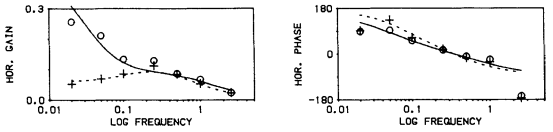
The vestibuloocular reflex (VOR) can adapt its size, direction, or dynamics in order to reduce retinal image motion that occurs during vestibular stimulation. In an accompanying abstract Harrison et al. describe adaptive modification of VOR direction by vertical optokinetic motion coupled to horizontal rotation. They show that adaptive changes in the VOR can occur during vestibular stimulation of horizontal semicircular canals only. Here we examine the VOR adaptation which occurs when pitch vertical rotation is coupled with horizontal optokinetic motion (Schultheis & Robinson, 1981). We show that rotational vestibular stimulation of either vertical canals or otoliths alone can produce modification of VOR direction.

Horizontal and pitch vertical eye movements were recorded from two normal alert cats and one cat with vertical semicircular canals inactivated by plugging (thanks to R. Schor). Whole body 0.25 Hz pitch oscillations of the cats were produced in two ways and coupled with optokinetic rotations that produced image motion in the horizontal direction on the retina: (1) Upright pitch (o) stimulated vertical canals and otoliths. The animal was placed in the normal upright position and rotated about a horizontal axis perpendicular to the cat's sagittal plane. Optokinetic motion occurred about a vertical axis. (2) Sideways pitch (+) stimulated vertical canals only. The animal was placed on its side and rotated about a vertical axis perpendicular to the cat's sagittal plane. Optokinetic motion occurred about a horizontal axis.

The gains and phases of horizontal VOR eye movements measured during pitch rotations in the dark after two hours of combined optokinetic and vestibular stimulation are shown below, with transfer function models. Each set of data shows the average adaptive smooth horizontal eye movements of the two cats after subtracting the very small horizontal movements measured before the adaptation. Sideways pitch gain was maximal at the training frequency. The added otolith stimulation in upright pitch produced a higher gain at low frequencies. The cat with plugged vertical canals developed horizontal eye movements during upright pitch adaptation, but had no VOR during sideways pitch. Thus, otolith stimulation contributes to adaptive changes in VOR direction.

Supported by NIH grants EY04058, EY05289.

$$o = \frac{0.7s}{(1+0.2s)(1+7.3s)}\left(1 - \frac{0.6}{s}\right) \qquad + = \frac{0.13s}{(1+0.3s)(1+1.2s)}\left(1 - \frac{0.5}{s}\right)$$



97.5 DYNAMICS OF DIRECTIONAL CHANGE IN THE VESTIBULOOCULAR REFLEX PRODUCED BY ADAPTIVE MODIFICATION. R.E.W. Harrison, J.F. Baker, Naoki Isu*, C.R. Wickland*, and B.W. Peterson (Spon. R.S. Eisenberg) Dept. of Physiology, Northwestern University School of Medicine, Chicago, IL 60611.

Through various neural networks, the vestibuloocular reflex (VOR) and the optokinetic reflex generate eye movements which assist retinal image fixation during angular rotation of head or environment. It is well known that alteration of the relation between visual and vestibular rotations in one plane produces plastic adaptive changes in VOR gain. More recently, Schultheis and Robinson (NYAS 374:501, 1981) showed that the direction of the VOR could be changed by pairing pitch vestibular rotation with horizontal optokinetic image rotation. These investigators used a complex vestibular stimulus where both the otoliths and the vertical canals were stimulated. We sought to determine if similar directional changes in eye movement occur when only canals are stimulated and to characterize dynamic properties of the change.

Prior to training, animals were tested in the dark for residual vertical eye movements produced by whole body constant velocity sinusoidal yaw rotation at .05-2.5 Hz. Cats were then trained by exposing them to yaw plane rotation which was coupled with simultaneous vertical optokinetic stimulation that had the same frequency and same or differing phase. After 2-4 hrs. training, the cats were again tested in the dark at the above frequencies for yaw-induced vertical eye movements. Pre- and post-adaptation vertical eye movements were compared by vector subtraction.

We observed the following: 1)After training, horizontal vestibular stimulation in the dark gave rise to vertical eye movements which were in the direction of the training optokinetic stimulus. 2)At .25 Hz, adaptation occurs rapidly within 15-30 minutes, followed by a prolonged but less rapid adaptation. In total darkness, an unrestrained animal will retain the post-adaptation effects for at least 14 hours. 3) Adaptation-associated vertical eye movements elicited by yaw vestibular stimulation alone are greatest at the training frequency. At test frequencies lower than the training frequency, the response was smaller and phase advanced; at test frequencies higher than the training frequency, the response was also smaller but phase lagged. These dynamic features could be modeled by a bandpass filter with time constants of 0.1 and 1.0 Hz. Studies with pseudorandom binary test stimuli confirm these results and suggest other mechanisms may be present at higher frequencies. 4)Preliminary observations with different training stimuli indicate phase lead or lag in vertical eye movements when the phase of the optokinetic portion of the training is correspondly advanced or retarded; training at 1 Hz. shifts induced VOR gain maxima towards that training frequency.

This work was supported by NIH grants EY04058 and EY05289

97.6 VESTIBULAR COMPENSATION: THE RELATIONSHIP OF EYE NYSTAGMUS AND NECK BENDING. David W. Jensen. Dept. Otorhinolaryngology and Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

A unilateral labyrinthectomy in the guinea pig induces profound spontaneous postural asymmetries that largely abate in two days. Data are presented here concerning the interdependence of two of the most prominent asymmetries (neck bending and eye nystagmus) and their compensations. This study expands upon unpublished observations mentioned by Schaefer and Meyer (in Memory and Transfer of Information, H.P. Zippel, ed., 1973).

A group of 14 guinea pigs received a unilateral peripheral vestibular neurectomy and labyrinthectomy which spared the vestibular ganglion. Five of the animals were suspended in a sling for 24 hours immediately after performing the surgery, in order to prevent standing contact with a solid surface. After the 24 hour suspension period, these animals were placed on a solid surface, and measurements of eye nystagmus and lateral head deviation taken over the next 2 to 3 days. Nine other animals placed on a solid standing surface immediately after the operation served as controls. Spontaneous eye nystagmus was measured using electro-oculography, and neck bending in yaw was measured with a goniometer. The mean rates of asymmetry abatements over consecutive two hour periods were also calculated and plotted.

The suspension period selectively delayed the compensation of neck bending in yaw, but did not affect the compensation of eye nystagmus. Twenty-five hours after the operation, the eye nystagmus intensity (beats/sec) of the suspension group was equal to that of the non-suspension group. However, the amount of neck bending in yaw as well as the ability to stand for the suspension group at 25 hours post-op was equal to that for the control group at only one hour post-op. The time course of neck bending compensation in the suspension group from 24-48 hours was the same as for the control group from 0-24 hours. Thus, the neck bending compensation was not "primed" during the delay period.

The results are consistent with the hypothesis that the central pathways that underlie the compensations for eye nystagmus and neck bending are distinct, with the capacity for independent operation. To date we have not been able to demonstrate that the compensation of eye nystagmus can be interfered with without affecting the compensation of neck bending. Therefore, we cannot rule out the possibility that there are some common pathways for the two compensations.

Supported by a clinical research center grant from the N.I.H.

97.7 EFFECT OF UTRICULAR AND SACCULAR MACULAR ABLATION ON FREQUENCY AND LATENCY OF MOTION SICKNESS-INDUCED EMESIS IN THE SQUIRREL MONKEY. K. R. Brizzee and M. Igarashi. Dept. of Neurobiology, Delta Regional Primate Research Center, Covington, LA 70433 and Dept. Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, TX 77030.

The etiological role of the vestibular labyrinth in the genesis of motion sickness has been demonstrated in a large number of studies spanning just over a century. These investigations showed conclusively that the vestibular labyrinth plays an essential role in the genesis of motion sickness. However, the relative role of the semicircular canals on the one hand, and the otolith organs on the other, in the elicitation of motion sickness has remained uncertain. It is the purpose of the present study to determine whether selective ablation of all of the otolithic sensors in the maculae utriculi and maculae sacculi but preserving the cristae ampullares of the semicircular canals, may eliminate the emetic response to normally effective motion stimuli in squirrel monkeys. In these studies three previously motion emetic-sensitive squirrel monkeys were rendered refractory to a standard motion-emetic regimen, consisting of both horizontal rotary and vertical motion, by a two-stage utriculo-sacculectomy procedure which left the semicircular canals, including the cristae ampullares intact. Three non-operated control squirrel monkeys tested on the same motion-emetic regimen time schedule as the operated animals remained motion-emetic sensitive with regard to incidence, frequency and latency of motion-induced emetic responses. Sham utriculo-sacculectomy (stapedectomy) later performed on two of the latter animals and one additional new animal did not alter the incidence, frequency or latency of motion-emetic responses to the same motion regimens.

Although several investigators have supported the view that motion sickness is generated by stimuli arising from both the otolith organs and semicircular canals, experimental proof of the necessity of having intact otolith organ structures has been lacking prior to the observations in the present study. The negative results in post-operative motion-emetic tests suggests strongly that the existence of the otolith organs (and their afferents which continually inform the direction change of linear accelerations) is essential, and the semicircular canal afferents alone are not sufficient for the elicitation of emesis by the combined rotary and vertical stimulus employed in the present investigation paradigm.

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97.8 CAT VESTIBULAR NUCLEAR NEURONS THAT EXHIBIT DIFFERENT RESPONSES TO ACTIVE AND PASSIVE HEAD ROTATIONS. F. Robinson, J. Hollerman*, and D. Tomko. Dept. of Physiology and the Center for Neuroscience, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261

When a cat moves through its normal enviornment its vestibular system is stimulated almost exclusively by voluntary movements of the head initiated by the animal. In contrast externally applied passive movements have almost always been used to study the properties of vestibular neurons. There are at least two plausible mechanisms which could cause neurons in the vestibular nuclei to respond differently to active movements than they do to passive movements. First, the vestibular nuclei receive a variety of non-vestibular sensory inputs including proprioceptive and somatosensory which could be active during a voluntary but not externally applied head rotations. Second, motor outflow could influence the sensitivity of neurons in the vestibular nuclei.

The current study was undertaken to determine how neurons in the vestibular nuclei of alert cats respond during active voluntary head rotations. Each cat was confined in a net bag and placed on a turntable so that the table's axis of rotation, which was oriented vertically, passed directly through the cat's C_1-C_2 joint. A head holder previously mounted to each cat's skull allowed the head to be fixed to the turntable or, when the head was free to move, made it possible to monitor the movements with a precision potentiometer attached by a rigid arm to the head holder. Single unit activity was recorded with etched tungsten microelectrodes driven through the cerebellum to the vestibular nuclei with a miniature mechanical microdrive which attached to a chronic recording chamber mounted over a craniotomy on the dorsal surface of the skull.

During voluntary movements the activity of most of the recorded neurons in the vestibular nuclei that responded to horizontal (yaw) rotation was predictable from their activity during externally applied rotation. A few vestibular nuclear neurons however showed little or no response to passive rotations but fired vigorously during active head rotations. The activity of these cells seemed related to velocity of the head rotation in one direction. Activity sometimes led the associated movement and sometimes followed it. To date these cells have been histologically confirmed in only the posterior region of the medial vestibular nucleus. Studies are continuing to determine the origin of the response of these neurons during active movement.

Supported by NASA grant NAG2-155, NIH grant NS17585 and a NASA Research Associate Award.

97.9 THE ACETYLCHOLINE RECEPTORS OF THE SEMICIRCULAR CANAL. Paul S. Guth, Charles H. Norris, and H. Williams*. Depts. of Pharmacology and Otolaryngology, Tulane University School of Medicine, New Orleans, LA 70112

Acetylcholine (Ach) is very likely the transmitter released by the efferents to the cochlea and lateral line. There is also evidence that Ach may be the transmitter of the efferents to the semicircular canal (S.C.). However, unlike the cochlea and lateral line, stimulation of the efferents to the S.C. produces both facilitation and inhibition of the firing rate of afferent fibers. This has been demonstrated in frog (Rossi et al., 1980), goldfish (Hartmann and Klinke, 1980)* and monkey (Goldberg and Fernandez, 1980). This biphasic effect can be mimicked by the application of Ach to the isolated S.C. of the frog. The facilitatory effect is mediated by muscarinic receptors, being mimicked by muscarine and carbachol and antagonized by low concentrations of atropine. The inhibitory effect is partially antagonized by d-tubocurarine and completely antagonized by strychnine and is therefore not a classical nicotinic receptor but is similar to the Ach receptor of the cochlea at which strychnine is also antagonistic. A receptor which is tonically exposed to Ach even in the isolated S.C. must be postulated because of our results with the anticholinesterase agents, eserine and echothiophate. The application of these substances causes an inhibition of the afferent firing rate. Since these agents are thought to have little or no intrinsic activity, acting only through the preservation of released Ach, we may presume that there is a tonically active cholinergic synapse in the isolated S.C. Caston (1972) reported that the spontaneous activity in the efferents is lost one hour after sacrifice of the frog. Our experiments are performed many hours after sacrifice. Therefore, we suggest that the reciprocal synapse, described by Dunn (1980) may be the site of possible tonic Ach release in this preparation and therefore may be a cholinergically mediated synapse. It appears from the existing evidence that there are at least three populations of cholinergic receptors associated with the semicircular canal. One of these mediates facilitation and the other two appear to mediate inhibition.

*Cat (Dechesne and Sans, 1980).

This research was supported in part by a grant from the Southern Hearing and Speech Foundation.

97.10 DIFFERENTIAL PROJECTIONS OF REGULARLY AND IRREGULARLY DISCHARGING VESTIBULAR PRIMARY AFFERENTS ONTO IDENTIFIED SECONDARY VESTIBULAR NEURONS IN THE BARBITURATE ANESTHETIZED SQUIRREL MONKEY. A. K. Moschovakis*, S. M. Highstein, and J. M. Goldberg Washington University, St. Louis, MO. 63110 and University of Chicago, Chicago, Il. 60637.

An electrophysiological paradigm was previously described (Soc. Neurosci. Abstr. 7: 39) that determined whether central vestibular neurons, monosynaptically related to the ipsilateral vestibular nerve (Vi), received their peripheral inputs predominantly from regular (R) afferents, from irregular (I) afferents, or else received mixed (M) inputs from both kinds of afferents. The paradigm is based on the fact that, when electric shocks are delivered via the perilymphatic space, there is an approximately 10-fold variation in thresholds systematically related to each afferent's discharge regularity (J. Neurophysiol.,47: 1236). I afferents have lower thresholds than do R afferents. The kind of vestibular-nerve input a secondary neuron receives is ascertained by plotting the size of the monosynaptic EPSP as shock strength is increased over a 16 to 32-fold range. The paradigm has now been used to characterize the peripheral inputs of secondary neurons contributing to various central pathways. Intracellular recordings were made in the superior, medial and lateral vestibular nuclei. Three classes of neurons were identified by antidromic activation and/or by intrasomatic HRP injections as projecting rostrally to the region of the oculomotor (Oc) nucleus, to the spinal cord (Sp), or to the flocculus (Fl). Twenty-five of 27 Oc neurons received either R or M inputs. All 13 Sp neurons received I or M inputs. Fl neurons could receive either an R input (7/25), an M input (9/25) or an I input (9/25). The difference in the monosynaptic Vi inputs of Oc and Sp neurons are consistent with the hypothesis that the response dynamics of vestibular nerve afferents are matched to the dynamic loads of the particular reflex pathways to which they contribute. Vc IPSPs were more commonly seen in Fl neurons (21/24) than in Oc (9/27) or Sp neurons (3/9). Latent periods of the Vc IPSPs were usually short (1.4-3.0 msec) and this was so for all classes of neurons, including those receiving I or R inputs. Virtually all neurons having a monosynaptic Vi EPSP also had a Vi disynaptic IPSP. Eighteen HRP-labeled neurons were recovered in the vestibular nuclei. Thirteen of these neurons were located in the superior and 5 in the lateral nucleus. Among the superior nucleus neurons, there was no apparent correlation in their soma location, type of Vi input and somadendritic morphology. (Supported NIH EY 05433 EY 01670 and NS 01330 and NASA NAG 2-148)

97.11 FINE STRUCTURE OF VESTIBULAR EFFERENT NEURONS IN THE ALBINO RAT. J.S WHITE Dept. of Anatomy, Creighton Univ. Sch. of Med., Omaha, NE 68178

As part of a study to determine the origins of the olivocochlear bundle in the albino rat (White, J.S. and W.B. Warr, J. Comp Neurol., 219:203-214, 1983), the locations of vestibular efferent neurons were also detected, using retrograde axonal transport of horseradish peroxidase following injections of the enzyme into the round window of the cochlea. In the present study, the fine structure of those HRP-labeled vestibular efferent neurons was investigated, particularly with regard to the types and distribution of their synaptic inputs, as a step toward understanding what drives this centrifugal bundle.

In all cases, two groups of labeled vestibular efferents were detected bilaterally both medial and lateral to the genu of the facial nerve. To date, examination of thin-sectioned material has concentrated on HRP-labeled vestibular efferents, detected using DAB as the chromogen, that were situated laterally, ipsilateral to the injected labyrinth. Most vestibular efferents tended to be small, fusiform neurons with major and minor axes measuring in the range of 15 x 10 microns. Usually, one or two primary dendrites could be seen arising from each pole. A nucleus with a slightly indented nuclear membrane largely fills a cytoplasm containing several small Nissl bodies consisting of four or five parallel cisternae and associated polysomes. Axon terminals are only occasionally seen in synapse with vestibular efferent cell bodies, and are only slightly more prevalent on the proximal parts of dendrites. Small terminals containing almost exclusively round vesicles were found on both the soma and dendrites. These terminals show a slight postsynaptic density adjacent to small synaptic junctions. A second, larger terminal, seen in synapse only with dendrites, contained small round or pleomorphic vesicles interspersed with elongate vesicles.

Anterogradely labeled axon terminals of primary vestibular afferent fibers were observed in synapse with small dendritic profiles near the labeled cells, but have not been seen to terminate directly upon a positively identified process of a vestibular efferent neuron. The presence of a primary vestibular afferent in synapse with a vestibular efferent neuron would suggest that a monosynaptic feedback mechanism exists between the vestibular labyrinth and the central nervous system. (Supported by NSF Grant BNS 83-20336)

97.12 EFFECTS OF IMAGINED TARGET DISTANCE ON VESTIBULOOCULAR RESPONSES. J. Goldberg and E. Icaza* Dept. of Otorhinolaryngology, Baylor College of Medicine, Houston, TX 77030.

We are studying factors that contribute to variability of vestibuloocular reflex (VOR) responses in humans. The VOR in the dark can be enhanced by imagining a stationary fixation target. It has also been shown that the gain of eye rotation required to fixate a target during head rotation increases above 1.0 as the distance between the target and the eye decreases. This report concerns the influence of location of imagined targets on VOR responses.

Eye movements were recorded electro-oculographically from the left eye of normal subjects seated in a chair rotating sinusoidally at 0.2 Hz and peak amplitudes from 8 to 48 degrees. The center of chair rotation was 10cm behind the centers of eye rotation. Subjects were asked to fixate a small stationary light spot that could be positioned as close as 10 cm in front of the eyes on the midline.

Calculations of VOR gains as a function of target distance have been in the past based on simplifying assumptions and resulted in overestimates of true gains. We used a geometric model to find the compensatory eye movements for an arbitrary target position exactly. Eye movements measured during fixation of near targets qualitatively confirm predictions of our model:

(1) the responses are distorted sinusoids for all amplitudes
(2) incremental gains at peak velocity points are asymmetrical
(3) these gains vary systematically with amplitude of rotation
(4) gains over 1.5 can be achieved for the 10 cm target distance

On some experimental runs, the target light was extinguished after several cycles of rotation. The subjects received prior instructions to remember target location and keep looking at it for the rest of the run, in the dark. The eye movement waveforms remained qualitatively unchanged except for an increase in the frequency of forward saccades. These types of saccades aid in tracking of a smoothly moving target as in pursuit, in contrast to anticompensatory quick phases normally present in vestibular nystagmus. Although the gain of compensatory eye movements decreased after the target was turned off, it still remained above 1.0 and often 1.2 until the completion of the 10-cycle run.

These results indicate that during passive rotations in the dark humans can boost the gain of compensatory eye movements to well over 1.0 through the effort of spatial localization. Distortion observed during localization of imagined near targets suggests that VOR circuitry is capable of nonlinear processing of incoming sinusoidal vestibular signals into eye movement waveforms appropriate for compensation.

Supported by the Clayton Foundation for Research.

STRUCTURE AND FUNCTION: CORTICAL AND SUBCORTICAL ORGANIZATION I

98.1 INITIAL FINDINGS IN A STUDY OF THE BRAIN MICROVASCULATURE OF HUMAN HYPERTENSIVES USING AN ALKALINE PHOSPHATASE STAIN WITH MICRORADIOGRAPHY AND LIGHT MICROSCOPY. M. A. Bell*, D. M. Moody*, and T. C. Johnston* (SPON: A. J. Sweatt). Department of Radiology, Bowman Gray School of Medicine, Winston-Salem, NC 27103

Staining thick (100 μ) celloidin sections of brain for alkaline phosphatase has proven to be an attractive and useful method for demonstrating the microvasculature of the cerebral cortex (Saunders and Bell, J. Neurosurg., 35: 128-140; Bell and Scarrow, Microvas. Res., 27: 189-203). Methods based on vascular red blood cell content or the introduction of injection media are liable to certain artefacts. The phosphatase enzyme on the other hand is active in arteriolar and capillary endothelium, and thus offers a more direct approach to the study of hypertensive vascular changes in human brains. We are currently extending the application of the phosphatase stain to deep grey and white structures of normal and hypertensive brains in an attempt to establish normal and altered vascular appearances in areas most vulnerable to damage. Preparations cover a wide range of magnifications and detail: 500-1000 μ celloidin sections are studied by microradiography (the standard metallic staining method for phosphatase is modified to produce a lead reaction product), while 100 μ celloidin and 1-3 μ resin sections are used with a light microscope to clarify vascular patterns and structure at low powers and at the cellular level.

In many tissues, arteries and large arterioles do not show phosphatase activity. Preliminary indications are that in the human brain relatively large penetrating arteries and arterioles do stain; variations in staining patterns, including a streaky effect, will be illustrated. The vascular patterns of all areas studied to date are well demonstrated, and some familiar and unfamiliar alterations of vascular structure have been observed; examples will be shown.

98.2 THE SPHENOID (Sph), DORSAL TEGMENTAL (DTg) AND POSTERODORSAL TEGMENTAL (PDTg) NUCLEI. G. Paxinos, I. Tork, T. Hokfelt*, L.L. Butcher, and A. Dorfman*. School of Psychol. and Anat., Univ. of New South Wales, Kensington, Australia, Karolinska Inst., Stockholm and Dept. Psychol., U.C.L.A.

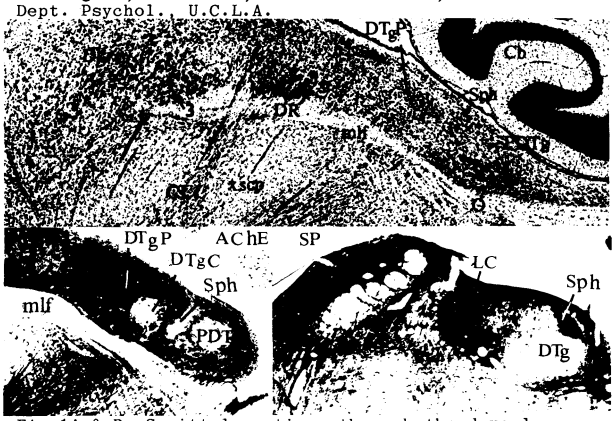

Fig 1A & B: Sagittal sections through the dorsal tegmentum of the rat, stained for Nissl and acetylcholinesterase (AChE); Fig 1C coronal section stained for substance P (SP).

Chemo- and cytoarchitectonic considerations were used to provide further evidence for delineation of nuclei in the dorsal tegmentum. AChE reactivity delineates the central (DTgC) and pericentral (DTgP) part of the DTg as well as the PDTg. An AChE poor nucleus wedged between the DTg and PDTg has recently been described (Paxinos and Butcher, In G. Paxinos, Ed., The Rat Nervous System, Academic Press, Sydney, 1985). The Sphenoid nucleus is densely stained in Nissl (A) as well as SP (C) and enkephalin preparations (Hamill & Jacobowitz, Soc. Neurosc. Ab., 10, 1984, 435). Nucleus O is densely stained in Nissl. The average cell sizes (in um) of the DTgC, DTgP, PDTg, Sph and O nuclei are 12.8 + 3, n = 52, 12.8 + 3, n = 142, 13.2 + 3, n = 48, 12.1 + 3, n = 67, 16.3 + 4, n = 18, respectively. For abr. see Paxinos and Watson (1982).

98.3 THE SEROTONIN-CONTAINING CELLS OF THE DORSAL AND MEDIAN RAPHE: A THREE-DIMENSIONAL ANATOMIC RECONSTRUCTION. B.E. Kosofsky, Eric S. Suchanek*, Loren E. Buhle* and M.E. Molliver. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

A three-dimensional reconstruction of the position of serotonin-containing cells in the midbrain of the rat is displayed in a motion picture. Transverse sections from the midbrain of rats were cut at 30 um and processed freely floating for 5-HT immunocytochemistry (antibody courtesy of H.G.W. Lidov). Fourteen reference levels (spaced 180 um apart) were analyzed. At each level, the positions of serotonin immunoreactive neurons in the dorsal raphe (DR) and median raphe (MR) nuclei were digitized and stored in a computer, as were the contours defining the ventricle, the periaqueductal grey, and the surface of the midbrain. The reference levels were aligned one with the other. The data set was loaded onto an Evans and Sutherland PS300 graphics workstation. Software was developed to dynamically rotate the reconstructed rat midbrain in order to obtain a 3-D appreciation of the size and shape of the DR and MR in relation to other brainstem structures. The DR was observed to be narrowest mediolaterally at its rostral limit (at the level of the III nerve nucleus) and narrowest dorsoventrally at its caudal limit (at the level of the dorsal tegmental nucleus of Gudden). In between, where the DR expands greatly in size, the "core" of the DR exhibits a clustering of 5-HT immunoreactive cell bodies; a dorsomedial subdivision, a ventromedial "fountain" subdivision, and two lateral wing subdivisions are discerned. Cells of the MR are more restricted in numbers and in mediolateral distribution. Clustering of MR cells into distinct subdivisions was not evident. The 3-D reconstruction served as a template for localizing the injection sites in a series of anterograde transport experiments. The bean lectin, PHA-L, was delivered iontophoretically into discrete DR and MR sites. Anterogradely labeled fibers in the cerebral cortex following every DR and MR injection form multifocal, widely distributed patches of innervation. Each site in the DR and MR projects to a specific set of cortical targets; cell clusters in different subnuclei project to different sets of targets with laminar patterns of innervation that are specific to each subnucleus. Frontal cortex is preferentially innervated by DR neurons, and there is a complex but systematic topography relating positions within the DR nucleus to additional sets of cortical targets. Taken together, topographic organization and regional differences in innervation pattern suggest that 5-HT neurons may have a selective and restricted effect in each area of cortex. This neuronal system provides an anatomic substrate by which subsets of raphe cells may independently influence particular combinations of cortical targets. (Support: NIH GM7303, NS15199, NS21011, and M552338)

98.4 DORSAL RAPHE PROJECTIONS TO FRONTAL CORTEX AND STRIATUM ARISE FROM DISTINCT POPULATIONS OF NEURONS. M.A. Wilson* and M.E. Molliver Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In order to determine whether different populations of cells within the dorsal raphe nucleus (DR) project to cortical and subcortical targets, the cells which project from the DR to frontal cortex and striatum were mapped in the rat. Two retrogradely transported, fluorescent dyes, true blue (TB) and diamidino yellow (DY), were injected via stereotaxically positioned micropipettes into the rostral pole of frontal cortex and rostral striatum, and labeled neurons were mapped in the midbrain raphe nuclei. The sites of TB and DY injections were reversed in some animals, to compensate for the different sensitivities of these tracers. Immunofluorescent labeling for serotonin or tyrosine hydroxylase was combined with retrograde tracing in some sections.

Distinct, partially overlapping populations of DR neurons project to frontal cortex and striatum, predominantly ipsilaterally, with few cells projecting to both forebrain structures. Striatally projecting neurons comprise a large diamond-shaped group in transverse sections of the DR, rostral and dorsal to the suprafascicular subdivision. Few striatally projecting neurons are found in the caudal DR. Neurons which project to the frontal cortex are found predominantly in the suprafascicular group, and in the caudal half of the dorsomedial group; scattered cortically projecting cells are found throughout the rest of the DR. Preliminary studies indicate that DR neurons projecting to occipital cortex are found caudal to the frontally projecting group, and primarily in the ventromedial division.

The cortically projecting cells in the ventral group are predominantly serotonergic, whereas almost half of those in the dorsomedial group are non-serotonergic. Both serotonergic and tyrosine hydroxylase immunoreactive neurons are present in the most rostral portion of the dorsal raphe, where the striatally projecting neurons are found. Most of the striatally projecting neurons are serotonergic; about one quarter are non-serotonergic, and very few of these (less than 5%) are tyrosine hydroxylase immunoreactive.

These results indicate that the DR can be fractionated on the basis of its projections and neurotransmitters. The rostral subdivision projects heavily to the striatum; the caudal dorsomedial subdivision, along with the ventral fountain, projects to the frontal cortex. Thus, DR projections to the telencephalon are topographically organized: morphologically different populations of cells project to striatum and cortex. (Support: NIH GM07445, NS15199 and NS21011.)

98.5 PARIETO-PREFRONTAL CONNECTIONS IN THE MONKEY: TOPOGRAPHIC DISTRIBUTION WITHIN THE PREFRONTAL CORTEX OF SECTORS CONNECTED WITH THE LATERAL AND MEDIAL POSTERIOR PARIETAL CORTEX. C. Cavada* and P.S. Goldman-Rakic (SPON: C. Avendaño). Sect. Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The posterior parietal cortex is an important link between several sensory areas and the prefrontal cortex. The goal of our ongoing investigation is to elucidate the full extent and topography of the cortico-cortical connections between posterior parietal and prefrontal areas. Our study has revealed a sizable new reciprocal connection between the medial parietal cortex and the principal sulcus, and a medio-lateral topographic arrangement of parieto-prefrontal connections. HRP-WGA was injected into various prefrontal and parietal sites in adult rhesus monkeys and the distributions of anterogradely labeled terminal fields and retrogradely labeled neurons were analyzed. Following injections of tracer in the dorsolateral prefrontal cortex, HRP-positive terminals and neurons were observed in Brodmann's area 7, both on the medial and lateral surfaces of the posterior parietal lobe. The topographic distribution of these connections was elucidated by systematic injections of HRP-WGA into the parietal cortex. After injections in medial area 7, transported label was observed mainly in the upper bank of the posterior half of the principal sulcus, whereas after lateral area 7 injections (convexity of the posterior parietal lobe), the heaviest anterograde and retrograde labeling in the principal sulcus was observed in the posterior half of its lower bank. Other differences between medial and lateral parietal connections with prefrontal regions outside the principal sulcus were also noted. Lateral area 7 is interconnected with the cortical convexities above and below the principal sulcus, with the anterior part of the orbitofrontal cortex, and with the cortex of both limbs of the arcuate sulcus. In contrast, the connections of the medial parietal cortex are largely limited to the convexity above the principal sulcus and to the upper limb of the arcuate sulcus.

The segregation within the prefrontal cortex of territories connected with the medial and lateral aspects of area 7 indicates that further evaluation of the morphological and functional characteristics of these posterior parietal sectors is needed in order to better understand the involvement of area 7 in visuo-spatial and visual attentional functions, and to shed light on the nature of the neural information transferred between parietal and prefrontal sectors.

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98.6 COMMON CORTICAL AND SUBCORTICAL TARGET AREAS OF THE DORSOLATERAL PREFRONTAL AND POSTERIOR PARIETAL CORTICES IN THE RHESUS MONKEY. L.D. Selemon and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Behavioral and clinical studies have long suggested a functional liaison of the prefrontal and parietal cortices. Lesions of either the dorsolateral prefrontal cortex (DPC; Brodmann's areas 9, 10) or the posterior parietal cortex (PPC; area 7) disrupt the performance of monkeys on a variety of spatial tasks and produce unilateral sensory neglect in both human and non-human primates. Moreover, the presence of dense, reciprocal corticocortical projections between the DPC and PPC provides an anatomical basis for this functional linkage.

Common efferent projections of the DPC and PPC were examined in three rhesus monkeys using a double anterograde labeling strategy. In two monkeys, horseradish peroxidase was placed in the PPC while tritiated amino acids were injected into the DPC. Placement of tracers was reversed in a third case. Terminal labeling originating from both DPC and PPC injection sites was observed in numerous ipsilateral cortical areas, including the posterior principal sulcal cortex, the dorsomedial (supplementary motor) cortex, the anterior bank of the arcuate sulcus, the anterior and posterior cingulate cortices, the frontal-parietal operculum, the fundus of the superior temporal gyrus, the parahippocampal gyrus, the presubiculum and the caudomedial lobule. In some of these common target areas, as for example in the cingulate cortices, DPC and PPC afferents terminate in an array of interdigitating columns, an arrangement much like that observed for callosal and associational projections to the principal sulcus (Goldman-Rakic and Schwartz,'82). In other areas, DPC and PPC terminals exhibit a laminar complementarity: e.g., in the fundus of the superior temporal gyrus, parietal terminals are densely distributed within laminae 4 and 6, whereas prefrontal terminals occupy mainly laminae 1 and 5 directly above the parietal bands. Several subcortical structures also receive efferents from both injection sites. Perhaps most prominent are the projections of the DPC and PPC to adjacent, longitudinal domains of the neostriatum as has been described previously (Selemon and Goldman-Rakic,'85). Similarly, DPC and PPC projections are in close apposition throughout the ventral anterior, dorsomedial, lateral dorsal and pulvinar nuclei of the thalamus. In the brainstem, DPC and PPC afferents terminate in the deep layers of the superior colliculus and the midline reticular formation of the pons.

The present study has uncovered a remarkably large number of cortical and subcortical areas that receive input from both the DPC and PPC. We propose that these common prefrontal and parietal efferent pathways constitute part of a complex anatomical circuit that mediates spatial behavior.

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98.7 TOPOGRAPHY AND LAMINAR ORIGIN OF CORTICAL VISUAL INPUT TO THE PREFRONTAL CORTEX OF THE RHESUS MONKEY. H. Barbas. Dept. of Health Sci. and Anatomy, Boston Univ. and Sch. of Med., Boston MA 02215.

The sources of ipsilateral cortical projections from visual association regions to peri-arcuate (Walker's areas 8 and 45), peri-principalis (area 46), and orbitofrontal (areas 11 and 12) regions were studied with horseradish peroxidase (HRP) in macaque monkeys. Projections from visual association regions to the prefrontal cortex originate in the depths and caudal bank of the superior temporal sulcus, and in and around the banks of the anterior and posterior middle temporal, occipitotemporal, parieto-occipital, inferior occipital, and lunate sulci. The above regions are included in area TE, and in peristriate areas OA and OB of Von Bonin and Bailey, or in areas IT, MT, V4, V3, V3A, VP and V2 according to more recent classification of the visual cortex.

Area TE is the principal source of visual projections to areas 11 and 12 and to the dorsolateral part of caudal area 46. On the other hand, input from peristriate regions is directed to the peri-arcuate cortex and particularly to its most caudal extent. In addition, the caudal bank of the intraparietal sulcus (area POa), where neurons have visual and visuomotor properties, projects to peri-arcuate and peri-principalis, but not to orbital regions.

Most visual association neurons projecting to the prefrontal cortex are situated in cortical layer III, and the rest are located in layers V and VI. The supragranular preponderance of labeling is most pronounced in peristriate parakoniocortical regions, where more than 90% of all HRP-labeled neurons are situated in layer III. There is a gradual decrease in supragranular labeling in progressively more rostral visual association regions, so that less than 70% of prefrontally directed neurons in rostral TE are situated in layer III. The regional decrease in the supragranular labeling closely parallels the cytoarchitectonic transitions observed in visual areas. Thus the proportion of labeled neurons in layer III progressively decreases from regions showing the highest, to those showing the lowest degree of laminar differentiation.

The data indicate that the most cytoarchitectonically differentiated peristriate regions project to the most differentiated prefrontal areas in the peri-arcuate zone. Similarly, the least differentiated portion of TE projects to the lesser differentiated orbital regions. In addition, cytoarchitectonic differentiation seems to be also closely associated with the laminar origin of corticocortical projections.

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98.8 ORIGIN OF AUDITORY CORTEX. K. K. Glendenning, M. Kudo*, S. Frost*, P. Hodges* and R. B. Masterton. Department of Psychology, Florida State University, Tallahassee, FL 32306.

Auditory thalamocortical projections as now seen in mammals are evolutionary derivatives of thalamo-noncortical projections of reptiles. In reptiles or birds the auditory thalamic nucleus, n. reuniens, medialis or ovoidalis, projects not to cerebral cortex but to the dorsal ventricular ridge--a dorsal outcropping of the striatal or basal ganglionic complex of the telencephalon. In sharp contrast, the mammalian medial geniculate projects mostly if not entirely to neocortex--giving rise to a true auditory konio-cortex with tonotopic organization. In the hope of viewing the evolutionary transformation of a noncortical to a cortical-based sensory system, we have examined the ascending projections of the medial geniculate in four of the most neurologically primitive mammals now alive, using first retrograde degeneration and then anterograde and retrograde axonal transport techniques.

The results show that these mammals retain a substantial reptile-like projection from their medial geniculate to basal telencephalic structures (including putamen, lateral amygdaloid nucleus, and caudate nucleus) in addition to a mammal-like projection to neocortex.

These two telencephalic projection targets are not as disparate in these mammals as they would be in some modern mammals because the basal ganglionic targets lie just ventromedial to the rhinal fissure while auditory cortex lies just above it. Thus, the two sets of geniculate efferents travel together, first forward and then laterally--the primitive one then ends promptly while the other continues dorsally into the internal capsule to end in nearby neocortex.

Because retrogradely transported fluorescent dyes applied to cortical and basal targets in opossum have consistently failed to double-label more than a few medial geniculate cells while single-labeling virtually all of them, the two projections probably arise from separate cell populations--the neocortical projection from rostral geniculate, the basal ganglionic projection from caudal geniculate.

The presence of these substantial non-neocortical projections in more primitive mammals suggests that the evolutionary bridge from reptile-like auditory systems to the mammal-like system can still be viewed in an arrested form among carefully selected extant mammals.

(Supported by grant NS07726.)

98.9 THE EFFECTS OF REVERSIBLE COLD LESIONS IN THE MIDDLE AND INFERIOR TEMPORAL GYRI AND SUPERIOR TEMPORAL SULCUS ON PERFORMANCE OF A VISUAL TASK. James A.Horel, Dorothy Joiner* and Mary Lou Voytko. Dept. of Anatomy and Cell Biology, SUNY, Upstate Med. Cntr., Syracuse, NY 13210.

Lesions in the inferotemporal cortex (IT) produce severe deficits in the performance of visual tasks. These lesions typically extend from the depths of the superior temporal sulcus (sts) to the occipitotemporal sulcus, encompassing both the middle (mtg) and inferior (itg) temporal gyri as well as the lower bank of sts. The cortex in sts is anatomically and functionally distinct from the gyri and there is evidence that the two gyri may differ from one another as well. In this experiment we compared the effects of separate reversible cold lesions to these three structures on the performance of delayed match to sample (DMS), a task that is particularly sensitive to IT lesions. Four Macaca fascicularis were trained using liquid reward and photographs of objects for stimuli. The animals were held in a restraining chair facing three rear projection screens and responded by pressing the screens. At sample, the animals responded to a stimulus that was projected to the center screen. This stimulus disappeared and there was a delay of either 0, 15, 30 or 45 sec. after which a stimulus that matched the sample appeared at one side screen and a nonmatching stimulus appeared at the other screen. Response to the match was rewarded. Cryodes were then implanted bilaterally over the anterior half of IT, one pair each over sts, mtg and itg. These cryodes were stainless steel loops of tubing approximately 3 x 20 mm and each was shaped to fit an area. The upper bank of sts was removed to allow a cryode to cover the inferior bank. Cooled methanol pumped through the tubing brought the metal to 0° C. The three structures were cooled separately in randomly presented blocks of 20 trials. Control blocks without cooling were randomly mixed with experimental blocks. The effects of the cold were in all cases completely reversed by removing the cold. Cooling sts or mtg was without effect; performance was indistinguishable from controls. Cooling itg produced powerful deficits at all delays. As a check on the cooling method, animals were trained on the same task and were given ablative mtg or itg lesions. The behavioral effects of the ablations duplicated those of the cold. We conclude that something critical for the performance of this task is in itg. The complete absence of an effect from mtg or sts lesions suggests that these structures may not normally participate in the performance of this task. Supported by NINCDS grant NS 1829-03.

98.10 THE PREMOTOR CORTEX OF MACAQUE MONKEYS: NEURAL ACTIVITY IN ANTICIPATION OF PREDICTABLE ENVIRONMENTAL EVENTS. Steven P. Wise and Karl-Heinz Mauritz*, Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, MD 20205.

The activity of premotor cortex neurons was studied in unanesthetized macaque monkeys that were operantly conditioned to perform a visually guided motor task. The monkeys were given a visuospatial instruction stimulus (IS) that provided the target for a limb movement, but the monkey was not allowed to execute the movement until the presentation, 1.5, 2.25, or 3 s later, of a trigger stimulus (TS). After its initial presentation, the IS was sometimes removed or changed during the interval between the IS and TS.

Three anticipatory patterns of activity were observed during performance of the task: (i) certain neurons became active during the 1 s intertrial interval preceding the IS, appearing to anticipate its location or time of occurrence; (ii) some of these and other neurons changed their discharge prior to a possible removal or change in the IS, which might occur 1 s after the initial presentation of the IS; and (iii) other cells showed neuronal modulation that preceded a time, during a trial, when a TS might occur. From a sample of 176 premotor cortex neurons from two monkeys, 52 showed the first pattern, 34 the second, and 24 the third.

The properties of some of these neurons were examined under conditions in which the timing of the behavior-guiding visual signals or their probability of occurrence was changed to make them less predictable. In general, the anticipatory neuronal activity was strongly influenced by changing event predictability. For example, increasing the intertrial interval led to a gradual decrease in the anticipatory activity preceding the IS. Further, if the probability that the IS would change during a trial was reduced to zero for a large number of consecutive trials, there were marked decreases in the anticipatory discharge preceding the time, during a trial, when a change in the IS might occur. The present findings suggest that neuronal activity within the premotor cortex reflects the anticipation of predictable environmental events.

98.11 CORTICOPONTINE PROJECTIONS IN THE TREE SHREW TUPAIA GLIS.
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There is a massive projection from the mammalian cortex to the pons. Corticopontine fibers arise from layer V pyramidal cells in the cerebral cortex and terminate on neurons in the pontine nuclei. Pontine cells in turn send their axons to the cerebellar cortex where they end as mossy fibers.

In rats, nearly all areas of the cerebral cortex project to the pons. In monkeys there is a projection from only about half of the cortex. Monkeys have few or no projections to the pons from temporal and frontal association areas or from striate and immediate prestriate cortex. We studied corticopontine projections in the tree shrew to compare the distribution of corticopontine cells in a non-primate mammal with a well demarcated striate cortex.

Multiple injections of horseradish peroxidase (HRP) were made in the pontine nuclei of three tree shrews. The injection cannulae were angled towards the pons from a caudal approach via the cerebellum. The animals survived for 2 days after which they were perfused and their brain processed to reveal the location and extent of the injection and the distribution of retrogradely labelled cells.

In a tree shrew with complete unilateral filling of the pontine nuclei, HRP labelled cells were found widely distributed in the ipsilateral neocortex. All retrogradely labelled neurons were layer V pyramidal cells. Like rats and monkeys there was a high density of corticopontine cells in motor, somatosensory and parietal association areas. Like rats but unlike monkeys all of frontal neocortex contained labelled cells. Also like rats but unlike monkeys the entire striate cortex and immediate extrastriate area all contained HRP labelled cells.

An area of the temporal lobe adjacent to the rhinal fissure contained few or no labelled cells. This area includes the thalamic projection fields of the medial geniculate and part of the pulvinar. There is an analogous region of sparse pontine projection from the temporal lobe in rats.

The distribution of corticopontine neurons in tree shrews closely resembles that seen in previous studies of rats. All area 17 projects to the pontine nuclei in both rats and tree shrews. In monkeys only that portion of area 17 which maps the far peripheral visual field projects to the pons. The results suggest that in these species there is a grossly different organization of cortical efferent pathways for the visual guidance of movement.

98.12 COMMISSURAL AND CALLOSAL CELLS IN PRIMITIVE MAMMALS.
E. Moreland-Granger*, K. K. Glendenning, P. Hodges* and R. B. Masterton. Department of Psychology, Florida State University, Tallahassee, FL 32306. (SPON: Friedrich K. Stephan).

In premammalian tetrapods (amphibians, reptiles and birds) and in pre-placental mammals (monotremes, marsupials), interhemispheric fibers are contained almost entirely within the anterior commissure. These animals have no corpus callosum whatever. With the appearance of the corpus callosum in placental mammals, both the corpus callosum and the anterior commissure share the interhemispheric fibers--the callosum containing most of the interneocortical fibers. In order to view the neocortical distribution of interhemispheric cells, we have cut and HRP-filled the commissural fibers in the common opossum (Didelphis virginiana) and the pouchless, short-tailed opossum (Monodelphis domesticus) and the callosal fibers in hedgehog (Erinaceus europaeus) and rat. Although there are a number of differences in the details of the cytoarchitectonic distribution of the cells in the two types of interhemispheric systems, one of the most striking differences is found in the cortical laminae of their origin. In most cytoarchitectonic areas of marsupial cortex, the commissural cells are most heavily concentrated in layer 3. In contrast, the callosal cells in hedgehog and rat are concentrated in layer 5 as well as in layer 3. This marked difference suggests that the evolutionary development of the direct neocortical-neocortical fibers in the corpus callosum was accompanied by a significant modification in their layers of origin. With many more interneocortical fibers emanating from layer 5 in placental mammals, a potentially significant shift in function is also implied.

(Supported by grant NS07726.)

TRANSMITTERS IN INVERTEBRATES I

99.1 LOCALIZATION OF ACETYLCHOLINESTERASE IN THE SYNGANGLION OF THE AMERICAN DOG TICK, DERMACENTOR VARIABILIS.
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Biochemical and pharmacological studies have provided evidence supporting the hypothesis that the tick nervous system includes cholinergic neurons. The presence of acetylcholine, choline acetyltransferase, and acetylcholinesterase (AChE) has been verified in tick nerve tissues. Very little is known about which compartments of the tick nervous system may contain cholinergic elements. Initial studies to localize the cholinergic components of the tick nervous system were undertaken using AChE ultrastructural cytochemistry.

Adult ticks were injected with fixative made up of 3% paraformaldehyde, 1% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4. The brains were excised and fixed by immersion for 3 hours at 4 degrees C. Following an overnight buffer rinse the brains were stained for 1-4 hrs to demonstrate AChE activity using a copper thiocholine procedure. The tissues were then fixed in Dalton's chrome osmium, dehydrated in ethanol, and embedded in epoxy resin.

Light microscopy of 1-2 micron epoxy sections revealed intense AChE stain in the synganglion (brain) and several peripheral nerves. The reaction product was particularly dense in the central neuropil area of the synganglion. The neurons were located around this central neuropil and appeared to be unstained although there was dense reaction product adjacent to the plasma membrane of most neurons. Electron microscopic observations showed that there were very large amounts of AChE reaction product in the neuropil region. This stain was in the extracellular space adjacent to profiles of neuronal processes and other neuropil structures. AChE activity was evident throughout the neuropil, and did not appear to vary a great deal between any anatomically distinct neuropil areas. In the outer neuronal zone of the synganglion there was intense AChE activity adjacent to the neuronal plasma membrane of the Type 1 neurons and large Type 2 neurosecretory neurons. The small, Type 3 globuli neurons had very little if any associated AChE activity. In addition, there was pronounced AChE activity associated with membranes of many axons in the rostral nerves. Preliminary data also indicates that AChE may also be associated with particular sensilla on tick appendages. Further information on neurotransmitter systems involved in tick sensory and reproductive processes may provide new strategies for control of ticks. Supported by NIH Grant AI 10986.

99.2 CHARACTERIZATION OF MUSCARINIC RECEPTORS IN CRUSTACEAN NERVOUS TISSUE BY L-[^{3}H]QNB BINDING.
D.L. Barker, T.F. Murray, G.J. Mpitsos and J.F. Siebenaller*. Dept. of Biochemistry and Biophysics, Coll. of Pharmacy, Coll. of Oceanography, Oregon State Univ., M.O. Hatfield Marine Science Center, Newport, OR 97365.

Acetylcholine is used in Crustacea as a primary sensory transmitter. Acetylcholine may also play a role as activator or modulator of bursting pacemaker activity, and physiological evidence suggests that this effect is mediated by muscarinic receptors. We find that the selective muscarinic antagonist L-[^{3}H]quinuclidinyl benzilate (QNB) binds with high affinity (K_d=0.2 nM) to a single population of specific sites in nervous tissue of the crab, Cancer magister. The density of L-[^{3}H]QNB binding sites is 105 fmol/mg protein in the membrane fraction prepared from the fused thoracic ganglionic ring. Other areas containing neuronal cell bodies and neuropil (brain and eyestalks) also have high densities, whereas specific L-[^{3}H]QNB binding is low or absent in membranes of peripheral nerve, artery and skeletal muscle. The binding site is stereoselective, as (-)QNB is over 200 times more potent than (+)QNB in competing with specific L-[^{3}H]QNB binding. The muscarinic antagonists scopolamine (K_i=2 nM) and atropine (K_i=5 nM) are over 10,000 times more effective inhibitors of L-[^{3}H]QNB binding than the nicotinic antagonists decamethonium and d-tubocurarine. In addition, muscarinic agonists have the expected relative potencies: oxotremorine > pilocarpine ≅ arecoline > carbamyl choline. This pharmacological profile of the L-[^{3}H]QNB binding site strongly suggests the presence of classical muscarinic receptors in crustacean nervous tissue.

This work was supported by Air Force Office of Scientific Research contract F49620-83-C-0063 to G.J.M.

99.3 IMMUNOCYTOCHEMICAL STUDY OF ARTHROPOD NEURONS WITH ANTIBODIES SPECIFIC TO MOLLUSCAN SMALL CARDIOACTIVE PEPTIDE AND SEROTONIN: A COMPARATIVE STUDY. Joseph C. Callaway, Boris Masinovsky and John S. Edwards, Department of Zoology, University of Washington, Seattle, WA 98195.

Small cardioactive peptide B (SCP_B), a nonapeptide in Aplysia californica (Morris et al., Nature 300, 1982) is a cardioaccelerator and modulates specific behavior, e.g. feeding (Lloyd, Fed. Proc. 41:2948, 1982).

With a monoclonal antibody against SCP_B (Masinovsky et al., 1985, in prep.) We have localized SCP-like and serotonin-like immunoreactive neurons in the CNS of the barnacle, Semibalanus cariosus, the cricket Acheta domesticus and the firebrat, Thermobia domestica (Thysanura).

SCP-like immunoreactivity of S. cariosus is limited to one pair of neurons in the supraoesophageal ganglion (SEG) and a cluster of four somata in the ventral ganglion (VG). The pair of neurons in the SEG appear to project neurites into the sheath of that ganglion. Acheta and Thermobia have SCP-like immunoreactive somata or neurites in each segmental ganglion and paired lateral posterior clusters of somata in each thoracic ganglion. Processes from these clusters in Acheta lead to the medial nerve.

Serotonin-like immunoreactivity in Thermobia, Acheta, and Manduca sexta (Lepidoptera) is conspicuously localized in two pairs of H-shaped neurons with posterior bilateral somata in each ganglion excluding the brain. Comparable neurons occur in the cockroach Periplaneta americana (Bishop and O'Shea, J. Neurobiol. 14:251, 1982). Fused ganglia contain sets of paired bilateral neurons equal to the number of fused segments. Insects and lobster ganglia typically have comparable bilateral H-shaped serotonin immunoreactive neurons; except that, in one thoracic and the abdominal ganglia of the lobster cell somata are submedial rather than lateral (Beltz and Kravitz, J. Neuroscience, 3:585, 1982). Sets of paired H-shaped neurons with paired midline somata, similar to those in the lobster, occur in the S. cariosus VG, each set associated with a presumed fused segment.

Though the location and morphology of serotonin-like immunoreactive neurons is apparently conserved in all arthropods studied, uniformity is greater among the insect orders than between decapod cirrepede crustaceans. No obvious homologies between insect and cirripede SCP-like immunoreactive neurons are evident. Insects of different orders have homologus serotonin-like immunoreactive neurons in each ganglion posterior to the suboesophogeal ganglion; homologous SCP-like immunoreactive neurons are only apparent in the thoracic ganglia. Supported by NIH grant NB0778 and the University of Washington GSRF.

99.4 IMMUNOCYTOCHEMICAL IDENTIFICATION OF THE NEUROSECRETORY CELLS THAT PRODUCE THE PEPTIDE ECLOSION HORMONE IN THE MOTH MANDUCA SEXTA. P.F. Copenhaver and J.W. Truman. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

We are interested in the mechanisms that control the release of Eclosion Hormone (EH), which triggers the stereotyped motor program of adult emergence in the moth Manduca sexta. This 4000 dalton peptide is produced in the animal's brain and transported out to a pituitary-like organ (the corpora cardiaca/allata, or CC/CA) for release at the culmination of adult development. The timing of this release event is tightly regulated by two factors: the declining titer of the steroid 20-hydroxy-ecdysone determines the day of EH release, while a circadian input determines the time of day (Truman J.W, Am. Zool. 21:655, 1981). This system thus presents an excellent opportunity to determine how these factors affect the individual neurosecretory cells within the central nervous system.

By dissection and bioassay of identified neurosecretory cell clusters from the moth brain, we have shown previously that a bilaterally paired group of neurosecretory cells in the dorsolateral protocerebrum produce EH, and that electrical stimulation of individual cells within this group will induce the release of bioassayable EH into the hemolymph surrounding the CC/CA (Copenhaver P.F. & Truman J.W, Soc. Neurosci. Abs. 14:153, 1984). To further characterize these putative EH cells, we have developed a serum antibody to the peptide. EH was extracted from the CC/CA of approximately 30,000 animals by high pressure liquid chromatography (a single CC/CA is estimated to contain 1-10 ng. of EH). The peptide was then conjugated to bovine serum albumin and delivered to a rabbit in a series of injections over the course of several months. The resulting polyclonal antibody possesses a strong affinity for EH as shown by immunoprecipitation. Pre-incubation of the serum with a sample containing the peptide blocks its ability to precipitate EH, while pre-incubation with homogenates of muscle, fat body, and optic lobes do not. In addition, immunohistochemical staining of serially sectioned moth brains consistantly reveals 5-6 of the large neurosecretory cells within the lateral clusters but does not produce any reaction in the other cell groups.

We are currently employing both intracellular recordings in a semi-intact preparation and patch-electrode recordings from isolated neurosecretory somata to characterize the physiological changes that these cells undergo around the time of adult emergence, in order to examine the cellular mechanisms underlying the regulation of this peptide hormone's release.

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99.5 CEREBRAL NEUROENDOCRINE SYSTEM OF MANDUCA SEXTA: LOCALIZATION AND RELEASE OF FMRFAMIDE-LIKE IMMUNOREACTIVITY. L.S. Carroll* and G.M. Carrow (SPON: J.L. Rosenbaum). The Biological Laboratories, Harvard University, Cambridge, MA 02138.

Immunoreactivity to the neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) has previously been localized to neurosecretory cells and corpora cardiaca of insects. We studied the distribution and possible neurohormonal function of FMRFamide-like substances in the cerebral neuroendocrine system of the tobacco hornworm, Manduca sexta. Indirect immunofluorescence in whole mount preparations was used to localize FMRFamide-like immunoreactivity (FLI) to about 25 bilaterally symmetrical pairs of somata in the brain. Processes in the cerebral neurohemal organs, the corpus cardiacum (CC) and corpus allatum (CA), also showed FLI. Preabsorption of the primary antiserum with an excess of synthetic FMRFamide eliminated staining.

The protocerebrum of Manduca contains two bilaterally symmetrical clusters of identified neurosecretory cells (NSCs) whose axons project to either the CC or the CA. The NSCs have been subdivided into four groups (Ia, Ib, IIa, IIb) on the basis of morphology (Carrow et al., J. Neurosci. 4:1034, 1984). NSCs within the Ib, IIa, and IIb groups were shown to contain FLI by combining intracellular injection of Lucifer Yellow with indirect immunofluorescence.

The amount of FMRFamide-like equivalents in homogenized extracts of prepupal brains, CC, CA, and combined CC and CA (retrocerebral complexes) was quantified by radioimmunoassay (RIA). The following values (mean $\pm$ SEM) were obtained: brain, 28.0 $\pm$ 3.8 fmol; CC, 2.5 $\pm$ 0.2 fmol; CA, 0.9 $\pm$ 0.1 fmol; retrocerebral complexes, 4.1 $\pm$ 0.2 fmol. The antiserum used throughout this study was specific for the C-terminus of FMRFamide (O'Donohue et al., Peptides 5:563, 1984). Inhibition binding curves generated from serial dilutions of synthetic FMRFamide and brain extracts were parallel, indicating the antiserum has a similar affinity for both antigens.

To determine whether FMRFamide-like substances could be released from the neurohemal organs, isolated retrocerebral complexes were incubated in a high potassium medium; the amount of FMRFamide-like equivalents released into the bathing medium was assayed by RIA. Approximately 16% of the FLI extractable from a retrocerebral complex was released. Release was shown to be calcium dependent.

These data suggest that FMRFamide-like substances are stored in cerebral NSCs and are released from terminals in the CC and CA, possibly to exert neurohormonal effects.

We thank T. O'Donohue and W. Watson for their generous gift of anti-FMRFamide antiserum. Supported by NSF grant PCM-8215638 and NIH grant R01 NS 21232-01.

99.6 A MONOCLONAL ANTIBODY ACTS AS A FUNCTIONAL BLOCKER OF CARDIOACCELERATORY PEPTIDE ACTIVITY IN THE TOBACCO HAWKMOTH, Manduca sexta. N.J. Tublitz, P.H. Taghert and P.D. Evans. AFRC Unit of Insect Neurophysiology and Pharmacology, Dept. of Zoology, Univ. of Cambridge, Cambridge CB2 3EJ, UK., and Dept. of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis MO 63110

Investigations into neuropeptide function have been hindered by a paucity of pharmacological compounds that specifically inhibit peptide action, usually by blocking the peptide receptor. In the absence of an amino acid sequence for the peptide under study, identification of a specific peptide antagonist is extrememly difficult and time-consuming. We have circumvented this problem by utilizing a monoclonal antibody that acts as a functional blocker of peptide action both in vivo and in vitro.

This role for a monoclonal antibody has been studied in the cardioacceleratory peptide (CAPs) system of the tobacco hawkmoth, Manduca sexta. Co-synthesized and co-released from the Manduca central nervous system, the two CAPs function as cardioregulatory neurohormones increasing heart rate during adult emergence and wing spreading behaviors (Tublitz and Truman, J. of exp. Biol., in press). Originally identified using a histological screening procedure, one of the monoclonal antibodies, 6C5, appeared to be specifically directed against both CAPs in several different ELISA and immunoprecipitation tests.

In vivo injections of 6C5 completely abolished the rise in CAP haemolymph titres that normally occurs in wing spreading animals. In addition, 6C5 treatment markedly depressed by 80% the increase in heart rate seen during wing spreading. Neither CAP haemolymph levels nor the CAP-induced elevation in heartbeat frequency were altered in controls injected with either saline or pre-immune mouse serum. Furthermore, the duration of wing spreading behavior was significantly prolonged by 6C5 injection, suggesting that wing spreading behavior is facilitated by the presence of the CAPs. Thus, by preventing the rise in CAP titres during wing spreading with its resultant physiological and behavioral effects, the monoclonal antibody 6C5 appears to act as a functional inhibitor of the CAPs. (Supported by an American Heart Association-British Heart Foundation grant to N.J.T.)

99.7 IMMUNOCYTOCHEMICAL AND CHROMATOGRAPHIC EVIDENCE FOR THE PRESENCE OF THE NEUROPEPTIDE PROCTOLIN IN THE CNS AND PERIPHERY OF DROSOPHILA. H.Keshishian, Biology Department, Yale University, New Haven, CT 06511.

Several neuronal precursor cells and their progeny in the fruitfly are homologous to cells previously identified in the grasshopper embryo (Thomas et al., Nature 310: 203-207). Here we show that there is conservation between these two species in the number, distribution and putative targets of several neurons expressing a common neuropeptide. Proctolin is a pentapeptide neurotransmitter found in cells of the CNS and periphery of a number of invertebrate species. The distribution of putative proctolin containing neurons was examined in the larvae of Oregon-R Drosophila using immunocytochemistry (PLI) and HPLC with a sensitive proctolin-specific bioassay. The CNS PLI reveals a small and conserved population of neurons whose distributions are restricted to the brain hemispheres and abdominal neuromeres of the ventral ganglion, and are stable through larval development. Within the brain hemispheres are two pairs of large (10 μ) neurons lying dorsal-laterally; other brain PLI consists of a bilateral pair of anterior dorsal-medial clusters consisting of 3-4 neurons each. In each neuromere from abdominal segment 1 through 7 there is a single prominently staining dorsal pair of neurons, along with several lightly staining dorsal-medial cells. In the 8th segment neuromere there is a cluster of usually six large (10-12μ) strongly staining neurons distributed between the dorsal and ventral sides. The cells are likely homologous to a cluster of 6 proctolinergic grasshopper motoneurons, that innervate the intrinsic hindgut muscles. Colabelling experiments in the fly are still in progress - we have to date succeeded in demonstrating that: 1) Lucifer yellow dye-fills of efferent neurons projecting into the gut nerve, and 2) somata revealed by cobalt backfills from the hindgut, colocalize to the proctolinergic cluster. The peripheral immunocytochemistry was also studied. Prominent axonal staining is observed in the intestinal region of the hindgut. The axons are varicose, and align with the longitudinal muscles. The midgut staining is characterized by occasional intrinsic multipolar proctolinergic neurons , as well as by very fine arborizations on posterior midgut. Also fine, proctolin staining motor endings are found on a number of abdominal bodywall muscles. To corroborate the immunoreactivity described above, proctolin was recovered by reverse phase C-18 acetonitrile gradient HPLC from tissue, and quantitatively biassayed. Recovered putative Drosophila proctolin from CNS coelutes with authentic proctolin, and shows qualitatively similar physiological effects on proctolin sensitive muscles. Sample tissue PLB levels for each 3rd instar larvae are: whole larvae range from 4-5 fmoles; isolated CNS, about 1 fmoles; bodywall muscles, 2-3 fmoles; hindguts, about .8 fmoles. Proctolin antiserum #9 was a kind gift to the author by Dr. M. O'Shea, Université de Genève.

99.8 NEURAL RELEASE OF A PEPTIDE CO-TRANSMITTER GREATLY ENHANCES TENSION GENERATION IN A CRAYFISH TONIC MUSCLE. C.A. Bishop, J.J. Wine, and M. O'Shea. Dept. of Psychology, Stanford Univ., Stanford, CA 94305 and Dept. of Animal Biology, Univ. of Geneva, Geneva, Switzerland.

The peptide proctolin coexists with a 'conventional' neurotransmitter in three of five excitatory motor neurons innervating the tonic flexor muscle of the crayfish abdomen, and is released from these motor neurons by nerve stimulation. Superfused proctolin acts postsynaptically to increase muscle tension in a membrane voltage dependent manner. However, proctolin has no detectable effect on the resting transmembrane potential or input resistance of the muscle fibers or on postsynaptic potentials (PSPs) (Bishop,C.A., Wine,J.J. and O'Shea,M. J. Neurosci. 4:2001-2009,1984; Bishop,C.A., Nagy,F., Wine,J.J. and O'Shea,M. Neurosci. Abst. 14:151,1984). We have now shown that proctolin release from neuronal terminals significantly amplifies muscle tension produced by the conventional transmitter.

Our experiments were made possible by the discovery that proctolin release can be significantly reduced without altering the release of the conventional transmitter. Proctolin release experiments, in which neurally stimulated peptide release from terminals of proximally severed motor neuron axons was monitored directly by a sensitive bioassay, indicated that low frequency stimulation produced a reduction in peptide release. Release from the proctolinergic motor neuron #1 (MN1) fell 32% after 5 min of stimulation at 10 Hz. A fifteen min, 10 Hz stimulation resulted in a barely detectable proctolin level.

To assess the effect of proctolin depletion on neurally evoked tension, MN1 was stimulated at a low frequency (5-10 Hz) for several sequential 5-10 min periods and short duration (5 sec) neurally evoked tension responses were recorded, after washes, before and after low frequency stimulation periods. PSPs were recorded throughout to monitor conventional transmitter release. Neurally evoked tension decreased after each low frequency stimulation period, and eventually plateaued at a level significantly lower than the initial level. Tension plateaued after stimulation periods totalling 30 min at 10 Hz stimulation and after totalling 45 min at 5 Hz. The amplitude of the EPSPs remained unchanged throughout all stimulation periods. Therefore, drop in tension did not result from lower levels of the conventional transmitter or changes in muscle conductance. The tension decrease is best explained by the reduction in proctolin release.

This is further indicated by the following: 1)No comparable drop in tension was detected after long term, low frequency stimulation of the non-proctolinergic motor neuron #6. 2)The tension change from the initial level to the reduced plateau level resulting from low frequency stimulation, demonstrated a voltage dependency similar to tension change due to proctolin perfusion. 3)Superfused proctolin amplified MN1 evoked tension to a much greater extent after tension was reduced to plateau level.

The percentage drop in neurally evoked tension for MN1 after tension was reduced to plateau level depended on the stimulation frequency of the evoked tension. The tension drop was 36% for 33 Hz, 63% for 20 Hz and 100% for 16.7 Hz. Thus, at physiologically relevant stimulating frequencies, neurally released proctolin is required before measurable tension is generated by MN1.

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99.9 PROCTOLIN IS A CO-TRANSMITTER FOR THE SETI MOTONEURON. M.K. Worden, J.L. Witten, and M. O'Shea. Lab. of Neurobiology, University of Geneva, CH-1211 Geneva 4, Switzerland and Committee on Neurobiology, University of Chicago, Chicago, Illinois 60637.

Here we provide our evidence that the identified slow excitatory motoneuron (SETi) of the locust extensor tibialis muscle (the ETi muscle) is peptidergic. All four of the neurons innervating the ETi muscle have been uniquely identified and transmitters have been assigned to each. These are the fast FETi motoneuron (glutaminergic), the slow SETi motoneuron (glutaminergic), the common inhibitory or CI motoneuron (GABA-ergic) and the dorsal unpaired median DUMETi neuron (octopaminergic). Several lines of evidence suggest that SETi uses the peptide proctolin (Arg-Tyr-Leu-Pro-Thr) as a neuromuscular transmitter in addition to glutamate. Staining with an antibody to proctolin reveals proctolin-immunoreactive axon collaterals and terminals on the slow fibers of the muscle. A proctolin-immunoreactive bilaterally symmetric pair of cell bodies is found in the CNS (metathoracic ganglion) co-positional with the paired somata of the SETi motoneurons. Proctolin is released from potassium-depolarized nerve terminals by a calcium dependent mechanism. Moreover proctolin can be isolated by HPLC from regions of the muscle innervated by SETi (2.5 fmoles/mg) where it is fifty times more concentrated than in non-SETi innervated regions. Physiological experiments also indicate a co-transmitter role for SETi-proctolin. Stimulation of SETi at a high frequency produces two effects that outlast the transient contractures normally attributed to glutamate. These are 1) an increase in the frequency of a myogenic rhythm of contraction and 2) an increase in tension that decays slowly. Both effects are mimicked by nanomolar amounts of proctolin but not glutamate. The postsynaptic effects of SETi stimulation are therefore consistent with the co-release of proctolin and glutamate.

We are now examining the interactions of other transmitters with the SETi-evoked proctolin effects and the modes of action of proctolin in this simple system. There may be differences in the mechanisms that underlie proctolin's two basic actions. For example, stimulation of the GABA-ergic CI motoneuron decreases the magnitude of the persistent or residual tension evoked subsequent to SETi stimulation but it does not affect the myogenic rhythm.

We hope this simple system consisting of four identified motoneurons will allow us to understand how different transmitter systems interact at a cellular level. Supported by NIH, NSF, E.I. du Pont de Nemours and Company.

99.10 CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN PHOSPHORYLATION APPEARS TO DETERMINE BEHAVIORAL SENSITIVITY TO A PEPTIDE HORMONE IN THE MOTH MANDUCA SEXTA. D.B. Morton* and J.W. Truman. Department of Zoology, University of Washington, Seattle, WA 98195.

Eclosion hormone (EH), a 4,000 dalton neuropeptide, acts directly on the CNS of insects to trigger ecdysis behavior (the stereotyped motor pattern used to shed the old cuticle at the end of each molt). Previous studies have shown that the action of EH on the CNS at pupal ecdysis in Manduca is mediated by cGMP (Morton and Truman (1984), Soc. Neurosci. Abstracts 10, 1040).

The CNS is behaviorally responsive to EH only at times that just preceed the normal time for ecdysis. Interestingly, EH is capable of elevating cGMP levels in the CNS at least 9 hours before the CNS will respond behaviorally to the peptide. This result suggests that EH receptors, the guanylate cyclase and the phosphodiesterase are present at this time, but some element in the cascade of events distal to cGMP is lacking, thereby rendering the system nonresponsive. Measurements of the levels of cGMP-dependent protein kinase in the CNS show that there are no differences in the levels of activity before and during behavioral sensitivity. This suggests that a substrate for the kinase may be involved in determining behavioral sensitivity.

Protein phosphorylation, using ^{32}P ATP, of CNS homogenates was carried out and the products separated using SDS polyacrilamide gel electrophoresis. Autoradiography of the gels revealed a protein, with a molecular weight of 54,000 daltons, which was phosphorylated in the presence of cGMP or cAMP. Exposure of sensitive animals to EH resulted in the disappearence of this labeled protein, presumably because it was phosphorylated in vivo, thus blocking its in vitro phosphorylation with labeled phosphate. Importantly, this protein only appears in the nervous system when the animals become behaviorally responsive to the peptide. Thus the synthesis of this protein may be the key step in rendering the CNS competent to respond to the EH peptide.

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99.11 FMRF-amide like peptides in Homarus americanus: distribution, immunocytochemical characterization and possible function L. Kobierski*, B.A. Trimmer*, B. Beltz, E.A. Kravitz, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

The molluscan cardioexcitatory peptide FMRF-amide is one member of a large peptide family found in many vertebrate and invertebrate species (Greenberg and Price, Ann. Rev. Physiol. 45:271-88, 1983). We have used immunocytochemistry to map the localization of FMRF-amide-like material in the nervous system of the lobster, Homarus americanus. Radioimmunoassay (RIA) methods also have been used to quantify the FMRF-amide-like material.

The FMRF-amide antibody, kindly supplied by Thomas O'Donahue (NIH), was generated against succinylated thyroglobulin conjugated to FMRF-amide. The antibody labels about 225 cell bodies in the nerve cord using wholemount immunocytochemical techniques. In addition staining is seen in: 1) brain neuropil regions similar in morphology to the olfactory and accessory lobes identified in other crustaceans (Hanstrom, J. Comp. Neurol. 38:221, 1925); 2) axons going out nerve roots to as yet unidentified peripheral targets; 3) a plexus of varicosities located in the connective tissue sheath surrounding the ventral nerve cord. This places endings in close proximity to the circulating haemolymph, suggesting a neurohormonal role for the FMRF-like material.

The specificity of the immunostaining was tested by preabsorbing the primary antisera with synthetic FMRF-amide and three FMRF-amide analogues: 1) Met-enkephalin-Arg-Phe; 2) bovine pancreatic polypeptide; 3) Phe-Met-Arg-Tyr-amide, all at 10^{-4}M concentrations. Preabsorption with FMRF-amide completely eliminates staining; preabsorption with each of the three FMRF-amide analogues results in approximately a 10% loss in the number of immunoreactive cell bodies while staining of other immunoreactive structures (neuropil, fibers) is not affected.

RIA studies in 1 lb lobsters show that: 1) the ventral nerve cord contains approximately 13.5 pmoles of FMRF-amide-like material/tissue; 2) about 1/6 of the total material found in the nerve cord is in the connective tissue sheath that surrounds the cord; 3) the haemolymph that surrounds and baths the nerve cord contains 1.8 x 10^{-11}M FMRF-amide-like material (3.15 pmoles total/1 lb. lobster). These findings further support a neurohormonal role for the FMRF-amide-like material. Present studies are: characterizing the FMRF-amide-like peptide, exploring its release from the sheath endings and examining its physiological role. (Supported by NIH).

99.12 CHARACTERIZATION OF IMMUNOREACTIVE FMRFamide- LIKE MATERIAL IN THE LOBSTER NERVOUS SYSTEM. B. A. Trimmer*, L. Kobierski and E. A. Kravitz. (SPON: M. Livingstone). Dept. of Neurobiology, Harvard Medical School, Boston. Mass. 02115.

The pattern and distribution of immunoreactive FMRFamide (Phe-Met-Arg-Phe-NH_2) - like material (FLI) in the nervous system of the lobster Homarus americanus has been mapped using an antiFMRFamide serum (Kobierski et al, Neurosci. abstr. 1985). These data suggest that FLI may play a role as a neurohormone. To facilitate the study of its functions we have begun to characterize and purify the immunoreactive material.

Antibody was kindly supplied by T.O'Donahue (NIH) and is specific for the amidated carboxyl dipeptide -Arg-Phe-NH_2. It shows only limited cross reactivity with -Arg-Tyr-NH_2 containing peptides such as bovine pancreatic polypeptide. Using this antibody as the basis for a radioimmunoassay (RIA) we have determined that the neurosecretory pericardial organs (PCO's) are the richest source of FLI. These organs show calcium dependent release of immunoreactivity when incubated in high (100 mM) potassium saline.

FLI can be extracted from PCO's by acidified methanol, and in our standard liquid phase competition RIA, displaces a labelled FMRFamide analogue from the antibody in a fashion parallel to authentic FMRFamide. FLI is a peptide or protein that is degraded by trypsin and in the process loses its ability to bind to the antibody, indicating that an arginyl or lysyl cleavage point lies within the antigenic determinant. Sephadex gel filtration chromatography reveals that lobster FLI has a larger apparent molecular weight than FMRFamide itself and that it consists of two separable components, one larger than 5000 daltons the other only slightly larger than FMRFamide. Both are more hydrophobic than FMRFamide and co-migrate in gradiant elution from a reverse phase HPLC column.

HPLC has also been used to fractionate FLI extracted from the nerve cord. This revealed, in addition to the material identified in the PCO's, a more hydrophobic substance which may be associated with the presence of cell bodies in the central nervous sytem. We are at present attempting to purify and identify the releasable FLI. The sequenced lobster analogue of FMRFamide will enable us to study the physiological role of FMRFamide related peptides in the lobster central nervous system. [Supported by a Harkness Fellowship (BAT) and an NIH grant (EAK)]

CONTROL OF MOVEMENT: ARM AND WRIST

100.1 RAPID WRIST FLEXION MOVEMENTS IN HUMANS: COMPENSATION FOR UNEXPECTED PERTURBATIONS. W. J. Becker*, R. Hayashi, R. G. Lee, and D. White*. Dept. of Clinical Neurosciences, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada T2N 1N4.

Rapid (ballistic) movements are thought to be generated by a central motor program which can produce the movement without sensory feedback. Even though pre-programmed, however, rapid movements can be influenced to some extent by peripheral feedback (Hallett and Marsden, J. Physiol., 294:33, 1979). We have investigated the influence of peripheral inputs on "pre-programmed" rapid movements further by introducing unexpected perturbations just before and during rapid wrist flexion movements.

The subject's wrist position and a target zone were displayed on an oscilloscope. Subjects were trained to perform rapid 30° wrist flexion movements to the target in response to an auditory signal. Wrist flexor and extensor EMG, wrist position, velocity and torque were recorded. After training, extensor and flexor wrist perturbations produced by a torque motor were randomly interposed in 10% of trials among control movement trials to alter the initial starting position of the wrist.

Wrist position during control trials rapidly moved to maximum movement amplitude (MMA), usually overshooting the target, and then returned to the target zone. With "early" extensor perturbations beginning during the reaction time to the auditory signal and before the onset of the first agonist EMG burst, subjects over compensated for the perturbations, with an increased MMA and an increase in movement peak velocity. In contrast, with "late" extensor perturbations beginning after the onset of the first agonist EMG burst, under compensation occurred with a reduced MMA and a reduced peak velocity. "Early" and "late" flexor perturbations produced less marked changes in MMA and peak velocity.

In conclusion, afferent inputs prior to the onset of the first agonist EMG burst can interact with descending motor commands to modify early motor output during rapid movements, leading to changes in MMA and peak velocity. In contrast, later afferent inputs have much less effect on MMA and the peak velocity of movement.

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100.2 HUMAN MOTOR UNIT RECRUITMENT DURING STATIC CONTRACTIONS IN DIFFERENT DIRECTIONS AND DURING DYNAMIC UNRESTRAINED MOVEMENT. B.H. Ross*, C.K. Thomas*, and B. Calancie (SPON: A. French). Dept. of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Spike triggered averaging (Stein, R.B. et al., Brain Res. 40: 187, 1972) for twitch amplitudes was used to determine the apparent recruitment order of 127 single motor units in the first dorsal interosseous muscle (FDI) of 5 subjects. Subjects produced static contractions in three directions: 1) flexion and 2) abduction of the index finger, and 3) flexion of the index finger coupled with adduction of the thumb. Twitch amplitudes for contractions in each direction were plotted against the respective recruitment thresholds. A similar procedure was used to study the recruitment of 48 motor units in the abductor pollicis brevis muscle (APB) of two subjects, who produced opposition and abduction contractions of the thumb. Finally the actual recruitment order of motor unit pairs in both FDI and APB was determined based on the relative onset of unit firing during a repeated scissoring movement in 3 subjects. These data from dynamic contractions were then compared to the apparent recruitment order of these same units as measured during static contractions.

The results show that the recruitment of motor units during various directions of static contraction of both FDI and APB muscles is strictly ordered. The units with the smallest twitch tensions (2-3 mN) were recruited at the lowest static forces (~5% maximum voluntary contraction), while those units with the highest twitch tensions (175-215 mN) fired at the largest static forces recorded. For the most part, this strict ordering of motor unit recruitment has been preserved during the dynamic scissoring task. That is, of 12 unit pairs studied to date, 10 demonstrated a recruitment order consistent with that observed during static contractions. Thus it appears from these data that the size principle of orderly recruitment adequately describes motor unit recruitment for both static and dynamic contractions of the FDI and APB muscles.

100.3 TRAJECTORY PROPERTIES OF PLANAR ARM MOVEMENTS AND THE EFFECT OF VISUAL GUIDANCE. Thomas Zeffiro and Deborah Claman. Neurology Service, Massachusetts General Hospital, Boston MA 02114.

We have investigated the influence of visual feedback on the spatial and temporal properties of visually triggered arm movements confined to a horizontal plane. Trajectory properties examined include error in final hand position, movement size, movement time, peak velocity, peak acceleration, peak deceleration, and the duration of accelerative and decelerative phases of movement.

Ten subjects performed a step tracking task in darkness, moving along the surface of a 20" by 20" digitizing tablet. They were instructed to move as quickly and accurately as possible to a target that would appear after they had reached the proper starting position. In one condition (no visual feedback) the target was extinguished as the movement commenced and in the other condition (visual feedback) the target remained lit until the end of the trial. The hand trajectory was sampled and stored for later analysis.

We examined relationships between various trajectory properties and movement size using linear and non-linear regression procedures. In both feedback conditions movement time, peak velocity, peak acceleration, and peak deceleration all increased monotonically with movement size. Although the duration of the accelerative and decelerative components of the movement increased monotonically at the same rate with increasing movement size, the ratio was less than 1, reflecting the asymmetry of the velocity profiles. Visual feedback invariably resulted in overall faster movements with larger peak velocities, accelerations, and decelerations. In addition, there was a tendency for the velocity profiles to become more symmetric, reflecting a shift in the relative duration of the accelerative and decelerative phases of movement.

While final position error increased monotonically with increasing movement size in the absence of visual feedback, this relationship was not observed when visual guidance was allowed.

The effects of visual guidance on the accuracy and kinematic properties of planar arm movements suggest that visual information about target and hand position, if available, is used to generate ongoing trajectory corrections. The kinematic effects of these corrections may be observed during the earliest components of the movement.

100.4 KINEMATIC VARIABILITY IN A THREE-JOINT FINGER-THUMB GRASP: DIGIT TRAJECTORIES AND JOINT ACTIONS ARE SUBORDINATE TO THE GOAL OF CONTACT CLOSURE. K.J. Cole and J.H. Abbs. Speech Motor Control Labs., Waisman Center, Univ. of Wisconsin, Madison, WI 53705-2280.

Reaching movements of the arm appear to be planned at the level of the hand trajectory, as indicated by straight-line hand paths, single-peaked tangential velocity profiles and joint kinematics that vary with target location (Morasso, P. Exp. Brain Res.,42, 1981). These data support the hypothesis that movements are planned at a goal level rather than at the joint or muscle level. Rapid grasp, if planned at a goal level, should show evidence of organization at the level of contact closure, rather than digit trajectory. As such, the constituent joint kinematics and digit trajectories toward closure may vary subordinately.

Movements of the thumb interphalangeal (IP) joint and index finger metacarpophalangeal (MP) and proximal interphalangeal (PIP) joints were monitored electro-goniometrically in humans producing rapid movements from an open hand position to a brief contact of the thumb and index finger. Subjects produced peak contact forces consistently between .75 and 1.25 N, as instructed. The finger tip position was monitored using an LED-distributed photodiode system. Intrinsic and extrinsic hand muscle EMG was recorded simultaneously.

Whereas finger-thumb contact was always achieved, the 2-D spatial location of contact varied significantly for repetitions of the task; the peak angular position of the thumb and the trajectory of the finger covaried to achieve distal pulpar contact. Finger trajectory variations were achieved via robust reciprocal actions of the MP and PIP joints, while the PIP and thumb peak positions varied directly. The observed motor equivalence and the specific joint pattern variations therein appeared to be controlled directly rather than being due to imprecision permitted by the oppositional nature of the task and/or to passive coupling. In this regard, the first lumbricalis muscle, which exerts only net extensor torques on the PIP joint and is normally inactive for hand closure, was phasically active for this task. Also, finger paths of greater distance and joint movements of greater amplitude were produced with greater peak tangential and instantaneous velocities, respectively.

Because there were striking motor equivalence variations in the constituent trajectories and joint kinematics, the redundant mechanical degrees of freedom afforded by the multijoint, multidigit action of grasp appear to be exploited as a control strategy. These results are consistent with goal level planning; the constituent trajectories and joint actions for precision grasp movements were subordinate to the goal of contact closure. (Supported by NIH grant NS-13274 and HD-03352.)

100.5 DRAWING MOVEMENTS IN THREE-DIMENSIONAL SPACE. J. F. Soechting and C. A. Terzuolo. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, MN 55455.

We have studied the angular motion at the shoulder and elbow during drawing movements performed in different planes in free space. Specifically, we asked subjects to draw simple geometric figures such as circles and ellipses with different inclinations. Subjects, while drawing such figures, produced motion at the wrist which was close to sinusoidal in two orthogonal directions in the plane of the motion. Furthermore, the angular motion at the shoulder and elbow, when described in a coordinate system which has been previously identified psychophysically, was also approximately sinusoidal. (Note that theoretically, a given motion at the wrist can result from infinite combinations of angular motions at the shoulder and elbow since the arm has four degrees of freedom while linear motion at the wrist has only three.) Thus, as is the case for other arm movements which have been studied, there is a unique relationship between motion at the wrist and angular motion at the proximal joints.

The data indicate that drawing movements in arbitrary planes are produced by regulating the phase relations among the sinusoidal motions of the angles. A fixed phase relation (180^{o}) between the angular elevation of the upper arm and of the forearm was found, irrespective of the plane of the motion. Instead, the phase of the yaw angle of the upper arm was related to that parameter, while the phase of the yaw angle of the forearm was correlated with the slant of the ellipses. In light of previous psychophysical experiments, we conclude that the topology of the sensorimotor map used for the production of a movement is the same as the one used for its perception. Furthermore, the mapping between wrist position and the orientation angles of the arm utilized for the production of drawing movements is not geometrically accurate and leads to predictable and consistent distortions of the motion of the wrist.

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100.6 GENERALIZED JOINT INTERPOLATION EXPLAINS STRAIGHT AND CURVED ARM MOVEMENTS. J.M. Hollerbach, S.P. Moore*, and C.G. Atkeson, Center for Biological Information Processing and Department of Psychology, M.I.T., Cambridge, MA 02139.

For unrestrained vertical arm movements measured with a Selspot system, it had been previously found that significantly curved end-point trajectories occur in certain regions of the workspace, but that in other regions the trajectories are approximately straight (Atkeson and Hollerbach, *J. Neurosci.*, in press). Straight-line trajectories in hand space have been taken as evidence for movement planning in hand coordinates, but the curved trajectories are a relatively new observation that were unexplained. When trajectories are specified as straight lines in joint space (joint interpolation), curved Cartesian trajectories could in principle result (Hollerbach and Atkeson, *Exp. Brain Res. Suppl.*, in press). We have studied whether the experimentally observed curved trajectories can be explained in this way.

Joint angles were inferred from positions of Selspot LEDs placed at various points on the arm, and the shoulder angle corresponding to upper arm flexion was plotted against the elbow angle. The results showed that most of the curved trajectories could indeed be characterized as straight lines in joint space.

Rather than accept a hypothesis that two different coordination strategies are in effect in different parts of the workspace, the straight trajectories in hand space were examined more closely for a possible alternative explanation. One class of straight hand-space trajectories actually intersected the shoulder joint, a known degenerate situation in which the joint-space trajectory is also straight. The other class of Cartesian straight-line trajectories could be generated if joint interpolation were generalized to allow one joint to be phase shifted and scaled in time relative to the other joint. Some remaining curved trajectories could also be accounted for similarly.

It is concluded that all trajectory classes can be explained by joint interpolation in its generalized form. Generalized joint interpolation can only approximate a Cartesian straight path if a joint does not reverse itself. The experimental curved movements correspond to regions where a Cartesian straight-line path requires joint reversal. It is hypothesized that subjects opt for strict joint interpolation for these movement regions, and that for the other movement regions find a good compromise to a straight line by judicious choice of interpolation parameters. Since it was not realized before that joint-based planning could yield relatively straight paths, these results illustrate again the dangers of arguing too closely from superficial features of data.

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100.7 FUNCTIONAL RELATIONSHIP BETWEEN TASK DEMANDS AND THREE-DIMENSIONAL MOVEMENT KINEMATICS. R.G. Marteniuk and S. Athenes*. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Simple arm movements involving pointing to a target or reaching and grasping for an object are not only products of a relatively complex movement system but also may be functionally related to task demands. In the present study, pointing and grasping movements were compared to determine if type of movement task affected the kinematic characteristics of the movement. The grasping movements were further examined to determine if there was a relationship between the initial reaching component and the grasp component. Moreover, an analysis was done to determine if the grasp component was functionally related to the size of the object being grasped.

Five subjects were instructed to move as quickly and accurately as possible to either place their index fingers on targets or grasp one cm thick objects that were 2 or 4 cm in diameter and either 20 or 40 cm from the hands' starting position. Five trials for each of the eight conditions were given with data derived from sampling (200 Hz) infrared emitting diodes placed on the index finger, the thumb, and two locations on the wrist. Movement times for both movement types generally followed Fitts' Law with the grasping movements requiring an additional 50 ms. In terms of peak speed (resultant velocity), however, both movement types produced amplitudes of equal value. Moreover, peak speed for the grasping movements occurred both relatively and absolutely earlier than for pointing movements resulting in a longer deceleration phase for the grasping movements. These results might indicate that the requirement to grasp an object not only places an additional level of difficulty on movement control but results in a motor control strategy that may optimize the use of visual feedback by lengthening the decelerative phase of the movement.

To determine if there was some functional relationhsip between the reaching and grasp components of the grasping movement, time of peak speed of the wrist was correlated with time of maximum grip aperture. The correlation was near zero and it appeared that each subject performed the task in different ways. Finally, size of object to be grasped affected grip aperture but maximum aperture was not invariantly related to object size. The small disk resulted in a larger relative aperture than did the larger one. Thus, the grasp component of the movement was not seen to be systematically related to the reach component nor to the physical dimensions of the objects to be grasped.

100.8 CONTROL PROPERTIES OF MOTOR UNITS IN HUMAN SYNERGIST-ANTAGONIST MUSCLES. C.J. De Luca, and J.L. Creigh*. NeuroMuscular Research Center, Boston University, Boston, MA 02215.

The electrical activity of several concurrently active motor units has been simultaneously recorded from the human extensor carpi radialis longus (ECRL) and the extensor carpi ulnaris (ECU). These muscles participate in controlling wrist movement and can function synergistically or antagonistically, depending on the task. The detected multichannel myoelectric signals have been decomposed into their constituent motor unit action potential trains using a computer assisted algorithm, allowing accurate calculation of the firing times of up to 11 concurrently active motor units.

The forearm and hand were secured in a specially designed device able to independently measure orthoganal forces isometrically generated by the wrist in a plane perpendicular to the forearm. The subjects were asked to track a circular force pattern, thus involving the muscles to be sequentially used in synergistic and antagonistic modes.

To date a total of 106 motor units in 12 contractions have been analyzed. The results indicate the following:

1) Small 1-2 Hz common fluctuations with no phase shift in the mean firing rates of motor units were noticed within the muscles studied; this was consistent with earlier work on other muscles. Common fluctuations also exist between the firing rates of ECU and ECRL motor units but with a variable phase shift. The variable phase shift suggests that the muscles are not necessairly considered as a functional unit by the CNS during synergistic function.

2) Cross-correlation analysis of the motor unit firing rates of either the ECU or the ECRL indicated that synchronization exists among the motor units within each muscle. No other significant interaction between the trains within a muscle were detected. Cross-intensity analysis performed across the muscles showed no significant interaction between the motor units of the two synergist muscles.

3) Analysis of the firing rate of motor units active during functionally related changes from wrist extension to adduction or abduction demonstrated a motor unit discharge behavior which is consistent with a notion indicating a multichannel input to sets of motor units in the two muscles.

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100.9 RESPONSE OF SYNERGISTS TO LOAD PERTURBATIONS IN A MULTI-JOINTED LIMB. F. Lacquaniti* and J. F. Soechting. Laboratory of Neurophysiology, University of Minnesota, Minneapolis, MN 55455.

A transient force applied to a limb segment will induce angular motion at joints proximally and distally to the point of application of the force. For example, a force applied to the upper arm in the backward direction results in extension at the shoulder and flexion at the elbow while a force acting on the forearm in the downward direction induces extension at both joints. We have begun to study the electromyographic responses of shoulder and elbow muscles to such perturbations. Last year, we showed that short latency responses in biceps and triceps were not correlated with the direction of the angular motion at the elbow joint resulting from such load perturbations. In fact, in both examples cited above, activation of biceps is observed. The observed behavior is not consistent with that expected from a feedback control of elbow angle; however it is consistent with a feedback control of elbow torque.

Since biceps and triceps span the shoulder joint as well as the elbow joint, there is one other possible interpretation of those results, namely that the patterns of activation of biceps and triceps reflect their dual action. Brachioradialis and brachialis are elbow flexors which do not span the shoulder joint. We have recorded EMG activity from those muscles in response to perturbations applied to the forearm and to the upper arm. As is the case for biceps, brachioradialis and brachialis responses are influenced also by motion at the shoulder joint and are not uniquely related to the motion at the elbow joint. However, the patterns of activity of the latter two muscles are not identical to that of biceps under all experimental conditions. Thus, the response of a given muscle to a load perturbation depends on the dynamical state of the entire limb and also on the particular function of that muscle, i.e. whether it spans one or two joints.

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101.1 THE ROLE OF VARIOUS AMYGDALA PROJECTION AREAS (BED NUCLEUS OF STRIA TERMINALIS, ROSTRAL LATERAL HYPOTHALAMUS, SUBSTANTIA NIGRA) IN FEAR-ENHANCED ACOUSTIC STARTLE. J.M. Mondlock* and M. Davis (SPON: D. Gallager). Depts. of Psychology and Psychiatry, Yale University, Conn. Mental Health Ctr., Ribicoff Res. Fac., 34 Park St., New Haven, CT 06508

We have previously shown that bilateral lesions of the central nucleus of the amygdala block fear-enhanced startle in the rat (increased acoustic startle in the presence of a light previously paired with shock; Mondlock & Davis, 1985), consistent with a large literature implicating the amygdala in fear conditioning. Previous work in this laboratory has attempted to delineate the neural pathway that mediates the acoustic startle reflex and the visual pathway that carries the visual conditioned fear information to the startle circuit (Davis et al., 1982; Tischler and Davis, 1983). The present study sought to begin to delineate the neural pathway by which the amygdala could influence fear-enhanced startle (potentiated startle).

Rats were given 10 light-shock pairings on each of 2 successive days. At 24-48 hrs following training, separate groups of rats received bilateral electrolytic lesions of the bed nucleus of the stria terminalis or interruption of the ventral amygdalofugal pathway at the level of the rostral lateral hypothalamus (using a knife cut transection) or at the level of the substantia nigra (using electrolytic lesions). Control animals were sham-operated. Approximately 3 days after surgery, the rats were tested for potentiated startle. Potentiated startle was blocked by lesions of the substantia nigra, but not by lesions of the bed nucleus of the stria terminalis or transection of the part of the ventral amygdalofugal pathway that projects to the rostral lateral hypothalamus and substantia innominata. These results indicate that the substantia nigra may be a component in the pathway from the amygdala to the startle circuit, since the amygdala projects to the substantia nigra and both are necessary for potentiated startle. Lesions of the intermediate and deep layers of the superior colliculus also block potentiated startle (Tischler & Davis, 1983). Therefore, the superior colliculus, which receives efferents from the substantia nigra and which projects to the ventral nucleus of the lateral lemniscus, a part of the startle circuit, may provide the final link between the amygdala and the startle circuit. Further studies will test the role of medial vs. lateral aspects of the superior colliculus in potentiated startle as well as the effect of more selective damage to the substantia nigra pars reticulata vs. pars compacta using electrolytic, 6-hydroxydopamine, or ibotenic acid lesions.

101.2 THE EFFECTS OF HIPPOCAMPAL AND SUBICULAR CELL LOSS PRODUCED BY INTRACEREBRAL IBOTENATE ON THE PARTIAL REINFORCEMENT EXTINCTION EFFECT. J.A. Gray*, L.E. Jarrard, J.N.P. Rawlins, and J.D. Sinden* (SPON: H.E. King). Dept. of Experimental Psychology, Univ. of Oxford, Oxford, England, and Dept. of Psychology, Washington and Lee Univ., Lexington, VA 24450.

Intracerebral injections of ibotenate were used to produce, in rats, extensive cell loss in the hippocampus and dentate gyrus ('complete hippocampal', CH), in the CH plus subiculum (SUB + CH), or in the subiculum plus entorhinal cortex (SUB + EC). These rats and sham-operate controls were trained to run in a straight alley for food reward delivered on a continuous (CR) or partial (PR) reinforcement schedule. In controls PR training gave rise to the well-known partial reinforcement extinction effect (PREE), i.e., greater resistance to extinction than that observed in CR-trained animals. The PREE survived CH lesions, which however caused generally greater resistance to extinction in both CR and PR training conditions, in the start and run sections of the alley only. Subicular cell loss (in both SUB + CH and SUB + EC groups), in contrast, abolished the PREE in the goal section only, partly by increasing resistance to extinction in the CR condition and partly by decreasing resistance to extinction in the PR condition. In addition, some of the effects of PR training on start and run speeds during acquisition were altered by the CH and SUB + CH lesions. These results confirm previous data showing that the hippocampal formation plays a role in mediating the behavioural effects of PR training, and demonstrate a double dissociation between the effects of hippocampal and subicular cell loss. The effects of complete hippocampal cell loss resembled those we have previously reported after medial septal electrolytic lesions; the effects of subicular cell loss resembled those seen after dorsolateral septal electrolytic lesions. A model is proposed to account for these findings, in which (a) nonreward usually leads to inhibition of behaviour via the medial septal projection to the hippocampus; and (b) a PR schedule attenuates such behavioural inhibition via a feedback loop (hippocampus → subiculum → lateral septal area → medial septal area).

101.3 SELECTIVE IBOTENIC ACID LESIONS OF HIPPOCAMPUS AND SUBICULUM: EFFECTS ON PERFORMANCE OF A COMPLEX PLACE AND CUE TASK. L. E. Jarrard. Dept. Psychol., Washington & Lee Univ., Lexington, VA 24450

In previous research we found that interrupting the intrahippocampal circuit either by lesioning the CA3 cells with kainic acid or the cells in the dentate gyrus with colchicine had minimal effects on performance of a complex place and cue memory task. In contrast with these results, performance was impaired when all of the hippocampus was removed by aspiration and when pathways and/or structures related to hippocampus (fimbria-fornix, entorhinal cortex) were lesioned (Jarrard et al., Behav. Neurosci., 1984, 98, 946-954). In order to study the effects of selectively damaging all the cell fields in the hippocampus (CA1-CA3, dentate gyrus) without interrupting fibers-of-passage, multiple injections of ibotenic acid (IBO) were used in the present research. In addition, the effects of IBO lesions of the subiculum were determined.

Using a within-subjects design, rats were trained before the operations to run on an 8-arm radial maze with a procedure that involves two kinds of learning (place, cue) and two memory functions (reference memory (RM), working memory (WM)). In the place task the same 4 arms were baited from trial to trial and room cues (door, cages, lights) remained in the same spatial location. In the cue task 8 removable inserts of different materials were placed in the arms but the location was changed in a random order from trial to trial -- 4 of the 8 cues were consistently baited.

After learning the two tasks, rats were divided into 2 control (operated, unoperated) and 3 lesion groups. In one lesion group the hippocampus was removed with aspiration. Multiple injections of IBO were used in the other groups to lesion either hippocampus or subiculum. Subsequent histology indicated that in some animals IBO spread to include damage to both hippocampus and subiculum -- these animals formed a combined Hippocampal-Subiculum group. Following recovery the rats were retrained to approach the last 4 arms (and 4 cues) that had been learned before the operations.

Even though histology indicated that most cells in the hippocampus were removed with the IBO injections, performance was minimally affected. Rats with lesions limited to the subiculum and those with aspiration lesions of hippocampus were impaired only on the place task. The most impaired animals were those that had combined IBO damage to both hippocampus and subiculum. Analysis of the pattern of errors indicated that animals with combined lesions were generally impaired on the place task making both RM and WM errors, whereas, errors on the cue task were limited to repeating arms already visited (WM).

These findings indicate that (a) if care is taken to avoid damaging fibers-of-passage, the hippocampus can be removed with only a minor, temporary effect on performance of the place and cue task; and (b) damage to subiculum, especially combined cell loss in both hippocampus and subiculum, results in impaired performance.

101.4 EFFECTS OF HIPPOCAMPAL LESIONS ON FEATURE-POSITIVE DISCRIMINATION CONDITIONING OF THE NICTITATING MEMBRANE RESPONSE IN RABBIT. K. J. Loechner*, J. J. Lo Turco*, and D. J. Weisz. Department of Psychology, Yale University, New Haven, CT 06520.

The specific aim of this experiment was to investigate the hypothesis that stimulus salience is an important variable in explaining the effects of hippocampal lesions on complex discrimination tasks. The paradigm chosen was simultaneous feature-positive discrimination conditioning of the nictitating membrane response in rabbit. Three groups of animals (hippocampal + cortical, cortical, and sham lesioned) were presented with two types of trials, a simultaneous tone-light compound reinforced with a corneal air puff (TL+) and a nonreinforced element (T- in the first experiment, or L- in the second experiment). Consequently, the feature-positive stimulus (the one presented only in the compound and never in isolation) predicted the unconditioned stimulus (UCS) with 100% accuracy whereas the other stimulus only had a 50% predictive value.

In the two experiments, the reinforced compound was the simultaneous presentation of a tone (1 kHz, 70 db) and a light (28 lx) for a duration of 850 msec co-terminating with the unconditioned stimulus, a 100 msec corneal air puff. Nonreinforced trials consisted of 850 msec tone or light presentations. We operationally defined salience as the rate at which conditioned responses are acquired to a particular stimulus in simple delay conditioning. In our laboratory conditioned responses to the 1 kHz tone appear in 150-300 trials in a simple delay conditioning paradigm, in contrast to the 400-500 trials required for the light. We predicted that hippocampally lesioned animals would perform as well as normals when the most salient stimulus in the compound was the feature-positive, but that they would fail to exhibit normal discriminative responding when the less salient stimulus was the better predictor of the UCS.

The results of the two experiments confirmed our prediction. In the first experiment, hippocampally lesioned animals were markedly disrupted in their ability to discriminate as evidenced by inappropriately high response levels to nonreinforced tone presentations. In the second experiment, in which tone was the feature-positive stimulus, there were no significant differences across the three experimental groups. In both experiments, there were no differences in acquisition rates to the reinforced tone-light compound. The results indicate that stimulus strength may be an important consideration in the interpretation of hippocampal lesion effects.

101.5 IBOTENIC ACID LESIONS OF THE MEDIAL SEPTAL AREA AND NUCLEUS BASALIS MAGNOCELLULARIS CAUSE DIFFERENTIAL IMPAIRMENTS IN TEMPORAL MEMORY. W.H. Meck, R.M. Church, G.L. Wenk and D.S. Olton. Depts. of Psychology, Brown University, Providence, RI 02912 & The Johns Hopkins University, Baltimore, MD 21218.

Three groups of ten rats were trained preoperatively on a 40-s peak-interval (PI) timing procedure. In this task white-noise signals are separated by a 120-s inter-trial interval. On a random half of the trials food is primed after the signal has been present for 40s and the rat's first lever press after the fixed interval causes the termination of the signal and the delivery of a 45 mg food pellet. On the remaining trials (peak trials) no food is given and the signal continues for 120s. On peak trials the rat's response rate increases as a function of time since signal onset until the time that food is sometimes available. The time of the maximal response rate during a trial is referred to as the peak time and the response rate at the peak time is referred to as the peak rate. Once the maximal level of responding is obtained the rat's response rate declines in a fairly symmetrical manner when response rate is plotted on a linear time scale. The peak time measure serves as an indicator of the rat's remembered time of reinforcement, a value that is stored in reference memory. The median baseline peak time for all three groups of rats was 40s ± 0.5s. Following initial PI training bilateral lesions of either the medial septal area (MSA) or the nucleus basalis magnocellularis (NBM) were made by injection of 25 nmoles of ibotenic acid or vehicle into each brain area. One week after surgery PI training was resumed. The effects of the various treatments were as follows: Rats given sham operations continued to maintain normal peak times at 40s ± 0.5s. Rats given MSA lesions gradually shifted their peak time values leftward on the time scale and eventually maintained a lateral shift in their peak functions at 34s ± 0.4s. In contrast, rats given NBM lesions gradually shifted their peak times rightward on the time scale and eventually maintained a lateral shift in their peak functions at 46s ± 0.6s. The conclusion is that ibotenic acid lesions placed in anatomically distinct cholinergic pathways produce differential impairments in temporal memory as evidenced by opposite effects on the remembered time of reinforcement. These quantitative changes in the content of temporal memory suggest a modification of the translation constant that determines the remembered duration of events.

101.6 IBOTENIC ACID LESIONS OF THE NUCLEUS BASALIS OF MEYNERT IMPAIR SPATIAL-REFERENCE MEMORY IN THE MORRIS WATER TASK. Carl P.J. Dokla*1, L.J. Thal*1, E.L. Gardner*1, David Hann*2, Janice C. Passabet*2, Larry Fitzgerald*2, and Mark Leiman*2. (SPON: J.J. Boitano). 1Departments of Neurology and Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y. 10461, and 2Department of Psychology, Fairfield University, Fairfield, CT 06430.

Ibotenic acid (IBO) lesions of the nucleus basalis of Meynert (NBM) produce significant depletions of cholineacetyltransferase (CAT) in the cortex of rats thereby disrupting working-memory tasks and providing an animal model for Alzheimer's disease. However, IBO lesions of the NBM also impair reference-memory tasks. Suction ablation of the frontal and parietal cortex has been shown to profoundly impair both spatial-reference memory (e.g., Morris water task) and working memory (Kolb, Sutherland, Whishaw; Behav. Neurosci. 97(1): 13-27, 1983). The present investigation examined the effects of nucleus basalis IBO lesions using a reference-memory task, the Morris water task, that minimized the effects of proximal cues and has been shown to be sensitive to frontal-parietal cortical damage. Male Fischer 344 rats were stereotaxically injected in two-stages (bilaterally) with 18 ug of IBO dissolved in buffered saline (1 ul). Sham-operated rats served as controls. Following postoperative recovery, rats were tested in the Morris water task. The rats were required to learn the position of a fixed, submerged platform in a small, circular pool filled with water rendered opaque by powdered milk. For the first two days of testing, trials were conducted in blocks of four with the rats started at random locations. For the next three days all rats were given a single trial which was started at a single fixed location (North). Rats in the IBO group displayed a profound impairment on the water task with significantly elevated escape latencies and repetitive swimming around the pool's periphery. On day 1 of testing the IBO group had significantly longer escape latencies than the sham-operated group (Median latency = 115 sec vs. shams 50 sec, $p<.005$). On day 2 a significant groups by trials interaction was found ($p<.05$); the IBO group had significantly longer escape latency than the sham-operated group on the first trial of the four trial block ($p<0.001$) but displayed progressively shorter latencies over the remaining test session. On day 4 the IBO group displayed significantly longer escape latencies than the sham-operated group ($p<0.02$). The present results very closely paralleled those reported by Kolb et al. (1983) for suction ablation lesions and indicate that IBO rats are significantly and profoundly impaired in a reference-memory task. However, IBO rats can display considerable learning within a day's session though impaired severely in long-term retention between days.

101.7 ACQUISITION AND RETENTION IN THE RADIAL ARM MAZE BY RATS WITH ISCHEMIC-INDUCED CA-1 HIPPOCAMPAL DAMAGE. H.P. Davis, W.A. Pulsinelli & B.T. Volpe*. Dept. Psychol., Univ. Colorado, Colo. Spgs., CO 80933 and Dept. Neurol., Cornell Univ. Med. Coll., NY, NY 10021.

Rats subjected to 30 min. of forebrain ischemia by 4-vessel occlusion demonstrate ischemic injury in the CA-1 zone of the hippocampus, an area implicated in the etiology of postcardiac arrest human amnesic syndrome. For some patients surviving cardiac arrest, cognitive disturbance and in particular a memory impairment may cause persistent morbidity. These studies examine whether ischemic brain damage in rats, particularly to CA-1 neurons, results in learning and memory deficits similar to those seen in humans.

In Experiment 1, control and post-ischemic rats (PI rats) were trained in a radial 8-arm maze task with 5 of 8 arms baited. By baiting the same 5 arms on all daily trials it is possible to measure reference performance (entering baited arms only) and working performance (not reentering a baited arm after the food is taken). On 95 trials, started 30 days after surgery, the PI rats with primarily CA-1 neuronal loss made significantly more reference and working errors than controls ($p<0.05$). PI rats and controls had comparable reference performance during the last 20 trials. However, PI rats demonstrated a persistent working memory deficit ($p<0.08$).

In Experiment 2, rats were pretrained for 70 trials and then sham operated or subjected to cerebral ischemia. PI and control rats did not differ on retention of the reference component when tested 30 days post-surgically. However, working memory of PI rats was impaired ($p<0.05$). Normal retention of reference memory by pretrained PI rats suggests that surgery per se does not induce a performance deficit. Consequently, the slower rate of acquisition by PI rats in Experiment 1 likely reflects impaired learning rather than a nonspecific impairment of performance.

Thus, rats with CA-1 loss after ischemic insult have impaired reference and working performance. Reference memory appears to eventually recover. PI rats demonstrate normal retention of the highly practiced reference component if learned prior to cerebral ischemia. The memory deficit of PI rats is similar to the human amnesic syndrome which may be characterized by impaired learning and memory for events after cerebral injury, but with normal retention of highly practiced premorbid skills such as language and social behavior.

101.8 REGIONAL GLUCOSE METABOLIC CHANGES IN AN ANIMAL MODEL OF POST-CARDIAC ARREST AMNESIA. B. T. Volpe*, H. P. Davis., W. A. Pulsinelli, Dept. of Neur.,Cornell Univ. Med. Sch.,NY,NY 10021.

We have been exploring an animal model for the human amnesic syndrome that may follow cardiac arrest (Volpe et al., Stroke 15:558, 1984). Most rats subjected to transient cerebral ischemia for 30 minutes survive indefinitely, and have selective injury to neurons in CA1 hippocampus and the dorsolateral (dl) striatum. We have shown previously that these post-ischemic (PI) animals eventually acquire and remember the "reference" aspects of a radial 8-arm maze as well as controls, but demonstrate delayed learning and persistently impaired memory for the "working" aspects of the task. We used 14C-2 deoxyglucose autoradiography to identify patterns of metabolic activity in PI rats that might correlate with permanent working performance impairment and recovery of reference performance. Four rats were subjected to 30 minutes of ischemic injury, and four rats were sham operated. All animals recovered and performed on a radial 8-arm maze in which 5 of 8 arms were baited. Behavioral results after 75 trials showed that reference performance was comparable between PI and controls, but working performance remained impaired for PI animals compared to controls. Months after finishing maze training, the regional metabolic rate for glucose (rCGU) was measured. The rCGU's in morphologically normal brain regions in PI and control rats were comparable (corpus callosum, sup. and inf. colliculus, medial geniculate, motor, entorhinal, subicular and cingulate cortex, and also CA2 and CA3). In areas of confirmed morphological damage (CA1 and dl striatum) the rCGU's of PI animals were significantly decreased compared to control values (see Table). In the mamillary bodies and the thalamus, regions with little or no morphologic damage, PI animals had significantly increased rCGU compared to control animals (Table). The decreased rCGU in CA1 and dl striatum probably represents neuron loss. We cannot determine whether the increase in rCGU in the mamillary and thalamic region is due to maze training or post injury modification of glucose metabolism. In either case, our results suggest that a lesion in CA1 participates in disinhibition of the mamillothalamic area, and further that the hippocampus may influence some forms of behavior through mechanisms of inhibition.

Regional Cerebral Glucose Metabolism (um/100g/min)

	Control (n=4)	Post Ischemic (n=4)
CA3	41	36 (p>.2)
CA1	38	25 (p<.05)
DL Striatum	55	35 (p<.01)
Mamillary Bodies	63	84 (p<.01)
VA Thalamus	56	76 (p<.004)

101.9 THE CONTINUING SEARCH FOR A ROLE OF THE CEREBELLAR CORTEX IN EYELID CONDITIONING. D. S. Woodruff-Pak, D. G. Lavond, C. G. Logan,* J. E. Steinmetz, & R. F. Thompson. Department of Psychology, Stanford University, Stanford, California 94305

We report here on cerebellar cortical aspiration studies on 17 rabbits run in several different eyelid conditioning paradigms to further examine the hypothesis that the cerebellar cortex is essential for the retention of the conditioned eyelid response. Twelve rabbits were first trained to a light CS, then trained to a tone CS then trained until they presented 80% CRs on alternating blocks of 8 tone and 8 light CSs. An additional 5 rabbits were trained to tone CS only. Left lateral cerebellar cortex was aspirated in 15 of the rabbits, and vermis alone was aspirated in 2. Rabbits were retested 5 days after aspiration. Histological identification of the lesions using Larsell's terminology revealed that in all 15 cases the entire ventromedial extent of HVI was removed. In 8 of the lateral cerebellar lesions all of HVI and HVIIA were removed, and in 7 animals complete removal of ventromedial HVI and medial HVIIA was achieved. Thirteen animals had CRs after the lesion. Of the 4 rabbits showing few or no CRs after aspiration, all showed clear evidence of interpositus nucleus deterioration. Five rabbits were retested 26 days post aspiration. Whatever behavior they had shown 5 days post-aspiration, they also showed 3 weeks later. Histological examination revealed slight to moderate inferior olive degeneration in the brains of rabbits with cortical lesions but no interpositus nucleus damage.

A subgroup of the above rabbits had lesions of the cerebellar cortical area involving lateral vermis and medial lateral cortex which projects to the interpositus nucleus (called zone C). These lesions ranged from a minimum of partial removal of vermian lobule IV and ventromedial HVI and medial HVIIA to partial removal of vermian lobules II-VI and complete removal of HVI and HVIIA. The interesting result from these lesions was that animals showed a deficit in CRs on the first day of retesting, but were able to relearn pre-aspiration levels of responding. These data indicate that the large portion of zone C lesioned was not essential, but it may have been involved as relearning was required. None of our zone C lesions, or in fact any lesions described here, eliminated all cortical input to the interpositus nucleus. Hence the possibility remains that there is cerebellar cortical tissue essential to conditioning.

We have pursued a number of hypotheses to examine when the cerebellar cortex might be essential for eyelid conditioning, and we still have found no essential role of the cerebellar cortex.

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101.10 DELAYED NON-MATCH TO SAMPLE WITH TRIAL-UNIQUE ODOR STIMULI IN INTACT AND FORNIX-DAMAGED RATS: A NEW TEST FOR RECOGNITION MEMORY AND MODEL OF TEMPORAL-LOBE AMNESIA. H. Eichenbaum, T. Parikh* & N. J. Cohen. Dept. Biol. Wellesley College, Wellesley, MA 02181, Center for Adaptive Systems, Boston University, Boston, MA 02215, and Dept. Psych., Johns Hopkins University, Baltimore MD 21218.

Modifications of the delayed match to sample (DMS) memory test by Mishkin & Delacour (J. Exp. Psych. 1:326, 1975) led to the creation of a recognition memory task that monkeys acquire rapidly and perform reliably. Their modifications of the paradigm included the use of trial-unique stimuli to eliminate inter-item interference and a non-match response requirement that monkeys adopt readily. Their paradigm has been used to develop a primate model of the human amnesic syndrome by demonstrating marked impairment of memory across delay in monkeys with medial temporal-lobe damage. We have adapted Mishkin & Delacour's procedures to our go, no-go odor discrimination paradigm and successfully developed a new recognition memory test for rats that exploits rodents' exceptional learning capacities in the olfactory modality.

Control rats and rats with bilateral fornix lesions were trained on a 10-sec delayed non-match to sample (DNMS) task using trial-unique odor cure chosen from a library of 200 pure chemical odorants. In the sample phase, a rat was required to maintain its nose in the odor sampling port for 2-sec (R+) during exposure to a novel odor in order to receive a water reward. During the match phase, the rat could sniff a second odor which was either identical to the sample or was another novel odor. Rewards were given only for R+ responses to the novel odor.

Control and fornix-damaged rats acquired the task at equivalent rates. On subsequent days, DNMS testing sessions at 10-sec delays were intermingled with sessions at 20, 30, and 60-sec delays. Control subjects consistently performed at 90% correct at all delays. In striking contrast, rats with fornix damage demonstrated significantly poorer performance at all delays beyond 10-sec, falling to chance performance at the 30-sec retention interval.

The development of measures for memory and models of amnesia in non-human subjects benefits by exploitation of stimulus modalities dominant in the species under study. This study has demonstrated for the first time, to our knowledge, rapid acquisition and high stable performance of rats in a recognition memory task with specific sensory cues. Furthermore, using this paradigm we have succeeded in modeling in rats with fornix damage the profound memory deficit observed in monkeys and humans with damage to the medial temporal-lobe. (Supported by HHS NS18744 & ONR N00014-83-K0337. We thank International Flavors & Fragrances and Naarden International for odor stimuli.)

101.11 INNERVATION OF THE SUBCORTICAL FOREBRAIN BY THE MEDIAL GENICULATE NUCLEUS: ANATOMICAL STUDIES OF AN EMOTIONAL CONDITIONING PATHWAY. J.E. LeDoux, D.A. Ruggiero, D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll. New York, NY 10021

The medial geniculate nucleus (MG) is generally described as a thalamo-cortical realy nucleus, receiving its major inputs from acoustic nuclei in the midbrain and projecting primarily to auditory cortex. However, lesions of the MG, but not of its cortical projection field, disrupt emotional conditioning to acoustic stimuli in rats (LeDoux et al, J. Neurosci. 4: 683-698, 1984). Since sensory signals ascending in the primary acoustic pathway to MG and then transmitted to a subcortical target may mediate emotional conditioning, we have sought to determine whether neurons in cytoarchitectural subregions of the MG give rise to subcortical projections and if so whether the same subregions are innervated by the inferior colliculus (IC), the major acoustic relay nucleus in the midbrain.

The rat MG was parcelled into three major subnuclei on the basis of gross morphological features and cellular cytoarchitecture: the ventral, dorsal and medial divisions. These are surrounded by several nuclei of the posterior thalamus, including the marginal zone, the posterior limitans nucleus, the posterior intralaminar nucleus, and the peripeduncular region.

Connections of the rat MG were studied using WGA-HRP as an anterorgrade and retrograde axonal marker. WGA-HRP (2%; 3-10nl) was microinjected into local brain areas. After 24h the rat was perfused transcardially and the tissue was processed histochemically using tetramethylbenzidine as the chromagen.

Following injections in the central nucleus (n=2) of IC anterograde transport was seen in the ventral and medial divisions of the MG. Injections of the external nucleus (n=3) or the dorsal cortex (n=2) of IC produced labeling throughout the MG, as well as in the surrounding areas of the posterior thalamus.

Large injections of MG (n=10) resulted in anterograde labeling of cortex and several subcortical areas, including the posterior caudate-putamen (CPU), the dorsal amygdala (AMY), the ventromedial nucleus of the hypothalamus (VMH), and the subparafascicular thalamic nucleus (SPF), as previously described (LeDoux et al, ibid). Injections centered in the ventral (n=2) or dorsal (n=1) divisions mainly resulted in cortical labeling, while injections in the medial division (n=5) produced anterograde transport in cortex and in CPU, AMY, and less consistently in in VMH and SPF. Injection of CPU or AMY retrogradely labeled neurons in the medial division of MG and in surrounding areas of the posterior thalamus. Injections in VMH or SPF only labeled the posterior thalamus.

The medial division of MG thus receives inputs from the central nucleus of IC and projects to the CPU and AMY. Recent behavioral studies indicate that selective interruption of connections between MG and AMY disrupts emotional conditioning (Iwata et al, this vol.). We conclude that neurons in the medial division of the MG constitute a pivotal link between acoustic and emotional processing systems of the brain. (Supported by Grants from the Am. Heart Assoc. and NIH/NHLBI).

102.1 THE IMMATURE ASTROCYTE:ITS ROLE DURING NORMAL CNS AXON TRACT DEVELOPMENT AND ITS ABILITY TO REDUCE SCAR FORMATION AND PROMOTE AXONAL REGENERATION WHEN TRANSPLANTED INTO THE BRAINS OF ADULTS.

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During normal development of the mouse brain, a "sling" of glial tissue that forms transiently along the presumptive path of the corpus callosum plays an important role in guiding the pioneering contingent of commissural axons across the cerebral midline (Silver, et al., J.C.N.210,1982). We have now discovered a similar structure in the embryonic cat brain using immunohistochemical localization of antibodies to GFAP. The "sling" is one of only 7 such structures in the cat forebrain that express GFAP at very early (E 39) stages. Each GFAP+ structure is associated with a major boundary in the brain, and several form at the borders of major developing axon tracts. The GFAP+ cells are radially arranged and collectively form beautifully organized sheets or plates. The 7 regions are (1) the midline "sling" (2) a tube of cells surrounding the anterior commissure at the midline (3) the glial "knot" (Silver, J.C.N.223,1984) at the rostral edge of the chiasm (4) a row of cells at the dorsal border of the olfactory tract (5&6) two plates of cells, one between the habenula and thalamus, the other between the thalamus (lower border) and hypothalamus, and (7) a collection of cells along the dorsal edge of the hippocampal fimbria. The role of these cells in creating boundaries for developing axon tracts will be discussed.

It has also been shown that when the "sling" is lesioned, the would-be commissural axons whirl into massive neuromas that persist indefinitely at the cerebral midline. Silver and Ogawa (Science, 220,1983) have shown that an untreated Millipore implant inserted between the neuromas in acallosal neonates can support the migration of immature astrocytes which, in turn, stimulate the denovo growth of callosal axons across the midline. Smith, Miller and Silver (Neurosci. Abs.,1985) have shown that only astrocytes within the brains of animals younger than P10 can serve this axon growth promoting function. In older mice astrocytes form dense scars at the implant surface. Glia in scars do not promote axon outgrowth. In the present study we have harvested immature astrocytes on Millipore from pre-critical stage mouse forebrains and have transplanted the glial coated prostheses into the brains of post-critical stage (P17 or P34) acallosal hosts. Such transplants greatly reduce glial scarring in the host, they inhibit bleeding and secondary necrosis, and again promote commissural axon growth across the midline in a malformed brain that normally would never reform a callosum with an untreated Millipore implant alone. Our results suggest that axonal regeneration in the post-critical CNS may be stimulated by recapitulating an embryonic glial environment at the lesion site. Supported by NIH (NS15731)and NSF(BNS8218700)

102.2 AFFINITY OF AFFERENT PIONEER GROWTH CONES FOR THE EPITHELIAL SUBSTRATE INCREASES PROXIMALLY WITHIN LEG SEGMENTS OF GRASSHOPPER EMBRYOS.

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The T1l pioneer neurons differentiate from the ectodermal epithelium at the tip of the embryonic limb bud. Their afferent growth cones follow a characteristic pathway along the inner surface of the epithelium. The dominant guidance cue for these growth cones is a set of non-adjacent, identified "guidepost cells". Another prominent cue is a limb segment boundary, along which the pioneer growth cones circumferentially reorient after completing an "initial path" of proximally directed growth (Cold Spring Harbor Symp. Quant. Biol. 48: 573-585).

In typical legs, the Fel and Trl guidepost cells contribute to proximal guidance on the initial path. However, several observations have suggested the presence of at least one additional proximal polarity cue along the initial path (ibid.).

One possible proximal guidance cue is an adhesion gradient on the epithelial substrate. Growth cones cultured on substrates of high adhesivity exhibit characteristic morphologies - increased degree of branching and spreading, growth cone lamellae, and filopodial retention - which produce increased neuronal apposition to the substrate. We have used these characterisics as criteria for assaying neuronal adhesion at different locations along the path. We find marked increases in degree of neuronal apposition at segment boundaries and in more proximal regions of segments.

The pioneer growth cones cross the proximal border of the tibia. Lateral lamellae or branches are extended, and lateral filopodia are preferentially retained there. As the leg segments develop further, the pioneer axons extend prominent lateral branches in the proximal region of the tibia, but not in the adjacent distal region of the femur.

The T1l growth cones exhibit a higher degree of branching in the proximal region of the femur than in the distal region when they are not in contact with guidepost cells there and can therefore respond to less dominant cues on the epithelial substrate. When they reach the distal boundary of the coxa, they abruptly reorient along the boundary, and do not extend branches proximally into the distal region of the coxa except along filopodia in contact with proximally located guidepost cells.

These observations suggest a pattern of proximally increasing neuronal apposition within each segment. This may reflect the differential expression of guidance molecules on the epithelial substrate. Guidance molecules could be adhesive in nature, or non-adhesion molecules which actively regulate growth cone extension.

102.3 NEURONAL GUIDANCE IN DISSECTED LIMB BUDS, IN CELL CULTURE, AND AT ECTOPIC LOCATIONS IN HOST LIMB BUDS.

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The first sensory neurons which extend axons in the embryonic grasshopper limb are the T1l pioneer neurons. Their axons grow proximally towards the CNS along a stereotyped, characteristic route. To investigate the cues which might guide axon growth, we have conducted the following experiments.

Limb bud manipulations: Prior to the onset of pioneer axonogenesis (29% of development), the right metathoracic leg was detached from the body, split open lengthwise along the posterior side, flattened and pinned. After two days in culture, the pioneer axon trajectories in both the experimental leg and the contralateral control leg were normal; axons extended proximally to the coxa/trochanter segment boundary and grew ventrally along the boundary. Normal pathway formation under these conditions is evidence against guidance by factors diffusing from the body, axial electrical currents within the limb, limb curvature, or local diffusable factors. These observations are consistent with the idea that local cues on the surfaces of cells with which the pioneer growth cones interact serve as proximally orienting guidance cues.

Cell culture: Embryonic limb buds were dissociated, and separated cells were plated in culture on poly-l-lysine coated cover slips. After 2 - 9 days in culture, neural, mesodermal and epithelial cells each differentiate and exhibit distinctive morphologies. These cell types can be unequivocally distinguished from one another on the basis of their morphology and cell surface properties. Neurons are recognized by anti-HRP antibody, while the lectin WGA binds to epithelial cells and not to mesodermal cells. Afferent neurons differentiated similarly both in situ and in vitro. Their axons were monopolar with a broad axon hillock, were as long as 90 µm and had lamellar growth cones, often with numerous filopodia. In mixed cell cultures, neurons contacted other neurons preferentially. Thus, neurons in vitro behave as they do in situ, where guidepost neurons serve as the highest affinity substrate for extending afferent axons.

Transplanted neurons: Dissociated neurons were isolated and labeled with a fluorescent probe. Labeled neurons were transplanted onto the inner surface of limb buds which were (a) un-opened but with the mesoderm removed, (b) split open and flattened. After two days in culture, transplanted neurons, in both preparations, adhered to the limb surface, produced growth cones with filopodia, and extended axons of up to 50 µm. The routes taken by axons of transplanted neurons serve as a test of several models of guidance features within the limb.

102.4 **PATHFINDING BY IDENTIFIABLE GROWTH CONES IN THE EMBRYONIC FISH SPINAL CORD.**

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Analysis of pathfinding in the CNS during embryogenesis can be highly successful when individual neurons are identifiable at all stages of development and the environment through which their growth cones navigate is simple. Such is the case with the early embryonic spinal cord of the fish. In Medaka oryzias the earliest neurons are relatively large, can be visualized in living embryos with Nomarski optics, and are accessible to single cell techniques. I have utilized intracellular injection of dyes and electron microscopic analysis of the fish embryo to study axonogenesis by these neurons.

At approximately 40-46 hr of development when 12-15 somites are present in the embryo, three kinds of neurons based on their initial growth cone trajectories and cell body locations are evident in the cord. These populations of neurons are distributed up and down the longitudinal axis of the cord, and are the dorsally located Rohon-Beard (RB) mechanosensory neurons, ventrally located axial motor neurons, and the laterally located commissural neurons. Additionally the cord contains smaller neuroepithelial cells which have numerous endfeet processes in contact with the basal lamina which delimits the neural tube. All three kinds of neurons project growth cones during this time period. So far the temporal sequence of axonogenesis has been followed for the RB neurons. These cells initially project two growth cones, one anterior and the other posterior, from the lateral pole of the cell body. Filopodia up to 10 um in length project longitudinally from these growth cones. At this time only one or two axonal profiles, which are in contact with the endfeet of the dorsal neuroepithelial cells, are present in the dorsal cord in EM sections. Later a well formed longitudinal tract is present in the same part of the cord. This indicates that RB neurons pioneer this dorso-lateral tract and raises the possibility that the endfeet of dorsal neuroepithelial cells may play a significant role in pathfinding by their growth cones. In the next 10 hr these growth cones extend up to 10 somites in each direction and the axons of RB cells fasciculate in the dorso-lateral tract. Also during this period a peripheral growth cone extends from the longitudinal axon near the cell body to begin innervating the skin. Another 60 hr later the RB central axons extend to fewer somites, project collateral longitudinal axons, and have become monopolar. Thus it appears that the earliest growth cones in this vertebrate CNS grow in a stereotyped manner. Experiments to elucidate how this is done are presently being pursued.

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102.5 AXON GUIDANCE IN ANEURAL WINGS OF DROSOPHILA. S.S. Blair and J. Palka, Dept. Zoology, NJ-15, Univ. Washington, Seattle, WA 98195

The wing of the fruitfly Drosophila melanogaster is formed during metamorphosis from the wing imaginal disc. This epithelial sac everts during the first hours of pupariation and then flattens, bringing the originally separate dorsal and ventral epithelia of the wing together to form the future wing blade. During this process a set of identifiable sensory neurons arise within the wing; by staining these neurons using immunohistochemical techniques, we have shown that they arise in a stereotyped sequence and lay down a characteristic set of nerve bundles which project proximally into the CNS.

It was demonstrated previously, using surgical and genetic techniques, that the initial, correctly oriented outgrowth of these neurons is not dependent upon the presence of other neurons ("guidepost" or "landmark" hypothesis) or upon physical constraints ("channel" hypothesis) within the developing wing. In fact, normal outgrowth can take place in a surgically altered wing which lacks both physical channels and putative guidepost neurons. Axons are thus being guided by some other cue or cues, either within the wing environment (extrinsic cues) or within the neuron itself (intrinsic cues).

To test for extrinsic cues, an experiment has been devised in which ectopic neurons are transplanted into a mutant, aneural wing, which is then reared in vitro; growing axons from the transplant in effect assay the inner surface of the aneural host wing for the presence of extrinsic guidance cues. The neuron-containing transplant was derived from the marginal bristle region of a wild-type wing, while the aneural host wing was from the mutant scute 10-1. Initial results indicate a marked preference for axonal outgrowth along the proximo-distal axis of the wing, in a position corresponding approximately to the site of the nerve of the third vein in normal wings. Since the transplants were oriented randomly in the host wing, axonal outgrowth into host tissue is apparently being directed by some form of extrinsic cue. As the host wing lacks neurons, transplant neurons cannot merely be following endogenous, intrinsically oriented nerve bundles. The nature and distribution of extrinsic cues is the object of further experimentation.

102.6 Results of Experimental Manipulation of the Neural Tube in Xenopus laevis Embryos on the Growth of Spinal Cord Axons. S M Bunt, T M Scott* and F G Inglis*, Department of Anatomy, University of Dundee, Dundee DD1 4HN UK, and *Health Sciences Centre, Faculty of Medicine, Memorial University of Newfoundland, St Johns, Newfoundland A1B 3V6, CANADA.

Many workers have described the pathways taken by fibres in the developing spinal cord and have attempted to deduce the guiding factors from observations of the normal neural tube. However, there has been little experimental manipulation of the system to test whether the mechanisms suggested can actually cause the fibres to grow in a particular direction. We have attempted to assess the role of spatially localised cues such as glial channels or fibronectin or laminin pathways in the guidance of the growth of spinal cord axons by rotating a section of neural tube and then investigating the effects this has on later growing axons.

A short section of the neural tube and surrounding tissues from a stage 22 Xenopus laevis embryo was excised and replaced by a section from another embryo labelled with tetramethyl rhodamine isothiocyanate (TRITC). The transplant was rotated around the vertical axis so that the previously rostral end of the transplanted section of tube was placed caudally in the host embryo. Scanning and Transmission electron microscopy had shown that no fibres were present at this time. Time lapse photography and the fluorescent labelling showed that the transplant healed in and survived without derotation.

The animals were left to grow to stages between 45 and 50 when HRP was applied to selected areas of the cord cross section. When processed as wholemounts these animals showed that the rotation only rarely disturbed the pathways of the later growing axons. In 20 cases out of 23 the fibres grew directly through the rotated region with no detectable disturbance to their normal parallel pattern of fibre growth. It therefore appears that the fibres cannot, on the basis of guidance cues present at stage 22, show a preference for the right or left side of the neural tube, nor for their direction of growth along the tube.

The existence of structural cues at the stages studied is also doubtful. We have examined developing neural tube by light microscopy, and by transmission and scanning electron microscopy to determine whether or not specialised structures capable of guiding growing axons are present. Only relatively unspecialised intercellular clefts were seen, with no evidence of grooves or channels. (Neuroscience Letters Suppl. 21,567, 1985).

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102.7 BASEMENT MEMBRANES AND NERVOUS SYSTEM DEVELOPMENT IN THE GRASSHOPPER EMBRYO. H. Anderson. Department of Zoology, University of California, Davis, CA 95616.

The growth cones of the first axons in the central nervous system of the grasshopper embryo progress along a basement membrane overlying the developing nervous system (Bate, C.M. & Grünewald, E.B., J. Embryol. exp. Morph. 61: 317, 1981). They do so in particular patterns characteristic for each axon, to produce a simple arrangement of nerves which later play the important role of providing the pathways for growth of later developing neurons. The growth cones of the first axons in the peripheral nervous system grow towards the CNS along the inner surface of the embryonic epidermis with their filopodia extending beneath the epithelial basement membrane (Bentley, D. & Caudy, M., Cold Spring Harbor Symp. Quant Biol. XLVIII: 573, 1983; Berlot, J. & Goodman, C.S., Science 223: 493, 1984). These axons also grow in particular patterns with a general tendency for growth to be directed proximally.

The basement membrane thus appears to act as a generally preferred substrate for the outgrowth of early axons in the grasshopper embryo. In addition to this role, particular components of the basement membrane could provide directional information for growth cone navigation.

Little is known about the structure and composition of any insect basement membrane. We have begun an investigation of the basement membrane of grasshopper embryos at stages between 25% and 35% of embryonic development - the period during which the first central and peripheral neurons establish their pathways.

We have used histochemical methods combined with light microscopy and electron microscopy to describe the overall appearance of the basement membrane. To investigate the distribution of individual molecular components within the basement membrane, we have examined the labelling patterns obtained after treating wholemount preparations and frozen sections of embryos with a variety of lectins and with affinity-purified polyclonal antibodies to identified components of vertebrate basement membranes which we have found bind to discrete bands on immunoblots of embryonic material.

We are now extending this investigation by generating monoclonal antibodies to embryonic grasshopper basement membrane itself.

Supported by NIH grant NS 21355.

102.8 GENES AFFECTING AXONAL GROWTH AND GUIDANCE OF LUMBAR NEURONS IN THE NEMATODE CAENORHABDITIS ELEGANS. S. S. SIDDIQUI and J. G. CULOTTI. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Earlier we have reported that antibodies to horseradish - peroxidase(HRP) stain a specific subset of identified neurons in the nematode C. elegans, including 4 pairs of lumbar neurons (PHA, PHB, PVN & PHC) in the tail of the animal. Using indirect immunofluorescence on wholemount squashes of the nematode with antibodies to HRP, we have screened more than 100 uncoordinated mutants of C. elegans. Mutants in 7 genes(unc-13, unc-33, unc-44, unc-51, unc-61, unc-71, unc-73, unc-98, and unc-106), show abnormal growth and guidance of postembryonic lumbar neurons PVN and PHC. A variety of axon guidance errors were observed in a fraction of the mutant animals. In most cases, the stained lumbar axons grew in abnormal lateral positions instead of running along the ventral nerve cord. Occasionally, these abnormal axons reached the ventral cord further anterior than in wild type and bifurcated randomly. Morphologies of the other two embryonic lumbar neurons(PHA/PHB) were determined previously by Hedgecock et al.(Develop.Biol.1985). This allows the axon growth mutants to be classified in 4 categories based on whether the mutant gene affects embryonic(PHA/PHB), the post-embryonic (PVN & PHC) lumbar neurons, or both; as summarized below.

Class	Embryonic neurons PHA&PHB (FITC uptake)	Post-embryonic neurons PVN&PHC (anti-HRP)
1. unc-106	misdirected	misdirected
2. unc-33, unc-44, unc-51	premature termination	misdirected
3. unc-13, unc-61, unc-73, unc-98	normal	misdirected
4. unc-76, unc-107	premature termination	normal

Interestingly, mutant unc-13 is resistant to Aldicarb, an inhibitor of AChE (J. Culotti) and the mutant unc-98 has a known defect in the body musculature(H. Epstein et al.)

(Supported by grants from March of Dimes and N.I.H.)

102.9 HOW THE FLY'S EYE FINDS THE FLY'S BRAIN. D.F. Ready, J.M. Goldsmith* and J.L. Ecker*. Department of Biology, Princeton University, Princeton, N.J. 08544.

To find their appropriate central targets, sensory neurons first have to find the CNS. We have used scanning electron microscopy of dis-sected *Drosophila* embryos to trace the development of the connection between the fruit fly eye and brain.

Nine hours after fertilization, the non-optic portion of the embryonic fly brain is a bilateral mass of neuroblasts that have budded from the dorsal and lateral head ectoderm. Optic brain development begins at about 9 hours with an infolding of the embryonic ectoderm along the posterior of the underlying brain (Poulson,D.F., in Biology of Drosophila, (M. Demerec, ed.), 1950; Turner, F.R. and Mahowald, A.P., Devel. Biol. 68:96-109, 1979). This ectodermal inpocketing fuses with the brain and later produces neuroblasts which will populate the optic lobes . Closure of the mouth of the optic lobe inpocketing erases its external opening, but preserves the connection between the ectoderm and the CNS. At about 10 hours, the brain begins to move posteriorly into the embryo, and the overlying head ectoderm is peeled away from the brain. At the site of contact between the ectoderm and optic lobe a thin cellular bridge, the optic stalk, is drawn out. As the brain continues its rearward migration, head ectoderm is rolled off the lateral face of the brain and the stalk is drawn foward over the surface of the brain. The trajectory of the stalk leads to a second region of adherence between the ectoderm and the front of the brain. Further backward migration of the brain drags a pocket out of the ectoderm at this adhesion site. This pocket is the future retinal epithelium, and is continuous with the future optic lobe via the optic stalk. Subsequent development retains this cellular bridge.

The precursors of the eye and optic lobe of *Drosophila* are thus connected from the outset on the two-dimensional ectodermal sheet of the embryo. This connectedness is preserved throughout the complex foldings of embryogenesis and subsequent development via the optic stalk. Consequently, axons of photoreceptors generated four days later in the retinal epithelium are directed to their appropriate central targets through this remnant of the ectodermal surface.

102.10 TRANSIENT EXPRESSION OF ANTIGENS IN THE PRIMARY OPTIC PATHWAYS OF THE FETAL MOUSE. M.A. Edwards and M. Yamamoto. Southard Lab., E.K. Shriver Ctr. for Mental Retardation, Inc., Waltham,MA 02154

The elongation of axons to their targets and the elaboration of terminal ramifications within them have been proposed to comprise two distinct modes of axonal growth that are under differential regulatory control (Jhaveri, Edwards & Schneider, Neurosci. Abst. 9, 702, '83). One way to begin to evaluate this hypothesis is to look for clear differences in the molecular species associated with axons or their substrates during the two phases of growth. Monoclonal antibodies (MAb's) were generated with conventional hybridoma methods following immunization of pigmented mice with fetal rat forebrain homogenates, and patterns of immunocytochemical staining of two such MAb's were assessed in serial cryostat sections of the fetal mouse primary visual system (fixation on slide with paraformaldehyde or acetone; HRP-coupled secondary antibody; DAB chromogen). The MAb 7C7 recognizes the disialo-ganglioside, GD3, as well two minor species of uncharacterized gangliosides (Yamamoto & Schwarting, in preparation); the other MAb, 4D7, recognizes two protein species of 110 and 140kD (Yamamoto et al, Neurosci. Abst.,'85). For comparison, alternate sections were stained with C2, a MAb recognizing a neurofilament component (op. cit.), thereby revealing the pattern of axonal outgrowth. A leading front of optic axon fascicles extends into the ventral peripheral optic stalk by E12, through the chiasm by E13, and along the optic tract into the superior colliculus by E15. Over the same interval, axons are recruited to the efferent outflow from a progressively greater proportion of the retinal surface, proceeding in a dorsocentral to peripheral progression. The MAb 4D7 selectively stains optic axons (and a few other CNS fiber systems) in the interval from E12 to E15. Subsequently, such staining diminishes and becomes undetectable by birth (E19). The MAb 7C7 similarly recognizes a determinant transiently associated with optic axons in the fetal period (along with numerous other CNS fiber systems). Against a background of diffuse perisomatic staining present in most CNS regions, dense immunoreactivity also occupies presumptive optic axonal pathways in advance of the arrival of the axons. As early as E12, 7C7 immunoreactivity delineates the marginal zones of the entire vitreal surface of the retina, the ventral optic stalk, the chiasmatic region, the lateral diencephalon, and the superior colliculus. As optic axons advance and accumulate on subsequent days, the dense staining appears to become associated with the axon fascicles themselves. From E15 to birth, staining in most regions of the CNS disappears, including the visual system. These results suggest a potential role for particular antigens in regulating optic axonal growth selectively during elongation to their targets. (Supported by EY404549 and HD05515).

102.11 AXONAL REGENERATION AND SPROUTING AFTER DAMAGE TO THE HEART. Ron Maron*, (SPON: U.J. McMahan). Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

The interatrial septum of the frog's heart is bounded on each of its two surfaces by endothelium and it contains scattered cardiac muscle fibers, the parasympathetic neurons that innervate them, and connective tissue cells. It is transparent so that the fine details of the cells can be clearly seen in a living isolated preparation. Individual post-ganglionic axons can be traced several hundred micrometers from their nerve cell bodies to where they make contact with cardiac muscle fibers. As in mammalian hearts, the terminal region of the axons consists of series of varicosities which lie at varying distances from the muscle fibers. The varicosities are probably transmitter release sites. Unlike axon terminals in skeletal muscles, the postganglionic axons in the septum have no Schwann cell covering. Indeed the axons directly face the extracellular matrix (McMahan and Kuffler, 1971).

Here, I describe the sequence of events following axonal damage which heretofore has been unknown for heart. Many of these events differ from those that occur after damage to motor axons in skeletal muscles. I observed these events in organ cultures of the septum by time-lapse videomicroscopy. In each preparation I crushed only a single parasympathetic axon. The following account is based on 5 preparations; all events were detected in each.

1) Within minutes after crushing a postganglionic axon over a length of 10-20 μm, the axonal continuity in that stretch was completely interrupted. 2) By 2 days after damage, one to three connective tissue cells migrated into the region of damage and hovered over the damaged stretch of the axon. 3) Beginning at about 5 day, the connective tissue cells left the site of damage and the portion of the axon that remained connected to the cell body began to elongate. The elongation was followed for several days. It covered several hundred microns. 4) The direction of the elongation did not follow the course of the severed segment of axon, but rather, it appeared to be random. The axon did not necessarily establish contact with the muscle fibers it originally innervated. 5) The regenerating axon formed varicosities before they reached myofibers. 6) Undamaged axons in the vicinity of the crush began to sprout processes at the same time the damaged axon began to regenerate. The regrowth of the crushed axon, the sprouting of nearby undamaged axons and the accumulation of the connective tissue cells was directly related to axonal damage; in all of several preparations crushing a connective tissue cell near a group of axons did not cause the axons to sprout or induce the accumulation of other connective tissue cells. Supported by NIH grant NS 14506 and a Weizmann Postdoctoral Fellowship.

102.12 An Integral Membrane Glycoprotein Complex that Participates In Axonal Guidance And Organization Of The Neuromuscular Junction. D. Bozyczko* and A. Horwitz* (SPON: R. Hogue-Angeletti). Dept. of Bioch. and Biophys. Univ. of Penn., School of Med., Phila, PA 19104.

We have used the CSAT monoclonal antibody which is directed against a laminin and fibronectin receptor to elucidate its role in axonal extension, guidance, and synaptogenesis. This antibody disturbs the adhesion of several classes of fibroblasts and muscle (Decker, et al. JCB (1984) 99: 1398-1404). Two peripheral nervous systems have been investigated, the dorsal root and ciliary ganglia. Both respond similarly. Neurons plated in the presence of CSAT do not adhere to the substratum and process formation is inhibited completely for at least 24-48 hours. When neurons are first allowed to extend processes, prior to CSAT addition, the results depend on the particular substrate. With some substrates neurites fasiculate and detach from the substratum, whereas with others, they retract and regrow to form bundles of processes. The antigen has been localized, using immunofluorescence, on the cell bodies, axons and growth cones. This distribution correlates with its biological effects on all parts of the neuron. The affinity purified antigen from fibroblasts, muscle, and peripheral nerve is very similar from all sources. It is a 110-160 kd integral membrane glycoprotein complex.

Using the CSAT monoclonal antibody and a polyclonal antisera directed against the affinity purified antigen we have also localized the antigen to concentrations of AchR hot spots and neuromuscular junction sites. On chick myotubes treated with brain extract, the antigen co-localizes with AchR clusters and laminin, as visualized with TMR-BGT and a monoclonal antibody against chick laminin, respectively. The antigen is also concentrated in regions of high AchR density on sections of post-hatch anterior latissimus dorsi.

Our observations implicate the CSAT antigen in the organization of AchR clusters at the neuromuscular junction and in axonal extension and guidance. With respect to the former, the observations presented here along with those on fibroblasts, where the antigen co-localizes with cytoskeletal and extracellular matrix components, suggests that the CSAT antigen contributes to the organization of the neuromuscular junction as a transmembrane link between the cytoskeleton and the basal lamina. In axonal migration, the antigen contributes not only to extension but to fasiculation as well.

103.1 THE USE OF THE VOLTERRA SERIES EXPANSION TO CHARACTERIZE DIRECTION SELECTIVITY. N.M. Gryzwacz* and C. Koch (SPON.: B. Dawson). The Center for Biological Information Processing, M.I.T., Cambridge MA, 02139.

Recently, psychophysicists have formulated different models of human motion perception. These models can be considered as arising from the simplest type of directional system, based on quadratic (2nd-order) interactions between neighboring channels and which are best described by a Volterra series expansion. Such an approach was pioneered by Reichardt and Poggio for the optomotor response of the fly. Moreover, it was shown that a localized interaction between excitatory and inhibitatory synapses, with a reversal potential close to the resting potential of the cell, in the dendritic tree may be the biophysical mechanism implementing these quadratic systems (Koch, Poggio, and Torre, 1982). We will discuss three properties of 2nd-order-models relevant for directional selectivity, comment on their physiological significance for the underlying circuitry and biophysical mechanisms and propose some critical experiments to test these properties. Phase invariance: the time averaged response of a 2nd-order system to a combination of several moving sinusoidal waves does not depend on the relative phase between the components. Thus, gratings which appear different but have the same Fourier components will lead to the same response of the motion system, as shown in the fly. Factorization: The response of the system to a single grating can be expressed as the product of a function depending on the spatial wavelength of the stimulus λ and a function only depending on the contrast frequency $\omega=v/\lambda$. Thus, for a fixed λ the normalized dependence of the response in ω is invariant. This property, however, holds only if the spatial and temporal characteristics of the receptive field of the motion system are independent, and if different inputs have similar receptive fields. Temporal behavior: The direction selective component of the response of the 2nd-order system does not depend on time, provided that either the spatial or the temporal filter making up the temporal-spatial receptive field is of the high-pass type.

We will discuss different biophysical schemes compatible with directional selectivity in the vertebrate retina (e.g., nonlinear interaction between synaptic inputs in the dendritic tree of the ganglion or the (starburst) amacrine cell; linear synaptic interaction followed by a synaptic rectification or by a spike threshold) emphasizing physiological experiments to distinguish between these different possibilities.

This research was supported by the Sloan Foundation and ONR.

103.2 SYNAPTIC ORGANIZATION OF ON-OFF DIRECTIONALLY SELECTIVE GANGLION CELLS IN RABBIT RETINA. E. V. Famiglietti. Dept. of Anatomy, Wayne St. Univ. Sch. of Med., Detroit, MI 48201.

Correlative morpho-physiological studies prompted the suggestion that type 1 bistratified cells are the ON-OFF directionally selective ganglion cells of rabbit retina (Famiglietti, E., Invest. Ophthal. 20 (Suppl.): 204, '81), recently confirmed by intracellular staining (Amthor, F. et al., Brain Res. 298: 187, '84). These cells have two dendritic arbors, narrowly stratified (2 um thick substrata), one each in the middle of sublamina a and of sublamina b of the inner plexiform layer (IPL), and are thus optimally positioned to receive both ON and OFF inputs. These are also precisely the same two strata occupied by starburst/cholinergic amacrine cells (Masland, R. and Mills, J., J. Cell Biol. 83: 159, '79; Famiglietti, E., Brain Res. 261: 138, '83). The recurving dendrites give rise to many short (5 to 20 um) branches, covering the plane rather evenly within the dendritic field perimeter. In the present study, Golgi-impregnated type 1 bistratified ganglion cells have been serially sectioned and reconstructed from montages of electron micrographs.

In one example, located about 2 mm ventral to the visual streak, more than 60% of the linear extent of the dendritic tree was reconstructed from > 3,000 electron micrographs. About 20% of the input comes from cone bipolar cells, and the remainder from amacrine cells. Quantitative analysis of the dendrites in sublamina b yielded 449 synapses equally distributed between 2 major dendritic systems originating from opposite poles of the cell body. Amacrine inputs were subdivided when possible into "probable cholinergic/ excitatory" and "probable GABAergic/ inhibitory" based upon ultrastructural criteria, but no more than 10% could be so classified. No input was found upon the cell body or proximal dendrites below the substratum of extensive branching. Inputs were high in density and increased from mainstem to terminal dendritic branches (up to 3.3 per 10 um^2), with concomitant increases in all classes of input. The following conclusions may be drawn: 1. there is no obvious, globally organized asymmetry of inputs in the dendritic tree which might account for the characteristic directionally selective response, 2. the high density of mixed inputs to thin (0.35 um) terminal dendrites maximizes the proximity of excitatory and inhibitory inputs at many points throughout the dendritic tree, in a manner consistent with a model in which local and distributed multiplicative interactions are proposed to underly the mechanism of directional selectivity (Torre, V. and Poggio, T., Proc. Roy. Soc. B 202: 409, '78); 3. to the extent that the model's optimal "on path" placement of inhibitory inputs (Koch, C. et al., P.N.A.S. 80: 2799, '83) is achieved, however, it appears to be a function of profusion of inputs, rather than selective placement during development.

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103.3 THE SHAPE AND DISTRIBUTION OF THE INDOLEAMINE-ACCUMULATING CELLS IN THE RABBIT RETINA. J.H. Sandell and R.H. Masland, Massachusetts General Hospital, Boston MA 02114.

Although indoleamine-accumulating amacrine cells are found in most vertebrates (Ehinger & Floren, 1976), the dendritic shape of these neurons is unknown. We have developed a method which allows systematic injection of these cells with Lucifer Yellow CH; and have used it to examine the individual morphology and dendritic mosaic of the indoleamine-accumulating cells in the rabbit retina.

The indoleamine-accumulating cells were labelled by intravitreal injection of 5,7 dihydroxytryptamine (5,7 DHT, 50 μg, 2 hr survival) followed by whole mount fixation in 2.5% paraformaldehyde/0.01-0.20% glutaraldehyde for 1 hr. 5,7 DHT produced green fluorescence in the sparse catecholamine-accumulating cells (which were not studied further) and yellow fluorescence in the indoleamine-accumulating cells. Lucifer Yellow was injected into the indoleamine-accumulating cells under visual control. Two cell types with distinct morphologies were revealed. Their somas were equally labelled with 5,7 DHT throughout changes in 5,7 DHT concentration, incubation time and fixation parameters.

Type 1 cells have large, irregularly shaped somas with 5-8 primary dendrites that branch sparsely in a radiating pattern. These processes have large distinctive varicosities separated by long regions of smooth dendrite. In vertical sections the dendrites curve down through the inner plexiform layer to form a dense plexus in sublamina 5. The shape of the Type 1 indoleamine-accumulating cell in the rabbit retina resembles that of A17 in the cat. The density of Type 1 cells is ~450/mm^2 in the visual streak and 146/mm^2 in the periphery. The dendritic field diameter averaged 376 μm for 48 Type 1 cells and was unrelated to retinal eccentricity. Thus the coverage factor for these cells decreased from the center to the periphery. This is the opposite of the coverage pattern for the ACh amacrines; and also differs from that of the horizontal cells and most retinal ganglion cells, which maintain uniform coverage at all eccentricities. Presumably the distinct coverage patterns of these cells reflect the different roles they play in retinal circuitry.

The Type 2 indoleamine-accumulating cells have small round somas with many fine dendrites. The dendrites have many small varicosities. Type 2 cells branch in the scleral portion of the inner plexiform layer, and join the Type 1 cells in forming the dense plexus of indoleamine-accumulating dendrites in sublamina 5.

As more cell types are studied, further examples of morphological diversity within homogeneously labelled populations may be observed. While the aldehyde-induced fluorescence of the amine-accumulating cells made them particularly amenable to the injection methods used here, any cells that are visible in the fluorescence microscope could be similarly injected.

103.4 EFFECTS OF DOPAMINE ON FROG GANGLION CELL RESPONSES. D.W. Rickman* and M.C. Citron. Neurology Research, Childrens Hospital of Los Angeles, Los Angeles, CA 90027.

We recorded responses of ON-OFF type (class 3) retinal ganglion cells in the frog, Rana pipens, to a white noise stimulus, and studied changes in the linear component after application of dopamine (DA).

The stimulus consisted of a grid of 16 X 16 square picture elements, each of which was independently modulated by a binary, random signal that changed every 16 msec. The resulting spatiotemporal random stimulus was videotaped and presented on a video monitor.

Extracellular spikes were recorded from an eyecup preparation. The stimulus image was reduced to 1 mm^2 and focused on the retina. Control recordings were made from retinas bathed in Ringers solution. The eyecup was then drained, and 5 ul of 100 uM DA in Ringers was applied. The cell's responses were again recorded, and linear spatiotemporal response properties of the receptive field were determined by first order cross-correlation (Wiener kernel analysis).

Spatiotemporal contour plots of first order kernels were constructed by cross-correlating the luminance values for the 256 spatial positions (16 X 16) of the stimulus with the ganglion cell spikes, binned in 16 msec intervals. These plots provide a comprehensive description of spatial and temporal aspects of the linear component of the receptive field.

Although cells treated with DA showed no spatial changes compared to controls, temporal changes were noted; the peak value of the kernel at the center of the receptive field occurring at a later poststimulus time. When kernels from DA treated cells were used to model the cell's response to a 0.5 sec flash, an increased ON response, compared to control, was found. Furthermore, individual first order kernels from treated cells showed greater correlation of the response with the stimulus, indicating an increase in the stimulus driven component of the response. Analysis of the effects of DA on the nonlinear response components is underway. (RO1 E04711 and KO4 EY00250)

103.5 EFFECTS OF GLYCINE, TAURINE AND MET-ENKAPHALIN ON DIRECTIONALLY SENSITIVE (DS) GANGLION CELLS IN TURTLE RETINA. M.Ariel* and A.R.Adolph, Eye Research Institute, Boston, MA 02114.

We have previously reported that, as in rabbit retina, GABA and ACh are essential elements in a mechanism by which many retinal ganglion cells respond to specific directions of stimulus movement. The pharmacological analysis of DS cells has been extended by studying effects of glycine, taurine, met-enkephalin and their receptor blockers in superfused turtle retinal eyecup.

Application of glycine and taurine totally suppressed the extracellularly recorded spike firing of DS ganglion cells. Millimolar concentrations of these amino acids were equally potent inhibitory agents whose actions were antagonized by micromolar concentrations of strychnine. GABA, on the other hand, was an inhibitory agent whose actions were not blocked by strychnine but were antagonized by picrotoxin. When tested in a low Ca++/EGTA perfusate which blocked synaptic transmission yet did not suppress spike firing, GABA inhibited ACh-induced activity but glycine had no effect. These results indicate that a strychnine-sensitive inhibitory receptor is not pressent on the DS cell membrane but may be elsewhere in pathways to DS cells.

Met-enkephalin and DALA- met-enkaphalinamide application occasionally caused small increases in the light responses of DS cells. Naloxone, a broad-spectrum opiate antagonist, moderately decreased these cells' overall responsiveness. Neither naloxone nor strychnine had any consistent effects on directional tuning of DS cells. Thus the functions of glycine, taurine and met-enkephalin are not yet known and may be revealed by studying other receptive field properties of DS cells. GABA and ACh remain the prime transmitter candidates involved in directional sensitivity. (Supported by EY-03383).

103.6 ANALYSIS OF DENDRITIC STRUCTURE OF CAT RETINAL GANGLION CELLS. Dennis Dacey. Committee on Neurobiology, University of Chicago, Chicago, Ill. 60637.

Previous morphological studies of alpha and beta cat retinal ganglion cells demonstrate a characteristic increase in dendritic field size with increasing distance from the central area of the retina for both of these cell classes. This study examines related changes in the detailed dendritic structure of alpha and beta cells with increases in eccentricity. Small, iontophoretic injections of horseradish peroxidase (HRP) were made directly into the retina and the morphology of HRP filled ganglion cells was studied in cobalt-enhanced, diamino-benzidine-reacted retinal wholemounts.

The dendritic tree of ganglion cells, including alpha and beta cells, exhibits two major components, the dendritic shafts and the dendritic appendages. The dendritic shafts comprise the primary dendrites that extend from the soma, and branch by successive bifurcation. They are principally smooth and tapered but bear a small number of fine spicules and other spine-like extensions that are less than 0.5 um in diameter. The dendritic appendages are short branchlets that arise commonly as thin (less than 0.5 um in diameter) side branches from the dendritic shafts. By contrast with the smooth dendritic shafts, the appendages are irregularly varicose and scalloped and are often recurved.

Changes in these dendritic components with eccentricity were examined by comparing changes in the length and numbers of dendritic shaft segments (a shaft segment was defined as the shaft length between branch points) and appendages at various distances from the area centralis. For both alpha and beta cells, dendritic shaft segments increased in length with increasing distance from the area centralis, accounting for the overall increase in dendritic field size. By contrast, the appendages for both cell classes showed a constant length (mean = 26 μm for alpha cells, 23 μm for beta cells) from 1 to 12 mm eccentricity. The numbers of appendages also remained constant for both cell classes (mean = 163 for alpha cells, 58 for beta cells) from 1 to 12 mm eccentricity. Thus with increasing eccentricity, the mean shaft length increases relative to the mean appendage length, so that central cells appear appendage-dominated while peripheral cells appear shaft-dominated.

It is hypothesized that the shafts and appendages represent functionally distinct components of the dendritic tree of alpha and beta cells.

103.7 CONE INPUTS TO CAT GANGLION CELLS. R.W. Rodieck and J. Dineen*, Department of Ophthalmology, University of Washington, Seattle WA 98195.

The great majority of cat ganglion cells show no spectral antagonism. Though most investigators have found these cells to receive from rods and a single cone type, some have reported that they can occasionally receive from two, three, or even seven spectrally distinct cone types.

We used a raster-based color stimulator to investigate the cone inputs to single X and Y cells in the cat retina. In the paradigm experiment, a blue spot, matched to the center of the receptive field, was flashed, thereby substituting for part of a larger green background. The blue intensity required to give a null response was determined. Neutral filters before the eye reduced this value, indicating a rod contribution. However, bleaching adaptation, or background adaptation outside the blue, which saturated the rod response also caused the null intensity to decrease. A change in cone pigment density would act to increase this value. Background adaptation to monochromatic lights showed that, in addition to the common 'green' cones, the ganglion cells were also receiving from cones having a peak spectral sensitivity consistent with the 'blue' cones that contribute to the rare 'color-coding' cat ganglion cells.

The receptive fields of ten X cells and thirteen Y cells were tested for their cone inputs. Every one showed a contribution of both blue and green cones to the center region. The surround region of ten of these cells were also tested, and all showed an input from both cone types.

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103.8 EVIDENCE FROM PERCEPTION SELECTS AVERAGE LOCAL CONTRAST OVER THE RETINEX THEORY R. Shapley (Spon. Floyd Ratliff)
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The Retinex Theory, originally proposed by Land and McCann (1971), was devised to account for the perception of brightness and color. A quantitative prediction of this theory is that equiluminant targets should appear equally bright, even when they are placed on unequally luminant uniform backgrounds. This Retinex prediction does not agree with the visual perception of normal humans, as I will demonstrate. This particular disconfirmation of Retinex prediction is not a special case. For example, equally luminant regions of so-called "Mondrian" patterns appear to be of different brightness if their average local contrasts are unequal, and appear equally bright if their average local contrasts are equal. These results suggest that (to a first approximation) the brightness of an object depends on the Average Local Contrast around the border of the object - the Theory of Average Local Contrast (TALC).

There is a plausable neurophysiological reason for TALC. I have found that the response amplitudes of retinal ganglion cells in the cat depend on local contrast rather than luminance or reflectance. Thus, the neural signal from the eye to the brain is coded in terms of contrast. Central visual cells combine these retinal contrast-dependent signals in order to compute the brightness of objects in a complex scene. The retinal calculation of contrast is a result of the neural and receptoral mechanism of light adaptation.

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The National Eye Institute

103.9 THE RELATION BETWEEN VISUAL RESOLUTION AND ECCENTRICITY FOR THE GANGLION CELLS OF THE MONKEY RETTINA. B.B. Lee, J.M. Crook* and A. Valberg*. Max Planck Inst. für biophys. Chemie, D-3400 Göttingen, West Germany.

The ability of ganglion cells to resolve fine gratings presumably sets a limit to the visual acuity of the intact animal. We measured the visual resolution shown by monkey retinal ganglion cells of various types as a function of retinal eccentricity. Cell activity was recorded directly from the inner plexiform layer, animals being anaesthetised with halothane and nitrous oxide. Stimulus luminance was 100 cd/m² with a 2 mm artificial pupil. Responses to black-white square-wave gratings of high spatial frequencies with a temporal frequency of 8 Hz were Fourier analysed and the amplitude of the first harmonic plotted against spatial frequency. In neither wavelength-opponent nor phasic cells was there a DC response component at high spatial frequencies. A response of 5 impulses/sec was defined as the resolution limit.

Visual resolving ability of both opponent and phasic cells showed the expected decrease with increasing eccentricity. Blue on-centre cells showed poor resolution. At a given eccentricity, wavelength-opponent (red on-centre, green on-centre) and phasic cells resolved gratings of comparable spatial frequency, despite the larger centre size of the latter cell type. This may be due on the one hand to the high contrast sensitivity of phasic cells and on the other to the poor responsiveness of most opponent cells to achromatic modulation of a stimulus. The results contrast with those from the cat where modulated responses are obtained from sustained cells at spatial frequencies at which neighbouring transient cells show no such modulation.

103.10 SPATIO-TEMPORAL VISION OF MACAQUES WITH SEVERE LOSS OF P_β RETINAL GANGLION CELLS. William H. Merigan and Thomas A. Eskin*, University of Rochester Medical Center, Rochester, NY 14642

Macaque monkeys treated with acrylamide, a neurotoxic chemical, show a pronounced loss of medium-sized (P_β) retinal ganglion cells, but sparing of the larger (P_α) retinal ganglion cells. Combined histologic, HRP transport and cytochrome oxidase studies reveal a deafferentation of parvocellular P_β-recipient layers of the lateral geniculate, but apparently normal input to magnocellular P_α-recipient layers. This preparation has allowed us to examine the visual role of the large cell retinogeniculate pathway in monkeys in the absence of the medium cell pathway. This study was of particular interest because physiological studies show different properties in these two pathways; the medium cell pathway shows higher spatial resolution but lower contrast sensitivity and temporal resolution than the large cell pathway.

Achromatic spatio-temporal vision was studied in two controls and three treated monkeys with standing and counterphase modulated gratings. A pronounced loss of contrast sensitivity (approximately one log unit) was found at all spatial frequencies for standing gratings. However, with moderate to higher rates of temporal modulation, reduced sensitivity was found only for higher spatial frequencies. The peak of the spatio-temporal contrast sensitivity function dropped and shifted from low temporal and middle spatial frequencies to higher temporal and low spatial frequencies.

These results suggest a close correspondence between the physiological and psychophysically measured spatio-temporal properties of large and medium cell visual pathways. However, they also suggest that the very high contrast sensitivities measured psychophysically depend on the medium cell pathway, in which the contrast sensitivity of individual neurons is rather low.

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INTERACTIONS BETWEEN NEUROTRANSMITTERS I

104.1 QUINOLINIC ACID AND TAURINE: INTERACTIONS IN THE BRAIN EXAMINED IN VIVO AND IN VITRO. A.Vezzani, E.D. French, W.O. Whetsell, Jr.[1] and R. Schwarcz, Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228 and [1]Dept. of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232.

Using an in vivo dialysis procedure in the unanesthetized rat, we have shown previously that intrahippocampal injections of 156 nmol of the excitotoxic brain metabolite quinolinic acid (QUIN) results in a rapid and selective increase in extracellular taurine (TAU) levels (Soc. Neurosci. Abst. 10, 11.6, 1984). We have now further investigated the interactions between QUIN and TAU using a spectrum of in vivo and in vitro paradigms.

Measurements of TAU were made in hippocampal dialysates following local co-application of 156 nmol QUIN and 468 nmol kynurenic acid, an antagonist of QUIN's excitotoxic effects. No TAU increases were found to occur under these conditions, indicating that QUIN-induced elevations of extracellular TAU levels were receptor-mediated. The question if the TAU response is related to the early stages of QUIN-induced neuronal degeneration was addressed by inserting a dialysis fiber into the cerebellum, an area which is quite resistant to QUIN-toxicity. Indeed, infusion of 156 nmol QUIN did not alter extracellular TAU levels in this area of the brain.

The mechanism of QUIN-TAU interactions was further explored in vitro. At 10mM, QUIN increased the efflux of ^{3}H-TAU from preloaded hippocampal slices by 56±8%(N=7). No such effect was observed with the non-excitotoxic QUIN-analog 3,5-pyridine dicarboxylic acid. 10mM QUIN did not, however, block the Na^+-dependent uptake of ^{3}H-TAU into hippocampal slices or into synaptosomal P_2 fractions.

Iontophoretic application of QUIN excited hippocampal pyramidal cells. This effect was completely inhibited by concurrently applied TAU.

Since the excitotoxic hypothesis postulates that inhibition of excitation be parallelled by a blockade of neurotoxicity, attempts were made to antagonize QUIN-toxicity by TAU. Intrahippocampal or systemic TAU, administered either acutely or chronically, failed to show such a protective effect in vivo, possibly due to the effective removal of TAU from the extracellular space. However, protection by TAU could be unequivocally demonstrated in organotypic hippocampal cultures (21 days in vitro) using 10^{-3}M QUIN as the degenerative agent and 10^{-3}-10^{-1}M TAU as the antagonist. While the specificity of these anti-excitotoxic actions of TAU with regard to excitatory receptor types remains to be examined, the possible role of TAU as an endogenous modulator of excitotoxic phenomena, which may take place in neurologic and/or psychiatric disorders, deserves further attention.

This work was supported by USPHS grants NS 16102 and 20509.

104.2 VASOPRESSIN-INDUCED NEURONAL MECHANISMS IN THE HIPPOCAMPUS. R.E. Brinton and B.S. McEwen Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

Vasopressin (AVP) is postulated to be a neuromodulator in the CNS and to influence psychological processes associated with the hippocampus. Discretely localized receptors for AVP have been detected in specific hippocampal cellular laminae (Brinton et al., PNAS, 1984; Biegon et al., NS. Lett., 1984). The purpose of our ongoing studies is to explore and define biochemically the neuronal mechanisms induced by AVP. These studies could lead to insights into how AVP modulates neuronal responses in the hippocampus and provide data relevant to adrenergic function and memory formation.

Initially we undertook experiments to characterize the dose response for AVP potentiation of adrenergic (NA) stimulated c-AMP accumulation, as originally reported by Church (Peptides,1983), in hippocampal slices from rat brain. We found a dose dependent AVP potentiation characterized by a bell-shaped dose response curve. Low concentrations of AVP, 5, 10, 25, and 50 nM, had no effect on NA stimulated c-AMP. 100 nM AVP showed a 30% increase over control while 250 nM AVP showed a maximal potentiation of 52%. At 500 nM and 1 uM AVP potentiation declined in magnitude to 33% and 20% over control respectively.

This potentiating effect was not shared by oxytocin at doses of 10, 100 nM or 1 uM and thus appears to be specific for the neurohypophyseal peptide AVP. The behaviorally active potent metabolite peptide AVP_{4-9} was ineffective at 10 and 100 nM in potentiating NA stimulated c-AMP. Interestingly, in autoradiography we found a distribution of AVP_{4-9} receptors within the hippocampus which was remarkably distinct from that of the parent peptide (Brinton et al., Life Sci. 1985).

Lastly we asked whether the AVP vasopressor antagonists, $d(CH_2)_5$tyr(Me)AVP, dPVAVP, and dEt_2VAVP, would block AVP potentiation of NA stimulated c-AMP. At an agonist concentration of 250 nM AVP and an antagonist concentration of 1 uM, approximately comparable to the agonist/antagonist ratio used in behavioral studies (Koob et al.Reg. Pep., 1981), the vasopressor antagonists did not block AVP potentiation and appeared to have alight agonist properties.

Studies are currently in progress to explore the mechanism by which AVP modulates NA stimulated c-AMP in the hippocampus.

The authors are very grateful to Dr. Maurice Manning for his generous gift of vasopressor antagonists. This research is supported by a Postdoctoral Research Fellowship Award (MH-09224) to R.E.B. from the NIMH.

104.3 CHOLECYSTOKININ AND NEUROTENSIN DURING INTRACRANIAL SELF-STIMULATION AFTER SYSTEMIC AND INTRACEREBROVENTRICULAR INJECTIONS: EFFECT OF SUBDIAPHRAGMATIC VAGOTOMY. Ph. De Witte and J.J. Vanderhaeghen. Lab. of Psychobiology, Université Catholique de Louvain, 1 place Croix du Sud, 1348, Louvain-La-Neuve and Lab. of Neuropathology and Neuropeptides Research, Université Libre de Bruxelles, Erasme Hospital, 808 route de Lennik, 1070 Bruxelles, Belgium.

Dopamine nerve fibers in the nucleus accumbens have been implicated in the mechanisms reponsable for intracranial self stimulation (ICSS). ICSS has been a fruitfull tool to study the action of several drugs having dopamine agonist (amphetamine, apomorphine) or antagonist (neuroleptics) properties. Since cholecystokinin (CCK) and neurotensin (NT) have been reported to be either, closely related to, or even, colocalized with dopamine in some nerve fibers of the mesolimbic system, we have thus investigated the relationship between dopamine, CCK and NT in that ICSS model. Rats with chronic electrodes implanted into the posterolateral hypothalamus were able to self stimulate during sixthy minute session. ICSS was estimated quantitatively by computing the rate of bar pressings for brain reinforcing electrical stimulation every five minute. CCK and NT were injected using the intraperitoneal (IP) or the intracerebroventricular (ICV) route. Given ICV, CCK and NT were effective at the dose of 100 picomole per kilo. Given intraperitoneally, NT was effective at the dose of 4 nanomole, i.e. five time more effective than CCK which work at the dose of 20 nanomole per kilo. With both peptides we observe decrease of ICSS immediately after ICV injection, only after five minute after IP injection. We observe this decreasing ICSS effect during approximately 20 minute. The potentiation of ICSS induced by 0.64 mg per kilo of d-amphetamine was reduced also for 20 minute by CCK given either IP or ICV. The fact that CCK and NT are respectively 200 and 50 time more potent using the ICV instead of the IP route, strongly suggests a central action for these peptides in ICSS modulation. It has been reported that the satiety effect on the feeding behaviour induced by CCK was mostly mediated by the periphery, since it disappeared after bilateral vagotomy. Quite the contrary eigthy per cent of the decrease of ICSS after CCK injection remains after the bilateral subdiaphragmatic vagotomy.

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104.4 HYPOLOCOMOTION INDUCED BY ACTIVATION OF DOPAMINE AUTORECEPTORS: EFFECTS OF NEUROTENSIN. S. T. Caln and C. B. Nemeroff. Departments of Psychiatry and Pharmacology, Duke University Medical Center, Durham, N. C. 27710.

The brain-gut tridecapeptide, neurotensin (NT) has a number of seemingly anomalous effects on dopaminergic neurotransmission. For example, centrally-administered NT inhibits amphetamine-induced hyperactivity (Nature, 291:73, 1981). In contrast, NT increases dopamine release from slices of striatum and nucleus accumbens (Eur. J. Pharmacol., 93:27, 1983). To explain these disparate effects, we have proposed that NT may antagonize the effect of dopamine (DA) activation of both postsynaptic and presynaptic receptors. As one test of this hypothesis, we have undertaken a series of studies designed to test the effect of NT on the hypolocomotion resulting from dopaminergic autoreceptor activation.

To determine the optimal conditions for observing a possible behavioral interaction between NT and DA autoreceptors, we first monitored the effect of autoreceptor activation on locomotor activity during both the light and dark phases of the circadian cycle. Either apomorphine (.048 mg/kg) or N-n-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP, 8.5 mg/kg) were administered IP and locomotor activity measured for 30 minutes in photocell cages. During the light cycle, apomorphine decreased ambulatory activity by approximately 40%. During the dark phase of the cycle, both apomorphine and 3-PPP decreased ambulatory activity by approximately 60%. To investigate the potential effects of NT on this hypolocomotion, we therefore conducted experiments during the dark phase of the cycle.

After the rats were lightly anesthetized with ether, NT (30 µg/10 µl) or saline was injected intracisternally (IC). Thirty min. later, the animals were injected IP with 3-PPP and locomotor activity monitored for 30 minutes. This protocol was chosen because the time period between 30 and 60 minutes post IC NT is the time of maximal increases in the concentrations of the dopamine metabolites DOPAC and HVA (J. Pharmacol. Exp. Ther., 222:1, 1982). There was an almost 80% decrease in locomotor activity in animals injected with saline IC and 3-PPP IP relative to animals with saline both IC and IP. NT did not significantly alter the hypolocomotion induced by 3-PPP. Because of the reported ability of NT to reduce the binding affinity of the dopamine agonist N-propylnorapomorphine (NNPA) (Acta Physiol. Scand., 119:459, 1983), studies are in progress to determine the behavioral and biochemical sequelae of combined NT/NNPA treatment. (Supported by NIMH MH-39415)

104.5 ROLE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) IN STRIATUM AND CULTURED STRIATAL NEURONS: RELATIONSHIP TO GABA, SOMATOSTATIN AND DOPAMINE. M.H. Makman*, J.A. Kessler, L.L. Brown, J.F. Cubells, L. Thal* and L.I. Wolfson. (SPON: E.L. Gardner). Departments of Biochemistry, Molecular Pharmacology, Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Previous studies of VIP and dopamine (DA) stimulated adenylate cyclases in adult rat striatum and in fetal rat striatal neurons cultured in vitro (Soc. Neurosci. Abstr. 10:762, 1984; Regulatory Peptides, 6:317, 1983), suggest that VIP and DA (at D_1 receptors) have partly similar and partly different functions in striatum. In the present study synaptosomes from adult striatum or cultures of intact striatal neurons derived from dissociated striatum of 17 day old rat embryos were first labeled with ^{3}H-glutamate. The preparations were washed and release of newly formed ^{3}H-GABA into the medium was measured. VIP (10 uM) stimulated the release of ^{3}H-GABA from both adult synaptosomes and fetal neurons. DA (10 uM) also stimulated ^{3}H-GABA release. In addition, both VIP and DA stimulated release of somatostatin immunoreactivity (SIR) from adult striatal synaptosomes. In preliminary studies forskolin and 8-thiomethyl cyclic AMP had no effect on release of either ^{3}H-GABA or SIR; hence these effects of VIP and DA may not be due to stimulation of AC. Injection in vivo of VIP (12.5 pmole/.25 ul) into a cannula chronically implanted in the dorsal striatum did not elicit rotational behavior. However, injection of VIP but not saline 10 min before intracannula injection of carbachol (1 ug/.25 ul) inhibited 78% of the contraversive rotational effect of carbachol. The rotational effects of carbachol and DA agonists acting at D_2 receptors in the dorsal striatum are qualitatively the same. We propose that VIP and possibly also DA at D_1 receptors antagonize striatal production of movement (rotation) by stimulation of GABA and somatostatin release from striatal interneurons. GABA and somatostatin may be co-localized in these neurons. Alternatively, VIP might release GABA from collaterals of neurons projecting to globus pallidus or substantia nigra. In preliminary studies ^{3}H-GABA release from nigral synaptosomes was also stimulated by VIP, but to a lesser extent than by DA. The ability of VIP to stimulate GABA release from fetal striatal neurons suggests a role for VIP-GABA interaction in striatum during development as well as in the adult (USPHS NS-09649, NS-20013, AG-00374 and GM-07260.)

104.6 RECEPTOR MEDIATED, Ca^{2+}-DEPENDENT RELEASE OF TYROSINE FROM BRAIN SLICES BY MET-ENKEPHALIN AND OTHER NEUROPEPTIDES. S.P. Arnerić, M.P. Meeley, M. Honig* and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We sought to establish whether the neuropeptide met-enkephalin (m-ENK) modulates the release of specific endogenous amino acids (AA) from slices of rat corpus striatum (CS), which contains very high levels of m-ENK (Neuropharmacol. 16:303, 1977). Slices (0.3-0.5 mm) of CS and, for comparison, cerbral cortex (Cx) were prepared from male rats and incubated with Kreb's bicarbonate buffer containing bacitracin (10uM) and physostigmine (100uM). The release of endogenous Tyr, GABA, Asp, Glu, Gly, Tau, Phe and Val was measured using HPLC.

Incubating m-ENK (1 uM) with slices of CS, but not Cx, increased not only the basal release of the putative transmitters Glu (+155%), Tau (+80%) and Gly (+50%), but also, surprisingly, Tyr (+81%) ($p < 0.05$; n=5-6). Facilitation of the basal release of Tyr, Glu, Tau and Gly by m-ENK was Ca^{2+}-dependent, and abolished in the presence of naloxone (1uM), an opiate receptor antagonist which, by itself, had no effect on the release of any of the AAs measured.

The increase in basal release of Tyr in slices of CS was dependent on the concentration of m-ENK present (0.1-10uM): release increased by 125% with 10uM m-ENK ($p < 0.001$; n=5). In contrast, depolarization with potassium (5-55mM) did not affect the release of Tyr in CS. The release of Val and Phe was not affected by incubation with m-ENK (10uM), or by depolarization with potassium (5-55 mM). However, the potassium-evoked release of Glu, Gly, GABA and Asp was both graded (5-55mM K^+) and Ca^{2+}-dependent. For all of the AAs measured, evoked release in the presence of 35mM K^+ was not altered by m-ENK (1 uM).

To determine the specificity of the m-ENK facilitation of Tyr release we examined the effect of three other peptides (1uM) in CS and Cx: cholecystokinin octapeptide (CCK-8), thyrotropin releasing hormone (TRH) and vasoactive intestinal peptide (VIP). Values are the mean % change from basal release of Tyr; * $p < 0.05$.

Treatment	m-ENK	CCK-8	TRH	VIP
Corpus Striatum	+81*	+49*	+44*	-7
Cerebral Cortex	+25	+18	+10	+69*

We conclude: a) Ca^{2+}-dependent, receptor-mediated release of Tyr is elicited by m-ENK in slices of CS, but not CX. b) CCK-8, TRH and VIP also increase basal release of Tyr in a regionally specific manner. c) Although the source of Tyr released in CS is unknown, in some brain areas it is conceivable that this amino acid functions as a transmitter. d) Alternatively, since it is a substrate for catecholamine biosynthesis, receptor-mediated release of Tyr may be a mechanism by which peptides modulate neurotransmitter synthesis.

104.7 SOMATOSTATIN-14 DIRECTLY DEPRESSES NEURONAL DISCHARGE BUT ENHANCES EXCITATORY RESPONSES TO ACETYLCHOLINE IN THE RAT CORTEX AND HIPPOCAMPUS J.R. Mancillas, G.R. Siggins and F.E. Bloom. Scripps Clinic and Res. Fdn, La Jolla, CA 92037

Interactions between somatostatin 14 (SS-14) and acetylcholine (ACh) were studied in areas CA1 and CA3 of the dorsal hippocampus, and in the parietal cortex, all areas which receive SS-14 and ACh innervation.

The activity of single identified CA1 and CA3 hippocampal pyramidal cells was recorded with five-barrel micropipettes, in rats anesthetized with halothane (0.75-1%). ACh (0.1 M, pH 4), Glutamate (GLU) (0.1 M, pH 8) and scopolamine (.002 M, pH 5) were applied by microiontophoresis, whereas SS-14 (3 mM, pH 6.5) was applied by electroosmosis. Twenty cortical, sixteen CA1 and fourteen CA3 pyramidal cells were studied.

SS-14, applied alone, had inhibitory effects on the spontaneous firing of 90% of all pyramidal cells tested, had no effect on 6% and a biphasic effect on 4%. No excitatory effects of SS-14 on pyramidal cells were observed in the absence of ACh in the drug barrels. SS-14 inhibitions displayed an extended time course, with a slow onset and the duration of the effect oulasting the pulse duration. ACh, on the other hand, produced facilitation of firing, with a much faster time course. When short pulses of ACh were applied within a sustained period of exposure to SS-14, the effects were dose-dependent. At small doses, SS-14 depressed the basal firing between ACh pulses, but did not affect the magnitude of ACh-induced excitations. At higher doses of SS-14, we observed an increased responsiveness in 78% of the test neurons to the ACh pulses (no effect in 22%), with much larger (up to 10-fold higher) and somewhat longer excitatory responses to ACh. At still higher SS-14 currents, cells initially showed an enhancement of responses to ACh followed by depression of basal firing and ACh responses. There were no such synergistic interactions between SS-14 and glutamate.

Pulses of SS-14, applied to a cell tonically driven by small (leak) amounts of ACh, caused an increase in neuronal firing by phasically "enabling" the ACh-induced tonic discharge. These increases in neuronal firing were blocked by scopolamine. Thus, iontophoretic application of SS-14 can yield qualitatively different effects on target cell activity depending on other presynaptic inputs or the presence of other chemical messengers. Such findings could explain the diverse neuronal effects of SS-14 reported in the literature. Supported by an APS fellowship to J.R.M., the USPHS (AA 06420 and AM 26741).

104.8 EVIDENCE SUPPORTING A ROLE OF ENDOGENOUS INTRA-NUCLEUS ACCUMBENS SUBSTANCE-P IN D-AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY: USE OF A MONOCLONAL ANTIBODY AGAINST SUBSTANCE-P.

P.J. Elliott, C.D. Kilts, and C.B. Nemeroff (Spon: T. Slotkin). Depts. of Psychiat. and Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Substance-P (SP) is found in high concentration in proximity to dopamine (DA) cell bodies in the ventral mesencephalon. In addition, SP binding sites have been identified in the terminal regions of DA pathways originating from the A-10 DA cell body group in the ventral tegmental area (VTA). Infusion of SP into the nucleus accumbens (NAS), a mesolimbic terminal area, has been reported to produce an increase in DA metabolism in this nucleus and to potentiate the locomotor hyperactivity of simultaneously infused DA (Kalivas and Miller, 1984, Neurosci. Lett., 48, 55-59). Electrical, drug and peptide stimulation of the VTA can all mimick these effects suggesting VTA-NAS dopamine system activation. However, 6-hydroxydopamine lesions of the VTA do not block the locomotor activity induced by infusions of SP into the lateral ventricles (Elliott and Iversen, unpublished observations). Thus it would appear that other neuronal mechanisms are involved in these behavioral and biochemical events. In the present experiments the effects of intra-NAS infusions of a monoclonal antibody directed against SP (Sera-Lab; NCL/34) on d-amphetamine-induced locomotor activity were investigated. Use of this antibody is preferential to the SP antagonists currently available because they have been found to be toxicand rather non-selective. Furthermore this antibody has been previously used successfully to demonstrate a role for SP in modulating meso-cortical DA neurons (Bannon et al., 1983, Nature, 306, 791-792). Adult male Sprague-Dawley rats with bilateral cannulae aimed at the NAS were infused with the SP antibody or a control antibody (Sera-Lab; rat monoclonal against mouse IgG) in a 2 µl volume/side. The effect on i.p. amphetamine (1.0 mg/kg) was investigated in photocell cages (Columbus Inst., Ohio) over a 90 min test period. Subjects receiving the SP antibody (n=9) exhibited a 40% decrease in d-amphetamine-induced locomotor activity when compared to rats infused with the control antibody (n=6). These findings suggest a role for endogenous intra-NAS substance-P in the locomotor activity induced by d-amphetamine. The effects of intra-NAS SP antibody on DA metabolism in this nucleus are currently under study.

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104.9 SUPRACHIASMATIC NUCLEUS SYNAPTIC RELATIONSHIPS: A DOUBLE ULTRASTRUCTURAL IMMUNOCYTOCHEMISTRY STUDY WITH PEROXIDASE AND GOLD SUBSTITUTED SILVER-PEROXIDASE. A. N. van den Pol and T.J.Gorcs*. Section of Neurosurgery, Yale Univ.Med.Sch., New Haven, Ct. 06510 and Dept.Physiol., UNC, Chapel Hill, NC 27514.

To investigate the neurotransmitter content of both the presynaptic and post-synaptic structures in the hypothalamic suprachiasmatic nucleus (SCN), the use of a double ultrastructural immunocytochemical analysis was tested. Several permutations of antisera against different putative neuroactive substances found in both cell bodies and axonal endings in the SCN were used; antigens studied included gastrin releasing peptide (GRP), vasoactive intestinal polypeptide, vasopressin-neurophysin, and the GABA synthesizing enzyme glutamate decarboxylase (GAD). The first antigen was stained with gold substituted for silver catalytically precipitated on polymerized diaminobenzidine. This method of immunostaining gave a punctate gold label distrubuted diffusely through the cytoplasm of immunoreactive cells; it also increased the sensitivity of the diaminobenzidine (DAB) reaction several fold and increased antigen detectability with both light and electron microscopy. The sensitivity of the gold substituted silver staining on HRP blots compares favorably with the use of tetramethylbenzidine in the detection of HRP; unlike TMB, the final gold immunolabel is stable in a wide range of pH and buffers. With ultrastructural analysis the metalic particles ranged in size up to 80 nm or more. The second antigen was labeled with HRP and DAB as the substrate, giving a non-granular reaction product.

GRP immunoreactive cells, dendrites, and axons labeled with gold substituted silver particles were found within the SCN, with the majority of immunoreactive profiles concentrated in the ventral part of the nucleus. Gold substituted silver particles were located only in immunoreactive neurons, and background metalic labeling was absent. GAD and GABA immunoreactive axons occurred in large numbers throughout the SCN, frequently making synaptic contact with dendrites and somata. Pre-synaptic GABA boutons had small clear vesicles, an occasional dense core vesicle, and a symmetrical contact zone. GRP immunoreactive axons contained both small clear vesicles and larger dense core vesicles, and made synaptic contact with unlabeled dendrites. GAD immunoreactive axons made synaptic contact with GRP immunoreactive cells and dendrites. Similarly, GAD immunoreactive axons made synaptic contact with vasopressin-neurophysin immunoreactive profiles within the SCN, mostly in the dorsomedial part of the SCN. GABAergic axons may play an important role in mediation of activity of both GRP- and vasopressin- containing SCN neurons.

(Supported by NIH NS16296 and 10174.)

104.10 TWO-COLOR IMMUNOFLUORESCENCE COMBINED WITH A FLUORESCENT RETROGRADE TRACER TO DEMONSTRATE POSSIBLE AFFERENTS TO DESCENDING MONOAMINERGIC NEURONS. M.W. Wessendorf and R.P. Elde. Dept. Anatomy, Univ. Minnesota, Minneapolis, MN 55455

The spinally-projecting monoaminergic neurons of the medulla have been implicated in the modulation of sensory, motor, and autonomic functions. In the present study, two-color immunofluorescence (Wessendorf and Elde, J. Histochem. Cytochem., in press) has been used to identify putative transmitters which might affect the activity of serotonergic (5HT) and catecholaminergic neurons. Additionally, fluorescence retrograde tracing has been used to identify possible afferents to spinally-projecting monoaminergic neurons.

The spinal cords of rats were hemisected at C1-C2 and a pleget of gelfoam soaked in 1% fast blue (FB) was inserted. After a survival time of 2-4 days, 5HT levels were elevated by the administration of 300 mg/kg tryptophan and 60 mg/kg tranylcypromine to make 5HT cells visible. Animals were then killed by perfusion fixation with Zamboni fixative. Ten micron sections were cut and stained for the simultaneous demonstration of 5HT-immunoreactivity (5HT-IR) and immunoreactivity for either enkephalin (ENK-IR), dynorphin 1-8 (DYN-IR), substance P (SP-IR), or the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH-IR). The protocol was such that 5HT-IR fluoresced red, and the other substances fluoresced green.

Green-fluorescing SP-IR fibers were found in apposition to red-fluorescing 5HT-IR cells in all areas where the latter were found, but were especially prominent in n. paragigantocellularis lateralis and n. raphe pallidus. The same pattern was also seen for ENK-, DYN-, and TH-IR. In addition, TH-IR fibers were also frequently found in close proximity to large calibre 5HT-IR processes, presumably dendrites, in n. gigantocellularis pars alpha. Conversely, 5HT-IR fibers were found in apposition to TH-IR cell bodies and dendrites in the A1 cell group.

In rats in which FB was administered, 5HT-IR somata labeled with FB were frequently observed. Fibers positive for ENK-, DYN-, SP-, and TH-IR were found in apposition to these cells in n. raphe magnus, n. paragigantocellularis lateralis, and n. gigantocellularis pars alpha. 5HT-IR fibers were also found in apposition to TH-IR, FB-labeled cells in the A1 catecholamine cell group.

These data suggest that the activity of descending serotonergic neurons could be modulated by a number of different neurotransmitter systems. They also suggest the presence of an anatomical substrate allowing interaction between 5HT neurons and descending catecholamine neurons.

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104.11 RESPONSES OF SMOOTH MUSCLE STRIPS FROM PENILE ERECTILE TISSUE TO DRUGS AND TRANSMURAL NERVE STIMULATION. W.G. Dail, L. McGuffee*, N. Minorsky*, and S. Little*. Departments of Anatomy and Pharmacology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

Histochemical studies of penile erectile tissue reveal acetylcholinesterase positive fibers, a dense adrenergic plexus and fibers which are immunoreactive for vasoactive intestinal polypeptide (VIP). Acetylcholine has long been regarded as the inhibitory substance which effects relaxation. However, contradictory evidence exists for acetylcholine and VIP, the more recent candidate for inhibition of penile smooth muscle. The present study examines the effects of putative neurotransmitter substances on the penile erectile tissue of the rat since more information is available on the chemical neuroanatomy of the erectile tissue in this species than in other animal models. Mechanical responses of smooth muscle strips from the penile crura were measured by a force-displacement transducer coupled to a polygraph. Norepinephrine (1 X 10^{-7} M) caused the muscle to contract while acetylcholine (1 X 10^{-5} M) did not alter the baseline tension or relax a norepinphrine-contracted muscle strip. The norepinephrine response was greatly reduced by the alpha adrenergic blocking agent phentolamine but was not altered by propanolol, a beta blocker. Muscle strips were not responsive to VIP. Stretch muscle strips and norepinephrine-contracted muscle strips were field stimulated. Field stimulation of the stretched muscle strips (3 Hertz, 12 volts) provoked a vigorous contraction which was totally blocked by tetrodotoxin (1 X 10^{-7} M) and greatly attenuated by the catecholamine-depleting drug, reserpine. Addition of acetylcholine during field stimulation of the muscle strip markedly inhibited the contractions. The muscarinic antagonist atropine blocked the inhibitory effect of acetylcholine while the nicotinic antagonist pentolinium was without effect. Contractions from transmural nerve stimulation were not affected by the addition of VIP. Field stimulation of norepinephrine-contracted muscle strips produced a relaxation. The relaxation was not affected by physostigmine or blocked by atropine. These studies support the role of norepinephrine as the excitatory neurotransmitter in penile erectile tissue. The failure of exogenous acetylcholine and VIP to alter tension of smooth muscle strips suggests that these substances are without postsynaptic effects. The data supports an initial hypothesis that acetylcholine indirectly causes relaxation of penile smooth muscle by inhibiting the release of the excitatory neurotransmitter norepinephrine. The mediator of the field stimulated relaxation of the contracted muscle strip is unknown. Supported by NIH 1RO11NS19839-02 and NIH RR08139-10.

104.12 INHIBITION OF AMINO ACID STIMULATED NEUROTRANSMITTER RELEASE FROM THE NUCLEUS ACCUMBENS BY PHENCYCLIDINE. S.M. Jones, L.D. Snell and K.M. Johnson. Dept. Pharmacol. & Toxicol., Univ. Texas Medical Branch, Galveston, TX, 77550.

We have previously reported that excitatory amino acids stimulate the release of ^{3}H-acetylcholine (ACh) and ^{3}H-dopamine (DA) from superfused rat striatal slices and that the release of both these neurotransmitters is inhibited by nanomolar concentrations of the psychotomimetic phencyclidine (PCP). This effect of PCP is selective for ACh release stimulated by action at the N-methyl-D-aspartate (NMDA)-sensitive glutamate receptor subtype and is shared by those members of the benzmorphan class of opiates and dioxolane dissociative anesthetics that demonstrate PCP-like properties in assays for the PCP/ sigma binding site and in several behavioral tests. We have now extended our findings to the nucleus accumbens septi (NAS) and the substantia nigra (SN).

Slices of rat brain NAS and SN were incubated in the presence of 50nM ^{3}H-choline or 50nM ^{3}H-dopamine for 30 min. Slices were superfused in plexiglas chambers supported by stainless steel mesh with physiological buffer containing 10μM hemicholinium-3 for ACh release experiments or 10μM pargyline and 1mM ascorbate for DA release experiments. Aliquots of superfusate collected in 3ml fractions were assayed for radioactivity by liquid scintillation spectroscopy. The amount of tritium efflux produced by 1mM NMDA alone was compared with that produced by NMDA in the presence of varying concentrations of PCP.

NMDA stimulated the release of both ACh and DA from NAS but only DA from the SN. NMDA-stimulated accumbens ACh release was completely inhibited by 0.5μM tetrodotoxin (TTX) and physiological concentrations of magnesium. Kainate (KA) and quisqualate (QA), agonists at the other glutamate receptor subtypes, also stimulated ACh release but were inhibited only 50% and 80%, respectively by TTX. Additionally, 1.2mM $MgCl_2$ did not significantly reduce the ACh release evoked by KA and QA. NMDA-stimulated ACh release was inhibited by PCP with an IC_{50} of 0.16μM. PCP (0.1μM) also significantly inhibited KA- and QA-induced ACh release.

The release of DA from the NAS by NMDA was inhibited by PCP with an IC_{50} of 0.11μM. PCP (0.1μM) did not significantly inhibit QA-stimulated DA release and significantly enhanced KA-stimulated DA release.

Similar to our previous work in the striatum, NMDA-stimulated ACh and DA release were potently inhibited by PCP with IC_{50}'s near 0.1μM and were selectively sensitive to physiological concentrations of magnesium. Unlike the striatum, PCP inhibited ACh release in the NAS stimulated by all three amino acid agonists. Interestingly, KA-stimulated DA release was enhanced by the inclusion of PCP in the superfusion buffer. Supported by DA-02073

FEEDING AND DRINKING IV

105.1 CONCENTRATIONS OF B-ENDORPHIN (BE) AND MET-ENKEPHALIN (ME) IN THE HYPOTHALAMUS OF SHEEP AFTER PERIPHERAL INJECTION OF CHEMICALS AFFECTING FOOD INTAKE. C.A. Baile, C.L. McLaughlin, D.H. Krestel-Rickert and M.A. Della-Fera. Washington University Medical School, St. Louis, MO. 63110.

There is increasing evidence that endogenous brain peptide concentrations may be related to degree of hunger and therefore may be affected by exogenous administration of chemicals which influence food intake. In the present experiments the effect of elfazepam (8 mg/kg), a food intake stimulant and of naloxone (.25 mg/kg), an opiate antagonist which decreases food intake, on concentrations of B-endorphin and met-enkephalin were measured in sheep. After a 4-hr fast following a meal, DMSO or the chemical were injected IV and sheep were fasted or allowed to eat a meal. Upon termination of the meal sheep were sacrificed and the following hypothalamic areas dissected out: anterior (AH), paraventricular (PVN), ventromedial (VMH), dorsomedial (DMH), lateral (LH) and posterior (PH). Tissue was extracted in .1N HCl and extracts assayed for peptide concentration. In the first experiment food intake was greater in elfazepam- than DMSO-injected sheep (290 vs 146g, p<.03). BE concentrations were lower in fed than fasted sheep in the AH (140 vs 155 pg/mg tissue, p<.04), LH (142 vs 174 pg/mg, p<.004) and PH (105 vs 137 pg/mg, p<.04). In addition, BE was lower in fed than fasted sheep injected with DMSO, but higher in fed than fasted sheep injected with elfazepam in the DMH (p<.02). ME concentrations were lower in elfazepam- than DMSO-injected sheep in the DMH and were higher in fed compared to fasted sheep in the AH (114 vs 87, p<.03), PVN (124 vs 90, p<.04), DMH (94 vs 50, p<.001), VMH (89 vs 53, p<.02) and PH (53 vs 33, p<.03). There were no interactions of drug and hunger condition in any area. In the second experiment, BE concentrations were lower in naloxone than saline-injected sheep in the PVN (148 vs 191 pg/mg, p<.05) and VMH (170 vs 194, p<.03) and were lower in fed than fasted sheep in the DMH (137 vs 164, p<.03) and PH (82 vs 96, p<.05). BE was higher in fed than fasted sheep injected with saline, but higher in fasted than fed sheep injected with naloxone in the AH (p<.05), PVN (p<.05), and VMH (p<.002). ME concentrations were not affected by drug or hunger condition alone, but analysis of significant interactions showed that ME was higher in fasted than fed sheep after saline injection and lower in fasted than fed sheep after naloxone injection in the AH (p<.03), PVN (p<.04), VMH (p<.002) and PH (p<.003). Thus, concentrations of opioid peptides were influenced not only by the degree of hunger, but also by peripheral administration of chemicals shown to influence food intake. Supported in part by NIH NS20000 and Monsanto Company.

105.2 PROLONGED NOREPINEPHRINE ADMINISTRATION FAILS TO STIMULATE BROWN FAT THERMOGENESIS IN SYRIAN HAMSTERS. J.M. Hamilton*, T.J. Bartness* and G.N. Wade. Dept. Psychology, Univ. Mass., Amherst, MA 01003 and Worcester Fdn. Expt. Biology, Shrewsbury, MA 01545.

Cold- and diet-induced nonshivering thermogenesis in rats are associated with increased sympathetic nervous system activity in brown adipose tissue (BAT). Daily injections of norepinephrine (NE) mimic these effects of temperature and diet on BAT of rats (Bartness, T.J. et al., unpublished; Rothwell, N.J. et al., Am. J. Physiol. 243:R339, 1982). Recently, however, Triandafillou, J. et al. (Am. J. Physiol. 247:E793, 1984) have reported a failure to elicit thermogenic responses in BAT of Syrian hamsters with daily injections of doses of NE which reliably stimulate BAT thermogenesis in rats (.4 mg/kg and .8 mg/kg). They concluded that the BAT response to cold acclimation or consumption of a palatable high-energy diet is not mediated by NE in Syrian hamsters.

Syrian hamsters require a higher dose of NE than rats to produce maximal thermogenic responses (Heldmaier, G. In: Effectors of Thermogenesis, eds. L. Girardier and J. Seydoux, 1978). It is possible that the doses used by Triandafillou et al. were insufficient to stimulate BAT of hamsters. We therefore conducted a partial replication of their study using .8 mg NE/kg and the recommended dose of 1.6 mg NE/kg for Syrian hamsters. In addition, we employed the twice daily injection regimen used for rats.

Adult male Syrian hamsters housed singly under a 16:8 h light: dark cycle with ad lib access to food and water were injected at 8 AM and 4 PM. Groups of hamsters (n=8) received two daily s.c. injections of vehicle or 1.6 mg NE/kg or an AM injection of .8 mg NE/kg and PM injection of vehicle. Hamsters were killed 14 days later and interscapular BAT (IBAT) removed and weighed. IBAT protein and NE content and mitochondrial GDP binding were measured.

Daily morning injections of .8 mg NE/kg had no effect on any of the parameters measured. Twice daily injections of 1.6 mg NE/kg resulted in significant decreases in body weight gain, food intake, and IBAT mass. Total IBAT GDP binding was decreased, but not significantly. NE content of IBAT was not affected.

These findings confirm those of Triandafillou et al. and therefore lend support to their conclusion that cold- or diet-induced changes in BAT of hamsters are not mediated by NE. This is consistent with the recent observation that thermogenic activity in BAT of high-fat-fed hamsters is not dependent on sympathetic stimulation (Hamilton, J.M. et al., unpublished). However, desensitization of BAT to sympathetic stimulation may also explain these results. We are currently investigating this possibility in hamsters with surgically denervated IBAT and in short day-housed hamsters in which BAT sympathetic activity might also be reduced.

105.3 POSTPRANDIAL INSULIN AND GLUCOSE ARE ELEVATED FOLLOWING AREA-POSTREMA ABLATION. Francoise J. Lacour*, Karon J. Sande*, Anne Robbins, Jon N. Kott, Daniel J. Porte, Jr., Stephen C. Woods and Nancy J. Kenney. (Spon. D. Finocchio) Department of Psychology, University of Washington and VA Medical Center, Seattle, WA 98195.

The area postrema and subjacent caudal medial nucleus of the solitary tract (AP/cmNTS) are reported to be closely connected with the autonomic nervous system. Thus, lesions of this region might be expected to alter gastric emptying rate, glucose absorption and/or insulin secretion. Therefore, in this study, the effect of meal ingestion on plasma glucose and insulin levels of fasted AP/cmNTS- and sham-lesioned rats was measured.

Twelve AP/cmNTS-lesioned and 11 sham-lesioned rats were studied beginning 25 wks after surgery. All rats were deprived of their standard laboratory chow for 15 hr prior to testing. Fifteen min after collection of 500 µl of tail blood for measurement of basal glucose and insulin (t_0), the rats were offered dilute sweetened condensed milk for 30 min. A second sample of tail blood was collected after the feeding session (t_{45}).

Lesioned rats did not differ from shams in either body weight at the time of testing or caloric intake during the test session. Lesioned rats weighed 418.3 ± 17.4 g and consumed 34.5 ± 2.6 kcal during the test. Sham-lesioned rats weighed 455.5 ± 16.4 g and ate 30.0 ± 2.3 kcal during the test session. Lesioned rats did not differ from the controls in basal levels of either insulin or glucose but had greater postprandial increases both of insulin ($F(1,21)=7.9$, $p<.01$) and of glucose ($F(1,21)=6.2$, $p<.05$) than did the sham-lesioned controls.

	Insulin (µU/ml)		Glucose (mg/dl)	
	t_0	t_{45}	t_0	t_{45}
APX[1]	6.8 ± 0.7	66.1 ± 14.1	144.8 ± 5.9	185.0 ± 7.8
Sham	9.6 ± 1.7	23.0 ± 4.2	147.7 ± 4.0	166.4 ± 5.9

[1] APX = AP/cmNTS lesioned

The increased glucose response and insulin release of the AP/cmNTS-lesioned rats may indicate a more rapid rate of glucose absorption following such ablation and suggests that this brainstem region may be involved in the physiological processes responsible for gastric emptying.

105.4 ATTENUATION OF HYPOPHAGIA FOLLOWING AREA-POSTREMA ABLATION BY CONTINUOUS DIET CHANGE. Naomi Tomoyasu and Nancy J. Kenney. Department of Psychology, University of Washington, Seattle, WA 98195.

Ablation of the area postrema and adjacent caudal-medial nucleus of the solitary tract (AP/cmNTS) results in a temporary reduction of food intake and a permanent decline of body weight. The reduction of food intake may be due, at least in part, to the development of an aversion to foods presented during the perisurgical period. Changing the lesioned rats' food results in a temporary increase of intake. However, intakes of the "new" food will decrease if access is continued for more than a few days. This study examines the possibility that continuous change of the lesioned rats' diet will attenuate the hypophagia which typically results from AP/cmNTS ablation.

Beginning immediately after surgery, 10 lesioned and 8 sham-lesioned rats were fed pelleted lab chow and one supermarket food (e.g., milk, salami, etc.) which was changed each day (ISM). For another 7 lesioned and 8 sham-lesioned rats (DSM), access to the supermarket foods was delayed for 5 days during which they were fed only the lab chow. An additional 7 lesioned and 7 sham-lesioned rats were fed only pelleted chow for the duration of the experiment. Intakes and body weights were measured daily.

Total daily caloric intakes of both ISM-fed ($F(1,16)=61.4$, $p<.01$) and DSM-fed ($F(1,13)= 6.9$, $p<.05$) lesioned rats were greater than those of lesioned rats fed only pelleted chow. But, intakes of the supermarket-fed, AP/cmNTS-lesioned rats remained below those of their similarly fed controls ($F(1,17)=34.8$, $p<.01$ for the ISM groups and $F(1,14)=20.4$, $p<.01$ for the DSM groups).

Whether the lesioned rats had access to the supermarket foods immediately after surgery or with some delay affected their choice of foods. The lesioned rats which had access to the supermarket foods immediately after surgery ate fewer calories from the supermarket foods than did their sham-lesioned controls ($F(1,17)=77.2$, $p<.01$). ISM-fed lesioned rats did not differ from their controls in the number of calories taken as pelleted chow. Lesioned rats for which access to the supermarket foods was delayed took fewer calories from the pelleted chow than did their controls ($F(1,13)= 20.0$, $p<.01$). These lesioned rats did not differ from their controls in the number of calories taken from the supermarket foods.

Thus, presentation of a choice of foods after surgery attenuates the hypophagia of AP/cmNTS-lesioned rats and results in a shift of preference for foods presented immediately after surgery.

105.5 PERSISTENCE OF GLYCOGEN DEPLETION IN CAUDATE AND HINDBRAIN PARALLELS PERSISTENCE OF FEEDING AFTER INSULIN-INDUCED GLUCOPRIVATION. L. Nzuzi* and S. Ritter, Dept. of Food Science and Human Nutrition, and College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520

It has been assumed that the glucoprivic control of feeding is activated by decreased availability of glucose to critical brain receptors in the caudal hindbrain. However, it has been demonstrated by R.C. Ritter et al. (Am J Physiol 234: E617, 1978) that if food is withheld after a high insulin dose, rats will still increase their intake even after the glucoprivic episode has abated spontaneously ("delayed glucoprivic feeding"). The fact that the delayed increase in feeding occurs when blood glucose, and presumably brain glucose availability, have returned to normal, suggests that the delayed feeding response may be controlled by some metabolic stimulus other than glucose itself. We speculated that brain glycogen levels may play such a role. Glycogen is the only form of stored energy in the brain and is stored in small amounts which are vulnerable to fluctuations in glucose availability. Although it is known that whole-brain glycogen levels are closely tied to blood glucose levels, regional glycogen levels during the development of and recovery from insulin-induced hypoglycemia have not been measured. For these reasons, we analyzed glycogen content in major brain regions during the 8 hrs following insulin injection.

Male Sprague-Dawley rats were injected with insulin (2.5 U/kg, i.p), and 6 rats were sacrificed by focused microwave irradiation 0, 2, 4, 6 and 8 hrs later. Plasma glucose was measured at each of these time points until sacrifice. Microwaved brains were dissected into the following regions: cortex, caudate, thalamus, hypothalamus, cerebellum and caudal hindbrain. Glycogen was measured fluorometrically, using a modification of the amyloglucosidase technique (Nahorski and Rogers, Anal Biochem 49: 492, 1972).

Plasma glucose levels fell to 25 ± 2.1 mg% by 2 hrs after insulin injection and recovered to basal values of approximately 100 mg% by 6 hrs. Glycogen (% of control) fell to the following levels by 2 hrs after insulin: 32.0% (hypothalamus), 13.9% (thalamus), 2.3% (cortex), 6.5% (caudate), 15.7% (cerebellum, and 4.6% (caudal brain stem). Recovery of basal glycogen levels closely paralleled recovery of blood glucose levels in all brain regions except caudate and caudal brain stem. In these two regions glycogen levels were still reduced at 6 hrs (63.5% and 62.7% of control) and 8 hrs (74.1% and 76.6%) after insulin. Thus, our results would be consistent with the possibility that delayed feeding after insulin may be controlled by glycogen depletion in the caudal hindbrain or caudate.

105.6 SUPPRESSION OF FOOD INTAKE BY LOW DOSE INFUSION OF BOMBESIN INTO THE FOURTH VENTRICLE. E.E. Ladenheim* and R.C. Ritter. Dept. of VCAPP, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843.

Several investigators have shown that bombesin administered into the lateral ventricles produces marked suppression of food intake. Suppression of food intake, however, is accompanied by enhancement of behaviors, such as grooming and increased locomotion, which are atypical of normal satiety. For example, Kulkosky et al. (1982) have shown that lateral ventricular infusion of bombesin increases grooming and scratching and decreases resting behaviors in rats. Therefore, it has been suggested that suppression of food intake by lateral ventricular bombesin results from activation of behaviors which compete with feeding and is not indicative of a direct effect of bombesin on neural substrates for the control of food intake.

Recently, however, high affinity binding sites for bombesin-like peptides have been described in several hindbrain structures implicated in the control of food intake. Therefore, we have examined the effects of fourth ventricular bombesin administration on food intake and motor activity of rats. Doses of 1, 5, and 25 ng were administered prior to presentation of a palatable solid food, cookies. Dose-dependent reductions in food intake were statistically significant by fifteen minutes after infusion, producing suppressions of 36.4 ± 8.6%, 55.0 ± 10.2% and 79.7 ± 7.1%, respectively. Computer-assisted monitoring of movement was examined and a behavioral inventory was obtained by direct observation following bombesin doses of 1 and 25 ng. Although significant reductions in food intake were apparent at both the 1 and 25 ng doses, increased motor activity was negligible at the 1 ng dose. The 25 ng dose, however, elicited a significant increase in activity confirming the increased grooming reported by Kulkosky et al.

These results demonstrate that bombesin administered into the fourth cerebral ventricle of the rat is a potent suppressor of food intake at doses which are lower than those previously reported for lateral ventricle infusion. In addition, increases in motor activity attributed to ventricular infusion of bombesin were not apparent at the lowest dose shown to produce significant suppressions of feeding. These findings suggest that the hindbrain may be a sensitive site for bombesin-induced suppression of food intake.

105.7 SUPPRESSION OF FEEDING BY GLUCAGON AFTER SELECTIVE HEPATIC VAGOTOMY IN RATS. S.C. Weatherford and S. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Selective hepatic vagotomy (HV) has been reported to abolish glucagon-induced suppression of food intake (Geary and Smith, Physiol Behav 31: 391, 1983). However, HV rats have been tested for glucagon satiety under a limited set of conditions: i.e., with a palatable liquid food during the light phase of the circadian light cycle. Therefore, we tested the effects of glucagon on food intake of HV rats (n = 12) and sham operated controls (n = 8) under several testing conditions. In agreement with Geary and Smith, glucagon (200 µg/kg ip) did not inhibit feeding in non-deprived HV rats tested 6 hr after lights on with a palatable liquid food. Controls ate 82 ± 7% ($p < .02$) and HV rats ate 99 ± 4% of their baseline intake under these conditions. Furthermore, after 10 hr food deprivation in tests conducted 2 hr after lights on, glucagon (100 µg/kg) did not suppress feeding in either HV rats or controls. HV rats consumed 95 ± 5% ($p > .1$) and controls consumed 120 ± 12% of their baseline intake ($p < .03$) under these conditions. However, when tested 2 hr after lights off following 8 hr of deprivation, glucagon reduced food intake in both HV rats and controls (83 ± 6% and 78 ± 9% of baseline, respectively, $p < .01$ for both groups).

We reported previously that hepatic portal injection of subdiabetogenic doses of alloxan impairs glucagon satiety. Alloxan-treated (AT) rats fail to suppress their food intake in response to glucagon when tested during the day with a palatable liquid food or when tested 1 or 2 hr after lights off with pelleted food. Although mechanisms responsible for alloxan's effect are still under investigation, our data are compatible with the possibility that alloxan damages hepatic vagal fibers. If so, glucagon-induced suppression of feeding might be observed in AT animals, as it is in HV rats, if the feeding test were moved farther into the dark phase of the light-dark cycle. Indeed, we found that when food deprived for 8 hr and tested 3 hr after lights off, both AT rats and controls suppressed their food intake in response to glucagon (74% and 76% of baseline, $p < .008$).

Our results suggest that the hepatic vagus participates in the behavioral response to glucagon, but does not account for all of glucagon's effects on feeding since glucagon inhibits feeding in vagotomized animals under some circumstances. Furthermore, the similarity of AT and HV rats in this respect supports the hypothesis that alloxan-induced impairment of glucagon satiety is mediated by vagal damage.

105.8 AN ANIMAL MODEL OF CHRONIC ANOREXIA NERVOSA? Michael Buck*, Steve Efthyvoulidis*, Mary Ann Marrazzi and Elliot D. Luby*. Depts. of Pharmacology & Psychiatry, Wayne State University School of Medicine, Detroit, Michigan 48201.

An auto-addiction opioid model of chronic anorexia nervosa has been proposed by our laboratory (Marrazzi & Luby, Int. J. Eating Disorders, in press, Winter 1986). According to this model, an initial period of dieting induces the release of endogenous opioids. These opioids are then the substrate for a positively reinforcing elation associated with dieting and induce an enduring life-threatening weight loss. An addiction to dieting results. Endogenous opioids may play a dual role in the response to starvation. 1) They increase food intake to correct it. 2) They adapt the organism for survival until nutritional repletion can occur. This involves down-regulating physiological and metabolic processes to the minimum essential. If these two responses become uncoupled, disorders of either anorexia or obesity can occur. Addiction to the second response may result in chronic anorexia nervosa. Evidence in the literature support these opioid actions and such opioid release and/or changes in the opioid systems with starvation. Evidence exists for disturbances of endogenous opioids in anorexia nervosa. The clinical picture of anorexia nervosa resembles that observed in the addictive disorders. Because endogenous opioids may mediate the auto-addictive state, narcotic antagonists may be therapeutically very useful. Clinical studies are beginning on the use of opiate blockade in the eating disorders and will be reported elsewhere.

We propose that the endogenous opioid systems of chronic anorexia nervosa patients may resemble those of mice as contrasted to those of most species including the normal human subject. Morphine causes sedation and increases food intake and blood glucose in most species. Mice are atypical in that they increase their locomotor activity in response to morphine. We report here that in female BALB/C mice, morphine decreases rather than increases both food intake and blood glucose, as well as increasing locomotor activity. Thus, the mice become hyperactive and anorexic as in anorexia nervosa.

105.9 THE ROLE OF FOOD INTAKE IN ADIPOSITY REDUCTIONS PRODUCED BY MORPHINE IN THE RAT. R. Ottaviani, J. P. MastroPaolo, & A. L. Riley. Psychopharmacology Lab, The American University, Washington, D.C. 20016

Although morphine reduces adiposity within a variety of paradigms, at present it is not certain that a single mechanism can account for this effect in every situation. In vitro reductions in adiposity may be reasonably attributed to a direct breakdown of fat by morphine, but the reductions in 24-h food intake following morphine administration (Heft, M., Daniels, G., Buller, A., & Riley, A., Neurosci, 1981; Riley, A., West, D., Sipol, A., & Woods, S., Neurosci, 1983) may account for the in vivo reduction in adiposity. Following the demonstration in Experiment 1 that both food intake and adiposity were concurrently reduced following in vivo morphine administration, Experiment 2 was conducted to examine the contribution of this reduction in 24-h food intake to the in vivo reduction in adiposity. Specifically, 24 rats in Experiment 2 were divided into two groups, one injected for 21 days with 40 mg/kg of morphine and the other with equivolume distilled water. Throughout these 21 days, immediately following the injection half of the morphine-injected rats and half of the distilled-water injected rats were presented with the amount of food consumed by morphine-injected rats in Experiment 1. The remaining rats in each group were presented with the amount of food consumed by control animals in Experiment 1. Prior to the daily injection, animals were gavaged with any food remaining from the 24-h ration. Both retroperitoneal and ovarian fat pads and gastrocnemius muscles were removed and weighed at the end of the study. Relative to the distilled-water injected rats, animals administered morphine had reduced fat pad weight. This reduction was independent of their daily food intake, i.e., there was an equivalent reduction in adiposity in morphine injected subjects given the morphine diet and the control diet. In addition, there was no reduction in adiposity evident in either of the control groups, even when the diet was restricted, i.e., the morphine diet. These data suggest that the in vivo reductions in adiposity are not related to changes in food intake and are instead a more direct effect of morphine, e.g., an increase in lipolysis or a decrease in LPL activity. Independent of the mechanism responsible for the morphine induced reduction in adiposity, these data are consistent with the possible role the endorphins play in both homeostatic feeding and stress reactions.

105.10 INTERACTION BETWEEN GLUCOSE AND LIPID METABOLISM IN THE CONTROL OF FOOD INTAKE IN RATS. M.I. Friedman*, M.G. Tordoff and I. Ramirez* (SPON: E. Kosar). Monell Chemical Senses Center, Philadelphia, PA 19104.

Changes in glucose and lipid metabolism are believed to provide separate but complimentary signals in the control of short- and long-term food intake. To determine whether glucose and fat metabolism interact directly in the control of eating, we examined the combined effects on food intake of intracellular glucopenia produced by injection of 2-deoxyglucose (2DG) and pharmacological inhibition of either fatty acid oxidation or lipolysis.

Male albino rats were given the fatty acid oxidation inhibitor, methyl palmoxirate (MP; 10mg/kg, p.o.) or vehicle (0.5% methyl cellulose) 2 hr prior to i.p. injection of either saline or 100, 200 or 400 mg/kg 2DG. Whereas MP did not affect food intake (6 hr test) in saline-treated rats, rats given MP ate significantly more than vehicle-treated rats after injection of 100 and 200 mg/kg 2DG. For example, relative to when they were given saline, vehicle-treated rats injected with 100 mg/kg 2DG failed to significantly increase intake in the first 2 hr (0.11 ± 0.04 vs. 0.72 ± 0.28). In contrast, MP-treated rats markedly increased food intake after injection of 100 mg/kg 2DG during the same interval (0.07 ± 0.03 vs. 3.46 ± 0.58). MP did not affect the response to 400 mg/kg 2DG, although intakes of MP-treated rats at the lower doses of 2DG were comparable to those at the 400 mg/kg dose, suggesting a maximal response to the low doses in MP-treated rats. MP did not alter plasma glucose levels at the time of 2DG injection nor did MP reduce the hyperglycemia produced by 100 mg/kg 2DG. This shows that the differential eating response of MP- and vehicle-treated rats was not due to differences in the competition between endogenous, circulating glucose and the glucose analogue. Consistent with its inhibitory action on fatty acid oxidation, MP reduced plasma ketone and elevated plasma free fatty acid levels in saline- and 2DG-treated rats.

The antilipolytic agent, nicotinic acid (NIC; 150 mg/kg, s.c.) given 10 min prior to i.p. injection of 100 mg/kg 2DG also potentiated the eating response to 2DG, whereas NIC alone had no effect on food intake.

These synergistic effects of combined treatment with 2DG and MP or NIC indicate that controls of food intake associated with changes in glucose and fat metabolism share a common mechanism.

105.11 HYPERPHAGIC AND ANORECTIC DRUG EFFECTS IN NONDEPRIVED RATS GIVEN A HIGH PALATABILITY DIET. S.J. Cooper, A. Jackson* and D.B. Gilbert*. Dept. Psychology, University of Birmingham, Birmingham B15 2TT, U.K.

A method which allows limited daily access to a high palatability diet has been used with male rats maintained on ad libitum food and water to investigate the effects on food consumption of kappa opiate receptor agonists, dopamine (D) receptor agonists and antagonists, and benzodiazepine (BZ) receptor agonists and inverse agonists. The aim has been to develop a sensitive and reliable method for detecting both hyperphagic and anorectic drug effects. The rats were first familiarised with the palatable diet, composed of powdered standard rat diet, sweetened condensed milk and water, before the drugs were administered.

A series of experiments was carried out with several kappa receptor agonists administered subcutaneously: the selective drugs, U-50,488H and tifluadom, each produced significant increases in food intake. Bremazocine and ethyketocyclazocine (EKC), however, both decreased food consumption. However, when rats were allowed to consume a pre-load before the drug administration and subsequent testing, 0.01 mg/kg bremazocine and 0.1 mg/kg EKC each produced hyperphagic effects. Morphine (0.3 - 10.0 mg/kg, s.c.) did not have a hyperphagic effect in either case. The degree of feeding satiation may be an important factor, therefore, in the sensitivity of the test to hyperphagic effects of some kappa agonists.

Hyperphagia was also produced by agonists (e.g. clonazepam, lorazepam, diazepam, zopiclone, CL218,872) acting at BZ receptors. Conversely, compounds which are believed to act as 'inverse agonists' at BZ receptors (e.g. DMCM, FG 7142, CGS 8216) produced a dose-dependent attenuation of food intake. These interesting data suggest a possibility of a bidirectional control of the appetite for palatable food operating through differential ligand actions at BZ receptors (Cooper, S.J. and L.B. Estall, Neurosci. Biobehav. Rev. 5: 5-19, 1985).

Anorectic effects were also obtained using either d- or l-amphetamine, and using the specific D1 receptor agonist SKF 38393. The anorectic effect produced by 0.3 mg/kg d-amphetamine (i.p) was reversed by the D1 receptor antagonist, SCH 23390 (0.01 - 0.1 mg/kg s.c.) but not by the D2 receptor antagonist, sulpiride (3.0 - 30.0 mg/kg, i.p). Such data suggest an involvement of D1 receptors underlying amphetamine-induced anorexia, in part at least, and more generally, in the control of feeding responses.

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105.12 BEHAVIORAL EFFECTS INDUCED BY QUALITATIVE CHANGES IN DIETARY FAT. D.V. Coscina, S. Yehuda and C.E. Leprohan. Sect. of Biopsychol., Clarke Inst. of Psychiatry, and Dept. of Nutr. Sci., Univ. of Toronto, Toronto, Ontario, Canada M5T 1R8.

Past research has shown that altering the nutrient composition of diets can produce a variety of biochemical and/or behavioral changes in experimental animals. Perhaps the best known of these changes are those associated with brain catecholamine and indoleamine metabolism following manipulations of their dietary precursors, tyrosine and tryptophan, respectively (e.g., J.D. Fernstrom Ann. Rev. Med., 32: 413, 1981). Contrasting to this, relatively little is known about the potential importance of dietary lipid to behavior and brain chemistry. We have begun to assess this question by measuring a variety of behaviors in rats fed diets differing only in the type of fat supplied. Subjects used were male albino (Sprague Dawley) or hooded (Long Evans) animals weighing 80 - 100 g at the outset of study. Across three separate replications, rats were offered ad libitum access to standard Purina Lab Chow (4% fat; n = 15) or one of two semi-purified diets containing equal protein (casein; 200 g/kg), carbohydrate (corn starch; 402 g/ kg), fat (200 g/kg) and vitamin/mineral mix (76 g/kg). However, the source of fat varied for the two groups (soya, n = 32; lard, n = 22). After 3 wk of eating these diets there were no differences across groups in total kcal consumed or body weight gained. Also, 15 min tests of activity in an open field revealed no differences in the number of horizontal crossings or vertical rears made. However, latencies to lick paws when placed on a 58° C hot plate were consistently longer for soya-fed rats (13.0 ± 4.8 sec., M ± SEM) compared to lard-fed (9.4 ± 3.3) or chow-fed (8.3 ± 2.1) animals. In addition, the ability of 10 or 15 mg/kg amphetamine sulfate i.p. to induce hypothermia in a 4° C ambient environment for 60 min was prevented in soya-fed (+1.3 ± 0.8° C colonic temperature change from baseline) but not lard-fed (-1.2 ± 0.8) or chow-fed (-2.7 ± 0.9) animals. Finally, preliminary results suggest that soya-fed rats show improved performance of a spatial learning task compared to rats in either of the other two groups. Taken together, these findings provide striking new evidence that the quality of dietary fat can produce robust changes in several rodent behaviors. Further work is underway to elucidate the behavioral and biochemical substrates of these modest dietary manipulations.

105.13 MONKEY LATERAL HYPOTHALAMIC ACTIVITY (LHA) DURING FEEDING TASK: PARTICIPATION OF CATECHOLAMINES AND OPIATE. Y. Oomura, H. Nishino, S. Aou and L. Lénárd*. Dept. of Physiol., Fac. of Med., Kyushu Univ. 60, Fukuoka 812; National Inst. Physiol. Sci., Okazaki 444, Japan; Univ. Med. School, Pécs, Hungary.

Correlation between single LHA neuron activity, bar press feeding, and modulation by electrophoretic catecholamines and an opiate were investigated. The paradigm was: i) Cue light on to start bar pressing. ii) Bar pressing (fixed ratio, FR, 30). iii) Short cue tone and food reward at end of bar pressing. iv) Taking and ingesting food. Of 171 neurons tested, 94 (55%) changed activity in one or more phases. Of 94 responding neurons, 15 (16%) responded at cue light on, 73 (78%) during bar press, 17 (18%) at cue tone, and 48 (51%) during reward. Bar press responses increased in trials for more palatable food, and decreased during no reward (extinction) or satiation. Dopamine (DA) excited 18%, and inhibited 26% of 200 neurons tested. Noradrenaline (NA) and morphine inhibited ½ of the neurons tested.

Glucose-sensitive (GS) neurons (38% of 143 tested) responded more than non-GS neurons in feeding tasks. Most GS neuron responses were decrease of activity during bar press and reward phases, but not at onset of initial light cue. Responses disappeared during high blood glucose level due to i.v. glucose injection. When non GS neurons responded, it was more often increase of activity at cue light on, before start of bar press and during reward. Reward responses of GS neurons (especially inhibitory) were probably opiate mediated, since they were blocked by naloxone, which did not affect other responses. Cue tone responses were suppressed by α-adrenoceptive blocker, and bar press responses were (in 3/11 cases) suppressed by β-adrenoceptive blocker. Non GS neurons that responded to cue light on and bar press initiation appeared to be DA sensitive, since DA antagonist blocked the responses. A mediator for bar press responses is not yet known.

Summary: Many LHA neurons responded during task; magnitudes and numbers of responses depended on motivational state. Opiate and catecholamines modulated effects, especially reward related responses. DA is important to external sensory integration and task initiation, NA to drive reduction (motor termination), and opiate to reward perception. DA sensitive neurons might integrate external information while GS neurons integrate internal information. A working hypothesis to guide later investigation presents GS neurons as integrating elements that combine various aspects of internal conditions and then project to effector neurons to control both initiation and termination of overt feeding related behavior.

106.1 ROLE OF KINASE C IN NEUROTRANSMITTER RELEASE. C.T. Giambalvo, Rhode Island Psychiatric Research & Training Center, University of Rhode Island and Brown University, Cranston, RI 02920

Protein kinase C is a Ca/phospholipid requiring enzyme that has been implicated in the release process. The experimental support for this hypothesis has been derived mostly from platelet studies and as yet, there is no direct evidence linking kinase C with the release of neurotransmitters in nervous tissues. In order to study the relationship between kinase C activity and the release of neurotransmitters, the effects of drugs on kinase C activity and release were examined in striatal synaptosomes in vitro and after injections in vivo.

In the release experiments, a striatal synaptosomal preparation was prelabelled with 0.01 μM - ^{3}H-DA and KCl (25mM) was added to induce depolarization. The amount of radioactivity released into the medium was expressed as a percent of total radioactivity. Kinase C activity was determined in tissue homogenates in an incubation medium containing Ca (16μM), phosphatidylserine (32μg/ml), diolein (3μg/ml), histones (0.2mg/ml), Tris buffer with $MgCl_2$ (6mM) (pH 7.4) and ^{35}S-ATP (12μM). After incubating 4 minutes at 30°C, the reaction was terminated with TCA. The precipitated protein was then counted.

Results indicated that: (1) there was a correlation between drugs affecting kinase C activity and the release of DA from striatal synaptosomes in vitro; (2) acute injection of drugs into rats that affect the firing rate of the nigrostriatal pathway and release of DA also affected kinase C activity, in both the striatum and substantia nigra; and, (3) chronic injections of neuroleptics into rats which inhibited release and firing rate also resulted in a decrease in striatal kinase C activity.

These results support the hypothesis that kinase C plays an important role in the release of DA in the nigrostriatal pathway.

106.2 A VINBLASTINE SENSITIVE HIGH AFFINITY CHOLINE UPTAKE SYSTEM M.T. Ivy* and J.G. Townsel. Department of Physiology and Biophysics, University of Illinois at Chicago, College of Medicine, Chicago, IL 60680 and Department of Physiology, Meharry Medical College, Nashville, TN 37208.

There is significant evidence that the high affinity choline uptake system (HAChUS) associated with cholinergic terminals may not be restricted to cholinergic neurons [Masland, R.H. and Mills, J.W., Proc. Natl. Acad. Sci. 77: 1671-1675, 1980]. Recent evidence implicates the HAChUS in the synthesis of phosphorylcholine (PCh), an intermediate of membrane phospholipid [Pu, G.A. and Masland, R.H., J. Neurosci. 4: 1559-1576, 1984]. We have recently characterized a non-cholinergic HAChUS in the cardiac ganglion (CG) of Limulus [Ivy, M.T., et al., J. Comp. Biochem. Physiol., (In Press)]. A physiologically significant property of the Cholinergic HAChUS is that of post-depolarization stimulation. Preincubation of the CG in 90 mM K^+ Chao's resulted in a 46% increase in high affinity choline uptake. The CG HAChUS appears related to the spontaneous electrical activity of this tissue. Micromolar concentrations of 5-hydroxytryptamine (5-HT) have been shown to reduce the spontaneous activity of the CG. The CG HAChUS was inhibited nearly 40% following 10 μM 5-HT preincubation. When the CG was preincubated in Chao's solution containing 10, 100 and 500 μM vinblastine (VB), high affinity choline uptake was inhibited 40, 51 and 64%, respectively. Similarly, 2, 4 and 8 hour preincubations of the CG in 500 μM VB Chao's resulted in a 52, 64 and 71% decrease in the HAChUS, respectively. In pulse-labelling experiments we examined the relative distribution of [^{3}H] in the soluble versus the insoluble fraction following high affinity uptake in the CG. The CG was incubated in 0.1 μM [^{3}H]choline Chao's for 60 min. Following several Chao's washes, the CG was incubated for 1, 2 or 4 hours with unlabelled choline (1 mM). After 2 hours, a 3-fold increase of radioactivity above basal level was measured in an insoluble (pellet) fraction of the CG. Subsequent studies revealed that prior exposure of the CG to 500 μM VB completely eliminated this increase of radiolabel in the pellet fraction. Treatment of the labelled pellet with trypsin resulted in the solubilization of a substantial proportion of the radioactivity. These results are interpreted as supportive of a possible role of the CG HAChUS in the supply of choline subserving the synthesis of membrane phospholipid associated with synaptic vesicle turnover.

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106.3 SUBCELLULAR STORAGE OF SEROTONIN IN A PARANEURON J.M. Barasch*, M.D. Gershon, E.A. Nunez* and H. Tamir. NY State Psych. Inst. and Dept. Anatomy & Cell Biol., Columbia Univ. P&S, NY 10032.

Serotonin (5-HT) is a neurotransmitter of central and enteric neurons. It is also found in parafollicular (PF) cells of the sheep thyroid. Within both neurons and PF cells, 5-HT appears to be stored in specialized subcellular storage vesicles, sometimes with a peptide, as for example calcitonin (CT) in PF cells. Neither the mechanism of uptake of 5-HT by vesicles, nor their means of retaining 5-HT, have been established. Although 5-HT-containing synaptic vesicles cannot be isolated from mixed populations of neural vesicles, purified PF cells can be obtained by the novel technique of phagocytic chromatography. Since PF cells, like neurons, are neurectodermal derivatives and contain the specifically neuroectodermal type of serotonin binding protein, we have used PF cells as a model system to investigate the properties of 5-HT storage organelles (5-HT/CT vesicles). 5-HT/CT vesicles were isolated by separating organelles by size and by density on two sequential Metrizamide gradients. Fractions were monitored using enzymatic markers, 5-HT and CT content, and by quantitative electron microscopy (EM). EM techniques included the demonstration of 5-HT and CT in vesicles by immunocytochemistry with colloidal gold. 5-HT/CT vesicles were found to take up ^{3}H-5-HT by a reserpine-sensitive mechanism. Luciferin-luciferase assays and uranaffin cytochemistry demonstrated that 5-HT/CT vesicles contain little or no ATP. Using acridine orange trapping, we found that 5-HT/CT vesicles have an ATP driven (N-ethyl maleimide sensitive, oligomycin insensitive) proton pump and can acidify their interior; however, the degree of acidification appears to be limited by the generation of a transmembrane potential difference (intravesicular positive) secondary to the translocation of H^+. Collapsing this potential with the K^+ ionophore, valinomycin, increased the pH gradient. Using acridine orange trapping with a fluorescence-activated cell sorter, PF cells were found to vary greatly in their content of acid granules. EM immunocytochemical detection of weak base accumulation confirmed the heterogeneous acidification of 5-HT/CT vesicles in intact PF cells. H^+ pumping into vesicles was increased by the K^+ ionophore, valinomycin, by the 5-HT secretagogues TSH and Ca^{+2}, and was blocked by the K^+/H^+ exchange ionophore, nigericin. This investigation represents the first isolation of a neuroectodermal type of 5-HT storage organelle. These vesicles appear to be different from other neurotransmitter vesicles in that they contain little or no ATP and have a proton pump that appears to be susceptible to physiological regulation. The effect of secretagogues suggests that vesicle acidification may be involved in the exocytotic release of hormones. Supported by NIH grants NS12969, AM19547, NS07062, NIMH37575.

106.4 EFFECTS OF AGE AND INCUBATION TIME ON NOREPINEPHRINE UPTAKE AND Na^+-K^+ ATPase ACTIVITY IN SHR AND WKY SYNAPTOSOMES. K.L. Hough, J.H. Rho, Y.T. Jeng, and A.A. Ross. Depts. of Medicine and Pathology, Schools of Medicine and Pharmacy, Univ. of Southern California, Los Angeles, CA 90033.

In order to examine the hypothesis that the transmembrane NE uptake system is affected by aging in spontaneously hypertensive rat (SHR) and Wistar Kyoto (WKY) controls, we studied the reuptake process directly in the synaptosomes from the hypothalamus of SHR and WKY between 4 and 41 weeks of age. The gradient-purified synaptosomes demonstrated NE uptake that was highly Na^+-dependent (90% average) and ouabain sensitive (50-60%). Initial uptake was measured using a 2 minute incubation at 30° C. In 13 paired experiments, from age 4 to 7 weeks, SHR had a significantly higher uptake of NE than WKY ($p < .05$), a trend which continued up until 10 weeks of age ($N=20$, $p < .02$). However, 13 additional paired experiments between ages 11 and 41 weeks failed to show any appreciable difference in initial uptake between SHR and WKY.

Our previous work utilized similar conditions but a 10 minute incubation. Under these conditions, 13 paired experiments of SHR and WKY between 8-10 weeks of age showed that WKY accumulates an average of 23% more NE than SHR ($p < .01$).

To better examine the effect of incubation time upon synaptosomal uptake and accumulation, synaptosomes from 3 SHR and 3 WKY rats (4 weeks old) were pooled, and tested for NE uptake and EM analysis. In two different experiments SHR took up more NE than WKY at 2 minutes incubation, but at all following time points WKY accumulated more NE than SHR. Minus Na^+ and 0° C. conditions remained low at all time points up to 30 minutes incubation, indicating minimal effects of vesicular or nonspecific uptake. EM analysis verified that synaptosomes remain remarkably intact at all incubation times up to 30 min.

In addition, Na^+-K^+- ATPase activity was examined in the same brain from which the hypothalamic synaptosomes were prepared. SHR enzyme activity is not only parallel to the rates of initial NE uptake but also age-dependent. Thus, the enzyme activity of SHR was higher than that of WKY (5-25%) during 5-9 weeks of age and then declined below those of WKY samples. We postulate that such an increased Na^+-K^+- ATPase activity in brain of juvenile hypertensives may be a compensatory increase for a reduced fractional sodium excretion by young SHR.

106.5 SYNEXIN INDUCES FUSION OF CHROMAFFIN GRANULE MEMBRANES BY A MECHANISM THAT IS INDEPENDENT OF CALCIUM AND ARACHIDONIC ACID. A. Stutzin*, P.I. Lelkes*, I. Cabantchik*, E. Rojas*, H.B. Pollard (SPON: C.R. Creveling). Laboratory of Cell Biology and Genetics, NIADDK, National Institutes of Health, Bethesda, MD. 20205.

Membrane fusion is a key event in numerous biological processes, including neurotransmission and exocytosis, and may be regulated by intracellular proteins, such as synexin, a calcium binding protein found in adrenal medulla and other secretory tissues. It has been shown that synexin promotes massive aggregation of chromaffin granules in a calcium dependent manner, followed by fusion if arachidonic acid is subsequently added. To study the mechanism of synexin-induced fusion we devised a novel on-line fluorescence assay in which chromaffin granule ghosts (CGG) were loaded with a self-quenched concentration of fluorescein-tagged Dextran (m.w. 20000) using a freeze-thaw technique. The labelled CGG were mixed with identically treated empty CGG in a ratio ranging between 1:5 - 1:10 and in the presence of specific rabbit antifluorescein antibodies in the medium. These antibodies quenched the fluorescent signal occurring during leakage, so that any increase in fluorescence came from dequenching of the dye due to dilution of the marker molecule into a space not accessible to the antibody, i.e. mixing of the CGG contents upon fusion. Under these conditions a rapid but clearly resolved increase in the fluorescence signal was observed after the addition of purified synexin. The changes were dependent on the synexin concentration and were inhibited by a specific polyclonal antisynexin antibody. Fusion occurred even at 10^{-9} M free calcium concentration and raising the free calcium concentration to 10^{-2} M had no influence on the reaction. The fusion event was pH dependent and the time course of the event was on the order of minutes, in agreement with other in-vitro fusion assays. The addition of other proteins like BSA or non-specific IgG failed to induce fusion events. The fusion event could also be coincidently followed by mixing of membrane lipids using octadecylrhodamine B, a non-exchangeable lipid analog. The validity of this fusion assay was confirmed using the well known terbium/dipicolinic acid fusion assay in a phosphatidylserine liposome system. The data support the notion that synexin could play an important role in membrane fusion during exocytosis, and that some aspects of synexin action may not depend on calcium.

106.6 INFLUENCE OF CALCIUM AND CYCLIC ADENOSINE MONOPHOSPHATE ON SEROTONIN SECRETION FROM THE HYPOTHALAMUS. H.A.F. Navarro*, R.F. Walker*, and V.J. Aloyo. Sanders-Brown Center on Aging, University of Kentucky Medical Center, Lexington, KY 40536.

Potassium (K^+) stimulates the release of monoamines from brain tissue in vitro. Calcium (Ca^{++}) and its binding proteins, as well as inositol phospholipid (PI) and cyclic nucleotide second messengers are thought to contribute to the secretory process. In the present study we examined the effects of Ca^{++}, A23187 (a Ca^{++} ionophore), phorbol 12-myristate 13-acetate (PMA; protein kinase C activator), and forskolin (adenylate cyclase activator) upon K^+-induced release of serotonin (5HT) from slices of hypothalamus (0.5mm) that were prepared rapidly from the brains of decapitated Long-Evans rats (male; 6 months old). Their endogenous 5HT pool was radiolabeled by incubating the slices in ^{3}H-5HT (1.0 mM; 30 min.). Afterwards, they were perifused with oxygenated buffer [Krebs-Ringer-Hepes (KRH), $CaCl_2$=2.5mM; KCl=6mM; ascorbate and pargyline-10^{-4}M] at a flow rate of 480ul/min. ^{3}H-5HT content of 1 min. fractions was monitored and a stable baseline efflux was achieved after perifusion for 30 min. When the concentration of KCl was increased to 45mM in osmotically equivalent buffer, ^{3}H-5HT release significantly exceeded baseline values. The magnitude of the K^+-induced ^{3}H-5HT release, but not the basal efflux was attenuated when Ca^{++}-free buffer plus EGTA was added. In contrast, preincubation of tissue slices in A23187 prior to K^+ stimulation potentiated ^{3}H-5HT release by the cation. However, the magnitude of ^{3}H-5HT release after perifusion with standard KRH buffer containing the ionophore was not comparable to that produced by K^+. Thus K^+ induced secretion does not occur solely as the result of Ca^{++} influx into the tissue. Instead, Ca^{++} may complement other factors which mediate ^{3}H-5HT release. PMA and forskolin were then used to determine if PI or cAMP second messengers, respectively, participate in the secretory process. Forskolin (100mM) but not PMA (0.1 or 1.0uM) significantly enhanced ^{3}H-5HT release in standard as well as EGTA containing KRH. These findings indicate that cAMP dependent processes contribute to ^{3}H-5HT release from hypothalamic slices in vitro. The inability of EGTA to block the forskolin effect suggests that extracellular Ca^{++} is not required for cAMP-mediated release of ^{3}H-5HT. However, synergistic interactions of intracellular Ca^{++} and cAMP dependent processes may occur and is the subject of ongoing investigation.

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106.7 **MECHANISTIC STUDIES OF ACETYLCHOLINE STORAGE AND DRUG INHIBITION.** S.M. Parsons, B. Bahr*, and D.A. Anderson*. Department of Chemistry, University of California, Santa Barbara, CA 93106.

Highly purified cholinergic synaptic vesicles isolated from Torpedo electric organ actively transport [^{3}H]acetylcholine(ACH) in the presence of MgATP. The steady state inital velocity uptake exhibits Michaelis-Menten like kinetics with an ACh K_m of about 0.3 mM and a V_{max} of about 1 nmol/min/mg, depending on the preparation of vesicles. Nonradioactive ACh is a competitive inhibitor with respect to [^{3}H]ACh. The potent tertiary amine inhibitor dl-trans-2- (4-phenylpiperidino)cyclohexanol (AH5183) is a noncompetitive inhibitor with respect to [^{3}H]ACh with K_i 41 nM. Inhibition is not readily reversed by washing of vesicles. The l-optical isomer of AH5183 is 55-fold more potent than the d-isomer. Some 60 analogs of the drug have been synthesized and evaluated. Drug potency is very dependent on analog structure. However, the more the analog structure resembles ACh the less potent it becomes. It is clear that a specific receptor for AH5183 exists, and based on the similarity of the K_i value to typical vesicle molarities used in these experiments it is likely that one or less AH5183 receptor is present per synaptic vesicle. Levorotary [^{3}H]AH5183 (2.9 Ci/mmol) recently has been synthesized and used in preliminary studies demonstrating that it is highly but reversibly bound by vesicles in the absence or presence of MgATP. Taken as a whole, the above data is not easily reconciled to a model where AH5183 acts as an analog of ACh by binding to the ACh transporters in a homogeneous population of synaptic vesicles to inhibit ACh storage.

106.8 RECEPTOR MEDIATED ENDOCYTOSIS OF BIOTIN-LABELED CRF ANALOGS BY PITUITARY OPIOCORTIN CELLS: ULTRASTRUCTURAL STUDIES. G. V. Childs (Moriarty)*, K.N. Westlund, G. Unabia*, J. L. Morell*, and G. Aguilera* (Spon: A. A. Perachio) Dept Anat. Univ. Texas Med. Br., Galveston, TX, 77550, NICHD, NIH, Bethesda, Md.

Biotin-labeled CRF binds to specific cells in the anterior pituitary. It is localized with avidin-biotin-peroxidase complexes (ABC). Double immunocytochemical stains for adrenocorticotropin or beta-endorphin are used to confirm the identity of the target cells (Westlund et al, Peptides 5: 627, 1984). In these ultrastructural studies, 2 biotin labeled CRF analogs were used. Bio-CRF-1 was conjugated to biotin as described in the previous publication. Bio-CRF-2 was synthesized with biotinylated-serine substituted for the N terminal serine. Binding assays showed that the biotinylated analogs exhibited no loss in binding affinity or potency for ACTH release when they were compared with unlabeled ovine or rat CRF. Extraction of the biotinylated analogs with avidin-sepharose resulted in no activity indicating that they were 100% biotinylated. Dispersed anterior lobe (AL) or intermediate lobe (IL) cells were stimulated in suspension for 1-60 min at 37°C with doses of 0.1-1 nM. In some experiments, the AL cells had been separated into fractions by centrifugal elutriation. Stimulated and control suspensions were fixed in glutaraldehyde, stained with the ABC technique, postfixed in osmium tetroxide, and embedded in a pellet for electron microscopy. Cell counts showed that both biotinylated analogs bound 7-10% of AL cells and 68 ± 4% of dispersed IL cells. Elutriation Fractions 5 and 6 showed a 5-6 fold enrichment in percentage of Bio-CRF reactive cells. Competition with excess (100-1000 fold) unlabeled CRF prevented binding by Bio-CRF and reduced staining to 1-2% of AL or IL cells. After 1-3 min of stimulation, patches of stain for Bio-CRF were seen on membranes near microvilli. Internalization in AL cells was evident in small vesicles 2-3 min after initial exposure to the Bio-CRF. Within 5 min labeling was seen in lysosomes, on membranes of the Golgi Complex vacuoles, and in the core of some of the immature secretion granules. At later times, stain was seen in multivesicular bodies (MVB) and in a subpopulation of secretion granules. IL cells showed staining in small vesicles as early as 1 min after exposure. At later times, stain was on secretory granule membranes, and in MVB. The ultrastructural analysis thus shows 2 internalization routes for the CRF. The lysosomal route may signify degradation or modification of the peptide whereas the MVB or secretion granule route may be the mechanism for receptor recycling to the surface. Cytochemical evidence suggests that the Golgi Complex is involved in the production of granules that contain the CRF receptors in the AL cells. In the IL cells, the granules may be labeled by direct contact with the small vesicles. Supported by RCDA HD-00395 (GVC) and NIH NS-0739 (KNW).

106.9 LABELLING IN VIVO OF SEROTONIN UPTAKE SITES J. Wolfe*, C. Goodman* and D.J. Brunswick*, (SPON: P. Conway). VA Medical Center and Dept. of Psychiatry, Univ. of Pennsylvania, Phila, PA 19104.

^{3}H-Cyanoimipramine (^{3}H-CN-IMI) has previously been shown to label sites associated with serotonin uptake in both platelets and brain homogenates. In this study we have investigated the binding properties of ^{3}H-CN-IMI following administration in vivo. Approximately 2uCi ^{3}H-CN-IMI was administered i.v. (tail vein) to two groups of rats; one received the potent serotonin uptake blocker fluoxetine (10mg/kg) 5 minutes before ^{3}H-CN-IMI; and the other received saline. The ratio of the radioactive concentration in the hypothalamus to that in the cerebellum (radioactivity ratio) was determined 15 minutes and up to 4 hours after ^{3}H-CN-IMI administration. These brain regions were chosen since previous in vitro studies using ^{3}H-imipramine have shown that the highest concentration of binding sites in the brain is in the hypothalamus, whereas the lowest is in the cerebellum. The radioactivity ratio thus provides a measure of receptor occupancy. The highest ratio in the saline pretreated animals was obtained 2 hours after ^{3}H-CN-IMI administration (1.80 $\pm$ .07, n = 19, mean $\pm$ S.E.). This ratio was significantly reduced ($p < .01$) in the fluoxetine pretreated animals (1.07 $\pm$ .07, n = 9). A number of other drugs which are potent serotonin uptake inhibitors caused a significant reduction ($p < .01$) in the radioactivity ratio including chlorimipramine (2mg/kg), sertraline (1mg/kg) and cyanoimipramine itself (0.3mg/kg). Other drugs which are not potent inhibitors of serotonin uptake did not reduce the ratio including dl-propranolol (5mg/kg), maprotyline (10mg/kg), metergoline (10mg/kg) and haloperidol (5mg/kg). These results indicate that ^{3}H-CN-IMI can be given to rats to provide an index of serotonin uptake sites in the CNS in vivo.

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106.10 A POTENTIAL PHOTOAFFINITY PROBE FOR THE CATECHOLAMINE/SEROTONIN PORTER OF SYNAPTIC VESICLES FROM MAMMALIAN BRAIN. J.A. Near*, S. Detwiller* and L. Shelton* (SPON: R.S. Gurd). Dept. of Pharmacology and Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47403.

Catecholamines and serotonin are sequestered in synaptic vesicles by an energy-dependent, stereoselective transport system. Tetrabenazine and Dihydrotetrabenazine (DH-TBZ) are potent competitive inhibitors of transport (K_i^{app}=1-10nM) and previous work in this laboratory has shown that [^{3}H]-DH-TBZ binds to both energized and unenergized bovine striatal synaptic vesicles with an apparent dissociation constant of approximately 3nM. Since the binding properties of DH-TBZ suggest it specifically blocks the substrate binding site of the porter, a photolabile nitroaryl azide derivative of tetrabenazine has been prepared and evaluated for use as a photoaffinity probe to covalently label this binding site.

ANP-TBZ (2-(4-azido-2-nitrophenoxy)-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-H-benzo[a]-quinolizine) was prepared from dihydrotetrabenazine and 4-fluoro,3-nitrophenyl azide. ANP-TBZ exhibits an optical absorbance peak at 350nm that is typical of nitroaryl azides. Irradiation (UVS Mineralite) causes a dramatic shift in absorbance at this wavelength ($T_{1/2}$<1min) that probably reflects photolysis of the azide to the nitrene and subsequent reaction with solvent.

In the absence of light, ANP-TBZ is a competitive inhibitor of dopamine accumulation by bovine striatal synaptic vesicles, with K_i^{app}=200nM. Competitive inhibition of [^{3}H]-TBZ binding is also observed with similar K_i. Energization of the porter by addition of Mg-ATP has no effect on inhibition of TBZ binding by ANP-TBZ. When vesicles are preincubated with ANP-TBZ (300nM) and subjected to irradiation prior to determination of dopamine transport activity, V_{max} is decreased 30-40% when compared with controls irradiated in the absence of ANP-TBZ, suggesting irreversible binding to the porter. In contrast, little difference in the K_m^{app} for dopamine was observed between samples irradiated in the presence and absence of ANP-TBZ, suggesting the K_i for inhibition of catecholamine transport is increased considerably by photolysis.

These results imply that ANP-TBZ binds reversibly to the substrate binding site of the catecholamine/serotonin porter in the absence of light, and that photolysis induces covalent coupling of the bound compound with the porter. Photolysis is rapid, and the product of photolysis appears to have a substantially lower affinity for the porter than ANP-TBZ itself. Because of these properties ANP-TBZ may be a useful photoaffinity probe for use in studies of neurotransmitter transport by synaptic vesicles in the CNS.

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106.11 ACCUMULATION OF ^{3}H-NOREPINEPHRINE, ^{3}H-DOPAMINE AND ^{3}H-5-HYDROXYTRYPTAMINE BY RAT BRAIN STORAGE VESICLES OCCURS IN KINETICALLY DISTINCT COMPARTMENTS DIFFERENTIALLY SENSITIVE TO NEUROTOXINS. J.A. Ruth, E.A. Ullman*, B.S. Liptzin*, J.V. Cuizon*, W.R. Wilson* and S.H. Park*. School of Pharmacy, University of Colorado, Boulder, CO 80309

We have previously shown that in membrane-impermeant media, the ATP-stimulated accumulation of ^{3}H-norepinephrine (NE), ^{3}H-dopamine (DA), and ^{3}H-5-hydroxytryptamine (5HT) by rat brain storage vesicles is saturable and stable for extended periods at 37°. The characteristics of NE, DA and 5HT accumulation were significantly different to suggest the possibility of selective amine accumulation by vesicle subpopulations. To further examine this possibility, the following studies were performed. Intraventricular administration of 6-hydroxydopamine (6OHDA) resulted in a 57% decrease in endogenous NE tissue content accompanied by a 40% decrease in ^{3}H-NE accumulation by rat brain vesicles. The effect was prevented by prior administration of desipramine. Vesicular ^{3}H-DA accumulation was unaffected by 6OHDA, although endogenous DA levels were reduced 80%. Intraventricular administration of 5,7-dihydroxytryptamine reduced endogenous 5HT tissue levels by 72% and reduced vesicular accumulation of ^{3}H-5HT by 40%, without affecting accumulation of ^{3}H-NE or ^{3}H-DA.

Competition kinetics demonstrated that accumulation of ^{3}H-NE (Km 5×10^{-6}) was noncompetitively inhibited by 5HT (Ki 3.5×10^{-6}) and DA (Ki 1.7×10^{-4}); accumulation of ^{3}H-5HT (Km 3×10^{-7}) was noncompetitively inhibited by NE (Ki 6×10^{-6}) and DA (Ki 6×10^{-6}); and that accumulation of ^{3}H-DA (Km 5×10^{-5}) was noncompetitively inhibited by NE (Ki 2.8×10^{-6}) and 5HT (Ki 2.2×10^{-5}).

Spontaneous efflux of endogenous NE (linear; t1/2 = 10 min), 5HT (linear t1/2 = 3 min) and DA (biphasic t1/2 = 1 min, 13 min) was paralleled by efflux of ^{3}H-amines.

The results further support selective amine accumulation by vesicles subpopulations in a rat whole brain vesicle preparation.

Supported by USPHS grant NS18752.

107.1 COMPARATIVE IDENTIFICATION OF NEUROPEPTIDES IN THE SPINAL CORD OF DOG, CAT, MONKEY AND SLOTH. S. Michener*, G. Harty*, D. Lucas*, D. Roddy*, V. Go* and T. Yaksh* (SPON: J. Szurszewski). Gastroenterology/Neurosurgical Units, Mayo Clinic, Rochester, MN 55905.

Despite many physiological studies which have shown that neuropeptides may function as neurotransmitters, no complete survey of neuropeptides in the spinal cord has been reported. Therefore, spinal cords and ganglia from cat, dog, owl monkey (Aotus trivirgatus) and two-toed sloth (Choloepus didactylus) were immediately removed post mortem and dissected into dorsal horn (DH), ventral horn (VH) and dorsal root ganglia (DRG) for each cord segment. Neuropeptides were extracted into boiling 0.1 N HCl, homogenized, neutralized and measured in the following specific radioimmunoassays: substance P (SP), met-5-enkephalin (met-Enk), vasoactive intestinal peptide (VIP), peptide histidine-isoleucine (PHI), cholecystokinin (CCK-8), neurotensin (NT) and bombesin (BLI). Results of four peptides are summarized below for the cat (n=5). The mean of the peptide concentrations for each region is reported (ng/g wet weight).

		Cervical	Thoracic	Lumbar	Sacral
SP	DH	110	106	232	369
	VH	39	40	47	107
	DRG	18	26	25	22
met-Enk	DH	51	46	87	257
	VH	24	27	30	89
	DRG	<4	<4	<4	<4
VIP	DH	<1	<1	6	210
	VH	<1	<1	2	7
	DRG	1	5	3	29
PHI	DH	2	2	8	271
	VH	3	2	3	11
	DRG	3	3	3	26

SP, met-Enk, VIP and PHI concentrations were highest in the sacral region, with the dorsal horn peptide concentrations higher than the ventral horn and DRG in all segments examined. Similar findings were observed for CCK-8, NT and BLI, and comparable distributions were observed in all animal species examined. Peptides were characterized using reverse phase HPLC, with detection by RIA. The principle peaks of immunoreactivity in dorsal-sacral samples co-eluted respectively with the following standards: SP-11; met-Enk5; VIP-28; PHI-27; CCK-8 and NT-13. The HPLC for BLI identified two peaks eluting shortly before the calibrations of bombesin 10 and gastrin-releasing peptide 27.

Conclusion: These results suggest that regulatory peptides in the spinal cord have different regional but comparable species distributions.

107.2 INTRA- AND INTERSEGMENTAL DISTRIBUTION OF THYROTROPIN-RELEASING HORMONE (TRH) IN RAT SPINAL CORD: TOPOGRAPHICAL VARIATION AND PRESENCE IN THE DORSAL HORN. D.H. Harkness* and M.S. Brownfield* (SPON:M. Javid). School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

TRH distribution was studied in spinal cords of control and pharmacologically treated adult male rats by immunocytochemistry (ICC) and radioimmunoassay (RIA). Anesthetized rats were perfused intracardially with 4% paraformaldehyde-0.2% glutaraldehyde in 0.10 M phosphate buffer, pH 7.5. Cryostat sections of all segments were processed for ICC using PAP ICC with and without nickel intensification. Antisera were usable at dilutions of 1:1,000 to 1:20,000 and were specific for TRH amide as shown by differential absorption tests with TRH acid, cyclo-His-Pro, LHRH and other peptides. Unfixed rat spinal cord segments were dissected on ice, extracted with 90% methanol, and dried. Samples were reconstituted in buffer, and TRH was measured by RIA.

ICC showed the unexpected presence of immunoreactive TRH-containing cells and fibers in dorsal horn lamina II and superficial lamina III throughout the spinal cord. Confirming other reports, the peptide was also present in the intermediolateral (IML) and ventral horns. Staining was intense along thoracic lamina V-VII border and in the IML, weak in these same intermediate lamina of the lumbar cord, and moderate to heavy at all levels of the somatic motor column. Colchicine treatment (100 ug, ivt), 24 hours before death, led to the demonstration of large numbers of cell bodies along the border of lamina II-III. Cell bodies were not found in any other lamina. Administration of 5,7-dihydroxytryptamine (5,7-DHT, 200 ug) 14 days before death resulted in a severe reduction of serotonin everywhere in the spinal cord as shown by ICC and HPLC. While TRH immunoreactivity was diminished in the ventral horn in parallel with serotonin, dorsal horn TRH staining was unaffected by 5,7-DHT. Despite the loss of serotonin in the IML staining for TRH was resistant to the effects of the neurotoxin.

RIA of extracts of whole segments showed a wide range of TRH concentrations (7.5± 1.2 to 59±3.7 pg/mg wet weight) and segmental specificity in its distribution that was generally reflective of ICC results. Highest levels (greater than 40 pg/mg wet weight) were measured in C8-T1, T7-T8, and T12-S3, while lowest levels (below 10 pg/mg wet weight) were observed in segments C2-C7. Other segements were intermediate in TRH concentration.

These studies show (1) an intrinsic TRH system exists in the dorsal horn separate from serotonin, (2) TRH in the IML and ventral horn is differentially responsive to 5,7-DHT, and (3) the segmental variation in TRH concentration suggests selective involvement of the peptide in sympathetic and somatic motor function. (Supported by UWGS)

107.3 CAPSAICIN INDUCES NEURONAL DEGENERATION IN THE BRAIN AND SPINAL CORD OF ADULT RATS. T.T. Dinh* and S. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164

Capsaicin is a potent neurotoxin which is highly selective for small-diameter primary afferent neurons. The prevailing view has been that capsaicin causes degeneration of these neurons only in neonatal animals. Silver stain studies of adult animals after systemic capcaicin treatment have not revealed evidence of degeneration. However, intracisternal administration of capsaicin to adult rats produces neuronal degeneration. In addition, systemic capsaicin treatment of adult rats decreases levels of certain peptides in the central nervous system and is associated with certain specific behavioral and physiological deficits, suggesting that neuronal damage has occurred. Therefore, we re-evaluated the neurotoxicity of systemic capsaicin treatment in adult rats, using a modification of the Carlsen-de Olmos cupric silver stain for degenerating neurons.

Adult male Sprague-Dawley rats were injected with a single dose of capsaicin (50 mg/kg, i.p.) or the capsaicin vehicle solution. Both vehicle solutions commonly used for capsaicin administration, 50% dimethyl sulfoxide (DMSO) in saline and 10% alcohol/10% Tween 80, were evaluated. Rats were sacrificed and perfused at 6, 12, 24 and 96 hrs after injection. Capsaicin-induced degeneration was present at all time points analyzed, but the greatest amount of staining was present in most regions at 12 and 24 hrs after the capsaicin injection. Capsaicin caused degeneration in areas previously shown to be affected by capsaicin in the neonatal rat. These areas include those associated with known projections of small diameter primary afferent neurons: the dorsal horn of the spinal cord, the spinal trigeminal nucleus and the nucleus of the solitary tract. In addition, we observed degeneration in several novel areas not previously described as being susceptible to capsaicin toxicity, including the inferior olive (medial nuc), interpeduncular nuc, supramammillary nuc, bed nuc of stria terminalis, and possibly the median eminence. The distribution of capsaicin-induced degeneration was similar for both vehicles, although DMSO tended to reduce the quality of the staining and predisposed to staining artifacts. In addition, DMSO itself appeared to cause degeneration in the subfornical organ.

Our results reveal that capsaicin produces degeneration in areas containing projections of primary afferent neurons in adult rats and in several areas of the brain not known to be recipients of primary afferent terminals.

107.4 SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE CAT SPINAL CORD. T.L. Krukoff, J. Ciriello, and F.R. Calaresu. Dept. of Physiology, University of Western Ontario, London, Canada. NCA 561

Recent anatomical and physiological evidence suggests that somatostatin likely plays a neurotransmitter role in the CNS, including the spinal cord. However, existing information about the distribution of somatostatin-like immunoreactivity (SS) in the spinal cord is incomplete. Therefore we have made a detailed investigation of the distribution of SS in the entire spinal cord of cats either treated or untreated with colchicine. In the treated group, 570 µg of colchicine were applied via an intrathecal polyethylene cannula to the spinal cord of cats anesthetized with ketamine (35 mg/kg i.p) and cats were allowed to survive for 24-48 hr. These animals and a group of untreated cats were anesthetized and perfused with Zamboni's fixative and frozen transverse and longitudinal sections (50 µm) from all segments of the cord were cut. Sections were placed into somatostatin-28 antiserum for 16-18 hr at 4°C. The biotin-avidin immunohistochemical procedure using a Vecta Stain Kit (Vector Labs, Burlingame, CA) was used to demonstrate SS.

In the dorsal horn SS was found in small neurons (less than 15 µm in diameter) within laminae II and III at all levels. Laminae IV to V contained smaller numbers of immunoreactive neurons at all levels that were medium (between 15 and 25 µm in diameter) to large (greater than 25 µm in diameter) in size. In addition, a small number of medium-sized neurons was found at the dorsal and dorsomedial borders of the gray and white matter in segments C1 to C5. In the sacral cord, a group of medium-sized bipolar neurons was found in the dorsolateral funiculus. In the intermediate and central gray matter, but excluding the intermediolateral nucleus and nucleus intercalatus (see Krukoff et al., 1985. J. Comp. Neurol. In press), multipolar neurons containing SS were found in lamina VII at all levels and in lamina X of the thoracic and upper lumbar cord. The former were medium to large in size and the latter were generally small in size. Finally, a small number of medium- to large-sized neurons with SS was found in the ventral horn of the cervical and upper thoracic cord. All neurons containing SS appeared to possess short processes. The distribution of nerve terminals and fibers containing SS was similar to that previously described in other species. Although the function of somatostatin in the spinal cord is not clear, its presence in neurons with short processes in numbers much larger than was previously reported suggests that it may act to modify local activity in the regions where it is found, including areas involved in sensory, visceromotor, and motor functions.

(Supported by the Medical Research Council of Canada and the Heart and Stroke Foundation of Ontario).

107.5 VIP IN GANGLIONECTOMIZED CAT SPINAL CORD. J. N. Young*, E. Rossitch, Jr.*, J. Ovelmen Levitt, B. S. Nashold, Jr. Division of Neurosurgery, Duke University Medical Center, Durham, NC 27710.

Vasoactive intestinal polypeptide (VIP) is a polypeptide which has been found in the GI tract, and in the central and peripheral nervous systems of various mammals. It has been found in small diameter sensory neurons, the superficial dorsal horns and the tract of Lissauer. Specifically, it has been localized to afferents at sacral levels and laterally projecting fibers at S2 and S3. This study attempted to exploit the high degree of localization of VIP to assess whether VIP-staining fibers sprout after deafferentation in the cat.

Ten cats were included in this study. Five cats underwent unilateral lumbosacral ganglionectomy of 3-5 dorsal roots: [L4, L5], L6, L7, and S1. They were sacrificed at varying times after surgery ranging from 2 weeks to 4 years. Five cats served as controls and received no surgery. All the animals were perfused by aortic cannulation with PBS, 4% paraformaldehyde in 0.1M phosphate buffer, and 5% sucrose in 4% paraformaldehyde buffer. Forty micron frozen sections were incubated overnight at 4° C in VIP antiserum diluted at 1:2000 in 1% goat serum/PBS. The secondary phase was performed using the ABC method. The sections were then stained with DAB and mounted on slides. Control sections were prepared with antiserum preabsorbed with VIP.

In the control cats, dense VIP immunoreactivity (VIPIR) was seen in the tract of Lissauer and surrounding the central canal in all sacral segments and less densely in L6 and L7. At the S2 and S3 level, VIPIR was also seen in ventrally projecting fibers at the lateral edge of the dorsal horns. These latter fibers occasionally extended as far as the posterior commissure and in a few sections were seen to cross the midline.

In the ganglionectomized animals, although there was increased nonspecific staining in white matter surrounding the deafferented dorsal horn, VIPIR of the laterally projecting fibers was qualitatively reduced on the deafferented side in cord segments below the level of the ganglionectomies. No increase in VIPIR was seen on the deafferented side at levels L6, L7, or S1.

The distribution of VIPIR in control animals is consistent with that found by other researchers. We found no evidence for increased VIP-fiber input to deafferented segments. In fact, our findings suggest that there is some VIP-fiber input from the S1 level, which was ganglionectomized. While this study does not show evidence for sprouting, it may be that lost input at the S1 level reduces our ability to demonstrate sprouting by this method.

107.6 NEUROPEPTIDE DISTRIBUTION IN THE SACRAL SPINAL CORD OF THE DOMESTIC FOWL, GALLUS DOMESTICUS. S.L. Freedman, L.F. May*, J.A. Morton*, D.C. Foss*, and R.M. Kriebel. Depts. of Anatomy & Neurobiology and Animal Sciences, Univ. of Vermont, Burlington, VT 05405.

Substance P, somatostatin, and met-enkephalin fibers have been localized by others in the spinal cord of the domestic fowl, Gallus domesticus (J. Comp. Neur. 213:406, 1983). In the present study the distribution of substance P and met-enkephalin fibers was confirmed. However, the primary objective of this investigation was to determine immunohistochemically the occurrence and distribution of gut-related and other peptides specifically within the sacral spinal cord. Hens were perfused intracardially with 4% paraformaldeyde in phosphate lysine-periodate buffer. The spinal cord and associated spinal nerves were carefully dissected and the spinal cord segmentally blocked according to each spinal nerve's contribution to the formation of the pudendal-pelvic nerve plexus (Anat Rec. 147:431, 1963). Thoracic and lumbar spinal cord were also obtained from each specimen for comparison with the peptide distribution in sacral segments. The tissues were immersed in fixative for 4 h followed by storage in sucrose phosphate buffer at 4°C. Frozen sections were cut at 30μm either horizontally or transversely and processed free-floating for immunohistochemistry; adjacent sections were stained with cresyl violet for orientation. Peroxidase anti-peroxidase methods were used to visualize antisera to substance P, leu- and met-enkephalin, LHRH, VIP, CCK, bombesin, and neuropeptide Y. Substance P and met-enkephalin fibers were distributed in the dorsal and intermediate areas of the gray matter at all levels of the spinal cord examined. Immunoreactive fibers for VIP, CCK, bombesin, and neuropeptide Y were observed only in sacral cord levels. In decreasing order, the immunoreactivity of nerve fibers in the sacral cord was VIP, CCK, neuropeptide Y, and bombesin. Each of these peptides was concentrated in the dorsal horn (laminae I-II) and around the central canal. The intermediate gray distribution corresponds to the bilateral intermediomedial cell column. In mammals it has been suggested that the gut-related peptides, such as VIP, are preferentially contained in pelvic visceral afferent fibers. The present study suggests that gut-related peptides are present also in the avian spinal cord at levels associated with pelvic visceral innervation.
Supported by BNS-8206452.

107.7 IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF THE CAUDAL NEUROSECRETORY COMPLEX IN THE PADDLEFISH (POLYODON SPATHULA) AND THE GAR (LEPISOSTEUS PLATYRHINCHUS). D. Onstott, K.E. Miller, and R. Elde. Department of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

The morphology of the caudal neurosecretory complex (CNC) and the pharmacology of its major hormones, urotensins I and II (UI,UII), have been extensively studied in teleost fish. More recently, the nature of its innervation has been explored and urotensins immunohistochemically localized within this system in teleosts. However, little is known about the CNC in the other major actinopterygian groups, the Chondrostei and Holostei, in which it may retain some ancestral characteristics. We have used an antiserum to corticotropin-releasing factor (CRF) that cross-reacts completely with UI and an antiserum raised against synthetic UII to localize UI/CRF and UII immunoreactivities within the caudal spinal cords of the paddlefish (Polyodon spathula) and the gar (Lepisosteus platyrhinchus). In addition, we have used electron microscopy to examine the ultrastructural organization of the CNC in these species.

For immunohistochemical studies the vertebral column was fixed in 2% paraformaldehyde and 15% saturated picric acid and decalcified. Cryostat sections were processed using the indirect immunofluorescence method. Two spinal cords were fixed and processed for electron microscopy.

In both species, the neurosecretory termination area was visible as a thin band of intense UII or somewhat weaker UI/CRF immunoreactivity closely apposed to the ventral surface of the spinal cord. Similar immunoreactivity was also found in numerous perikarya, which, however, differed between the two species in their morphology, number, and distribution. Partial co-containment of UI/CRF and UII immunoreactivities was observed in cells of both species. Many rostro-caudally and dorso-ventrally oriented UI/CRF- and UII-immunostained fibers were also visible. The ultrastructure of CNC neurons was similar to other species with the cytoplasm containing numerous large granular vesicles (150nm). CNC perikarya were often apposed by glial processes and also received synaptic contacts. Synaptic terminals most commonly contained small clear vesicles (40nm) or small clear vesicles and small dense core vesicles (70nm). A thin layer (2-4um) of neurosecretory terminals was observed on the ventral and ventro-lateral surface of the spinal cord. Unlike teleosts, the neurosecretory terminals did not contact capillaries, but terminate at the spinal cord surface. The neurosecretory granules appear to release their content into the meninx of the spinal cord.
Supported by Graduate School, Univ. MN and ImmunoNuclear Corp.

107.8 LUTEINIZING HORMONE-RELEASING HORMONE (LHRH)-IMMUNOREACTIVE (IR) CELLS OF THE TERMINAL NERVE, BUT NOT OTHER LHRH-IR NEURONS IN THE GOLDFISH BRAIN, ARE ALSO FMRFAMIDE- AND TACHYKININ-IR. Ann L. Kyle*, W.K. Stell, and O. Kah*. Dept. of Anatomy and Lions' Sight Centre, University of Calgary, Calgary, Alberta, Canada. T2N 4N1

Several cell groups within the teleost brain are immunoreactive (IR) for luteinizing hormone-releasing hormone (LHRH). In the goldfish, the LHRH-IR cells of one of these groups, the terminal nerve, are also IR for the molluscan cardiac excitatory peptide, FMRFamide (Stell, Walker, Chohan and Ball, PNAS, 81: 940), and a tachykinin-like peptide (TK) that is similar to, but not identical with, substance P (Stell, Chohan and Kyle, Invest. Ophthalmol. Vis. Sci. Suppl. 1984). We asked whether colocalization of LHRH- with FMRFamide- and/or TK-IR was a general feature of LHRH-IR cells or a characteristic specific to the terminal nerve.

Alternating, serial sections of goldfish brain were incubated with antiserum to teleost LHRH (Sherwood GF4) and adjacent sections with antiserum to FMRFamide (O'Donahue 232); other brains received antisera to LHRH and substance P (ImmunoNuclear AB 353, lot 8352022). IR was visualized using the avidin/biotin immuno-peroxidase method (Vector Labs.).

LHRH-IR was found consistently in three locations: 1. in the terminal nerve ganglion, consisting of large bipolar and multipolar cells embedded within the olfactory nerve; 2. in a loose array of ventro-laterally located bipolar cells extending through the telencephalon, preoptic area (majority of cells), and hypothalamus to the pituitary stalk; and 3. in large, multipolar cells between the posterior commissure and the midbrain tegmentum (synencephalon). Neither the ventro-lateral bipolars nor the large cells of the synencephalon were IR for either FMRFamide or TK. In contrast, as previously reported, the terminal nerve was IR for all three peptides. This suggests that the LHRH-IR cells of the terminal nerve represent a population different from the other LHRH-IR neurons in the goldfish brain.
This work was supported by The Natural Sciences and Engineering Research Council of Canada, and The Alberta Heritage Foundation for Medical Research.

107.9 ULTRASTRUCTURE OF LUTEINIZING HORMONE-RELEASING HORMONE(LHRH) NEURONS IN INTACT AND CASTRATED MALE HAMSTERS. M.N. Lehman and A.-J. Silverman. Dept. Anatomy & Cell Biology, Columbia Univ. Coll. Phys. & Surg., New York, NY 10032.

As preliminary observations to examining possible photoperiodic influences upon the structure and synaptology of LHRH neurons, we studied the morphology of these cells, using ultrastructural immunocytochemical procedures,in intact and long-term (3-6 months) castrated male hamsters housed under a 14L:10D photoperiod. Animals were perfused with 4% paraformaldehyde/0.2% glutaraldehyde. Vibratome sections (60um) were incubated with primary antiserum LR-1 (gift of R. Benoit) containing .02% saponin and processed using an avidin-biotin-immunoperoxidase kit (Vectastain). Areas of interest were osmicated, and embedded in Epon. Thick sections (1 um) were cut to locate immunostained perikarya, and subsequently serial thin sections were collected. LHRH cells in the medial septum, vertical limb of the diagonal band, and medial preoptic area were identified by a granular reaction product which was unevenly distributed within the cytoplasm of each neuron. Reaction product was most often associated with the rough endoplasmic reticulum (RER), particularly that RER adjacent to the nucleus, and was also associated with secretory granules, but not with the Golgi apparatus, nor the nucleoplasm. The nuclei of immunoreactive (IR) cells in both intact and castrated animals had several prominent identations and contained multiple nucleoli; dense reaction product was seen in aggregates along the cytoplasmic face of the nuclear membrane, and within their identations. The soma and dendrites of LHRH cells in this species receive extremely few, if any synaptic inputs. Of a subset of nine cells extensively analyzed through serial sections, we found only one example of synaptic input to an LHRH neuron. In that case two non-IR axon terminals containing clear round vesicles contacted the distal end of a tortuously bent dendrite and formed symmetrical synapses. In contrast, adjacent non-IR soma and dendrites in the surrounding neuropil were richly innervated. The plasma membrane of LHRH neurons was often separated from the adjacent neuropil by thin glial processes. Occasionally close somatic appositions were seen between LHRH cells and non-IR neurons, and in one instance between two LHRH perikarya, but no evidence of gap junctions between adjacent plasma membranes was seen. The extreme paucity of axosomatic and axodendritic synapses onto LHRH neurons in this species is in striking contrast to the synaptic arrangements of LHRH cells in the guinea pig (Silverman, JCN 227:452) and suggests that the regulation of their pulsatile activity may occur either at the level of their terminals within the median eminence, or by humoral signals. Supported by HD 10665 (AJS) and MH 15174 (MNL).

107.10 IMMUNOCYTOCHEMICAL LOCALIZATION OF LUTEINIZING HORMONE (LH) IN RAT BRAIN. G. Hostetter, A. Eaton* and M. S. Brownfield*. Department of Cell Biology and Anatomy, Medical School, Oregon Health Sciences University, Portland, OR 97201, and Department of Structural and Functional Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

The distribution of luteinizing hormone (LH) in rat brain was traced by immunocytochemistry. Immunocytochemical processing was conducted on tissue from ten adult male Sprague-Dawley rats, six untreated and four pretreated with intracerebroventricular injections of 100µg colchicine 24 hours before sacrifice. The animals were sacrificed under deep anesthesia by perfusion through the aorta with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.5. Brains and pituitaries were removed, post-fixed, and cut into 50µm-thick sections. The sections were processed by pre-embedding immunocytochemistry using the peroxidase anti-peroxidase (PAP) and avidin-biotin (ABC) methods. Four LH antisera were employed as the primary antibody, two generated against the intact LH molecule and two against LH beta (generous gifts of Drs. H. Papkoff and B. Kerdelhue). Immunocytochemical specificity was established by pre-incubating the LH antibodies with rat LH, TSH, prolactin, growth hormone, ovine FSH, and synthetic ACTH, as well as by examining the LH staining in the pituitary. The primary antisera were used in brain at a dilution of 1:250-1:1000 for 16 hours at 4°C. Following incubation in the primary antibody, the bridging antibodies and PAP or ABC were applied sequentially. Bound peroxidase was visualized with 30mg % diaminobenzidine tetrachloride with 0.01% hydrogen peroxide in Tris buffer.

Cell bodies, immunoreactive for LH, were localized within the hypothalamic arcuate nucleus, ventromedial nucleus, and the periarcuate area ventral to the ventromedial nucleus. Immunopositive fibers were traced to numerous structures, including discrete regions of the hypothalamus, thalamus, septal area, amygdala, and the brainstem, including periaquaductal gray, raphe nuclei, brainstem reticular nuclei, and the locus ceruleus. This pattern reflects the distribution shown by previous radioimmunoassay studies of LH in brain extracts (Hostetter et al, 1981; Emanuele et al, 1981). Continuing research in our laboratories focuses on the function of this widespread system of brain LH.

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107.11 IMMUNOHISTOCHEMICAL EVIDENCE FOR A SUBCHIASMATIC PATHWAY CONTAINING LUTRINIZING HORMONE RELEASING HORMONE, SOMATOSTATIN, NEUROPEPTIDE Y AND TYROSINE HYDROXYLASE. Clive W.Coen[1], Peter R.C.Howe[2]* & Nihal C.de Lanerolle[3]. [1]Dept. Anatomy, King's College, London University, London WC2R 2LS, [2]CSIRO Division of Human Nutrition, Adelaide and[3]Section of Neurosurgery, Yale University.

The presence of a subchiasmatic pathway in the female rat containing luteinizing hormone releasing hormone (LHRH) immunoreactivity was first described by Flerkó, Sétálo and associates (Brain Res. 152: 401, 1978 and 198: 63, 1980; Acta Morph. Acad. Sci. Hung. 29: 259, 1981); this was subsequently confirmed in the male rat by Hoffman & Gibbs (Neuroscience 7: 1979, 1982).

We have undertaken further immunohistochemical investigations of this pathway in females of various species. Rats and rhesus monkeys were perfused with 5% acrolein; human tissue was fixed by immersion in the same solution. Serial sections (50um) were cut by vibratome coronally or parasagittally through the hypothalamus and attached optic chiasm. Using a modified PAP method, the immunoreactive distribution of LHRH, neurophysin, somatostatin (SOM), neuropeptide Y (NPY), tyrosine hydroxylase (TH), dopamine β-hydroxylase and phenylethanolamine N-methyltransferase was examined in the rat; in the human and monkey tissue immunohistochemistry for only LHRH was performed. Fibres containing immunoreactive LHRH were found running on the inferior surface of the optic chiasm in the midline in each of the three species. Subchiasmatic fibres containing TH- or, but more rarely, SOM- or NPY- immunoreactivity were also identified in the rat; no staining occurred at this site with the primary antisera against the other substances.

The possibility of a functional relationship between the LHRH-, SOM-, NPY- and TH- containing processes identified in this study remains to be examined. Considerable evidence exists implicating hypothalamic catecholamines, especially adrenaline, in LH release (Coen & Coombs, Neuroscience 10: 187, 1983) and both dopamine and noradrenaline in the secretion of SOM (Chihara et al. Endocrinology 104: 1656, 1979); nevertheless, in this particular pathway only the enzyme for dopamine synthesis (TH) was observed.

These results indicate that a remarkably superficial subchiasmatic pathway, possibly involved in the regulation of luteinizing hormone release from the anterior pituitary gland, is common to rats and primates. In this context, it should be noted that attempts to disrupt the menstrual cycle in monkeys by hypothalamic deafferentation may fail when a dorsal approach is made (Krey et al., Endocrinology 96: 1073, 1975) but be successful with a transorbital procedure (Cogen et al., Endocrinology 107: 677, 1980); the latter approach (from the inferior aspect) would necessarily sever the subchiasmatic LHRH fibres in addition to those in more dorsal planes. This study indicates the importance of assessing hypothalamic deafferentations immunohistochemically.

The functional significance and the sites of origin and termination of the subchiasmatic fibres containing LHRH-, SOM-, NPY- or TH- immunoreactivity are under investigation.

107.12 THE ORIGIN OF IMMUNOREACTIVE SOMATOSTATIN FIBERS IN THE DENTATE GYRUS OF THE RAT I Bakst*, JH Morrison, CA Avendano*, DG Amaral. (SPON:G.Banker) The Salk Institute, La Jolla,Ca 92037.

We have immunohistochemically localized somatostatin immunoreactivity in the dentate gyrus of the rat. Antiserum (SS320) directed against somatostatin 28(1-12), a 12 amino-acid fragment of somatostatin 28, stains a dense plexus of fibers in the outer half of the molecular layer in the rat as well as the monkey. Antiserum SS309, which is directed against somatostatin 28, a NH_2 -terminally extended form of somatostatin, stains neuronal cell bodies in the hilar region, but there are no observable somatostatin positive processes that extend to the outer molecular layer. We have performed a series of experimental manipulations to determine the cell bodies of origin of the somatostatin immunoreactivity in the molecular layer.

To exclude that the somatostatin in the molecular layer of the dentate gyrus originates from cell bodies in the entorhinal cortex (the origin of the major projection to the somatostatin dense region) ten rats underwent unilateral deafferentation of the entorhinal projection to the dentate gyrus, and were allowed a two week survival, before being processed. This resulted in unilateral shrinkage of the molecular layer on the side of the lesion, but an apparent increase in somatostatin(SS320) immunoreactivity, possibly due to the fibers being more densely packed in a narrower area. In contrast, the injection of 0.5 microliters of 0.1% kainic acid into the hilus of 12 rats followed by a 10-14 day survival period, resulted in a general loss of hilar neurons as determined by Nissl staining, a severe loss of somatostatin immunoreactive neurons in the same region, aaccompanied by a virtual depletion of somatostatin (SS320) immunoreactive fibers in the molecular layer at the level of the injection site. Acetylcholinesterase histochemistry of adjacent sections showed a normal distribution of acetylcholinesterase fibers in the dentate gyrus including the molecular layer. Six additional rats were injected with 50 nanoliters of a 2% WGA-HRP solution in various regions of the dentate gyrus and processed 24 hours later, for the concurrent demonstration of the retrograde tracer, and somatostatin (SS309) immunoreactivity in the same tissue section using a modified histochemical method for HRP. In cases where the injection of WGA-HRP filled the hilus at septal levels, double labeled neurons (HRP and SS309) were found temporal to the injection, but confined to the hilar region; a smaller number of double labeled neurons was found in the contralateral hilus but no double labeled neurons were found in sub-cortical regions. Thus the major source of somatostatin positive fibers in the molecular layer of the dentate gyrus is from cells in the hilar region. There is evidence of a commissural somatostatin projection to the dentate gyrus as well. Supported by grant NS-20004 to DGA.

107.13 SOMATOSTATIN IMMUNOREACTIVITY IN SECOND-ORDER AUDITORY NEURONS OF THE RAT, M. von Krosigk*, S.R. Vincent, J.C. Brown* and E.G. McGeer (SPON: J. Steeves). Division of Neurological Sciences, Depts. of Psychiatry and Physiology, Univ. of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

The immunohistochemical localization of somatostatin in the central auditory system of the rat was examined using monoclonal antibodies to synthetic cyclic somatostatin. Large numbers of somatostatin-positive neurons were present in the temporal cortex, the inferior colliculus and the ventral nucleus of the lateral lemniscus. Also, a large number of intensely stained neurons were found in the ventral cochlear nuclei. Large multipolar somatostatin-positive neurons were sparsely scattered in both the anterior and posterior ventral cochlear nuclei. In addition, a great many small immunoreactive cells were present throughout the anterior ventral cochlear nucleus. Many of these had eccentric unstained nuclei and resembled the globular cell type (Osen K.K., J. Comp. Neurol. 136: 453, 1969).

The globular cells send thick axons to the contralateral medial nucleus of the trapezoid body, which terminates in large calyces. Large somatostatin-positive terminals were found surrounding the principal neurons of the medial nucleus of the trapezoid body and in thick sections these appeared to correspond to the calyces of Held. The morphology of these terminals was also examined using electron microscopy. Large somatostatin-positive terminals were observed, often in symmetric contact with cell bodies and major dendrites. The terminals contained round vesicles and the contacts were often interrupted by extracellular spaces. These features are consistent with the morphology of the calyces of Held.

Injections of the fluorescent tracer True Blue into the nucleus of the trapezoid body retrogradely labelled many of the neurons in the anterior ventral cochlear nucleus. These retrogradely labelled neurons could be simultaneously stained immunohistochemically for somatostatin.

These results suggest that somatostatin is contained in second-order auditory fibers arising in the anterior ventral cochlear nucleus and terminating in the medial nucleus of the trapezoid body of the rat.

In the cat, somatostatin-positive neurons were present in the temporal cortex, the inferior colliculus, and the ventral nucleus of the lateral lemniscus. However, only a few of the large multipolar cells of the ventral cochlear nuclei were stained. The small globular cells in this nucleus and the calyces of Held in the medial nucleus of the trapezoid body were not stained. Thus, there may be important species differences in the transmitters present in central auditory pathways. (Supported by the Medical Research Council of Canada.)

107.14 DISTRIBUTION OF SOMATOSTATIN IMMUNOREACTIVE NEURONS IN FRONTAL NEOCORTEX AND UNDERLYING "WHITE" MATTER OF THE HUMAN FETUS AND PRETERM INFANT. I. Kostović and A. Fučić*, Section of Neuroanatomy, Department of Anatomy, Medical Faculty, University of Zagreb, Šalata 11, 41001 Zagreb, Yugoslavia.

Somatostatin immunoreactive neurons are present in all cell-rich layers and underlying white matter of the primate adult cortex (Sorensen, Neurosci., 7:1227, 1982; Morrison et al., Brain Res., 262: 344, 1983; Hendry et al., J. Neurosci., 4:2447, 1984). In order to determine developmental appearance of somatostatin immunoreactive neurons in cortical layers we have investigated laminar distribution of somatostatin immunoreactive neurons in frontal neocortex of human fetus and preterm infant with immunohistochemical peroxidase-antiperoxidase and avidin-biotin conjugate systems. The examination of telencephalic wall in late fetuses (4,5-6 months of gestation) revealed the presence of somatostatin immunoreactive neurons below the cortical plate in the transient fetal, synapse-rich subplate zone. In addition, somatostatin immunoreactive neurons were found in the fetal "white" matter (defined as a zone of large bundles of parallel fibers). The vast majority of somatostatin immunoreactive neurons have relatively large cell bodies (13x9 micrometers in diameter). The main morphological feature of subplate somatostatin immunoreactive neurons are long, radiating dendrites. In preterm infants (6-8 months of gestation) there is an increase in number of small (8x4 micrometers in diameter) somatostatin immunoreactive neurons in the zone of transition between the deep cortical plate (prospective layers VI and V) and subplate layer. The major part of the cortical plate (prospective layers II-IV) does not contain somatostatin immunoreactive neurons at this age. In newborn infant somatostatin immunoreactive neurons were occasionally found within the layer III of the superficial cortex.
In conclusion, somatostatin immunoreactivity of the intrinsic neurons in the subplate zone and white matter appears before immunoreactivity of neurons in the cortical plate. This earlier appearance of somatostatin immunoreactive neurons within the deep cortical anlage and "white" matter may simply reflect an earlier differentiation of deep cortical layers. However, subplate and interstitial neurons may play a developmental role as special, fetal type of local circuitry neurons.
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107.15 NEUROPEPTIDE LIKE IMMUNOREACTIVITY IN HUMAN SPHENO-PALATINE AND TRIGEMINAL GANGLIA.
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Studies have shown the widespread occurrence of Vasoactive Intestinal Polypeptide (VIP), substance-P (SP), and Leu-Enkephalin (L-ENK) immunoreactivity in sensory or parasympathetic ganglia from a number of different animal models. The distribution, function and pathological significance of neuropeptides in human ganglia remains essentially unexplored. Neuropeptide studies in humans have been hampered by the problem of post mortem cellular autolysis evident within 12 hours of death. We have been able to obtain human specimens incidental to autopsy within 4-10 hours of death. In a number of these cases, fixation with phosphate buffered 4% paraformaldehyde produced tissue with adequate cytology. We have examined the sphenopalatine and trigeminal ganglia for VIP-like, SP-like and L-ENK-like immunoreactivity. Many of the cells in the sphenopalatine ganglia show VIP-like and L-ENK-like immunoreactivity diffusely throughout the soma cytoplasm . Reactive fibers can be seen occasionally within the ganglia. SP-like immunoreactivity was seen in a few small cells scattered among the larger cells. The trigeminal ganglia showed little or no VIP-like immunoreactivity but intense L-ENK-like and SP-like immunoreactivity was present in a number of cells. These results are similar to those obtained in animal models.

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107.16 STRUCTURE AND PEPTIDE-CONTENT OF THE PARACERVICAL GANGLION INCLUDING ATRIAL NATRIURETIC FACTOR- AND ENKEPHALIN-IMMUNOREACTIVE TERMINALS. R.E. PAPKA, H.H. TRAURIG AND P. KLENN*. Dept. Anatomy, Univ. KY. Sch. of Med., Lexington, KY 40536.

Some of our recent studies have examined the presence, distribution and origin of peptide-containing nerves in the female rat reproductive system, as well as the neuropeptide content of the dorsal root ganglia, paracervical ganglia (PG) and inferior mesenteric ganglion (IMG); these are the major sources of fibers innervating the reproductive organs. Neuron cell bodies in the PG project axons immunoreactive for neuropeptide tyrosine (NPY) and vasoactive intestinal polypeptide (VIP) to the reproductive tract whereas IMG neuron cell bodies project NPY-immunoreactive axons. Peptide-containing nerve fibers and terminals in the PG include those immunoreactive for substance P, VIP and Leu-enkephalin (L-ENK). The present study was undertaken to further characterize the structure of, and neural interactions in, ganglia which have inputs to the reproductive tract. Standard immunofluorescence methods, electron microscopic immunocytochemistry employing colloidal gold techniques and routine transmission electron microscopy were used to examine additional synaptic inputs to the PG and IMG and to examine the ultrastructure of the PG. VIP-immunoreactive fibers and terminals were present in the IMG. Atrial natriuretic factor (ANF)- and L-ENK-immunoreactive terminals were identified in the PG with both fluorescent and electron microscopic immunocytochemistry. These terminals were found adjacent to and in synaptic contact with PG neurons. Immunoreactivity was localized exclusively to large (90-100nm) dense-core vesicles in these terminals. Small intensely fluorescent (SIF)-catecholamine containing cells were numerous throughout the PG and adjacent nerve trunks. The SIF cells were present as isolated cell clusters and as individual cells closely adjacent to PG neurons. Specialized contacts between the SIF cells and PG neurons have not been observed. L-ENK- or ANF-immunoreactive terminals have not been observed contacting the SIF cells. These data have further defined the complex synaptic relationships and transmitter content of autonomic neurons which innervate the female reproductive tract. These complex interactions may provide a neural substrate which could play a significant role in the regulation of the female reproductive tract. (Supported in part by NIH-BRSG #RR05374).

107.17 **ANATOMICAL LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDE ON CEREBRAL ARTERIES.** S.H. Tsai, J.H. McLean, J.M. Tew and M.T. Shipley, (SPON. R. Cardell), Departments of Anatomy/Cell Biology and Neurosurgery, University of Cincinnati Medical Center, 231 Bethesda Avenue, ML 521, Cincinnati, Ohio 45267-0521

Calcitonin gene-related peptide (CGRP) is a fascinating molecule recently discovered by recombinant DNA technology (Amara et al Nature, 1982). In thyroidal 'C' cells the calcitonin gene produces an mRNA that encodes a precursor to the hormone calcitonin. However, in neural tissues alternative processing of the same gene leads to an mRNA that encodes CGRP and antibodies to CGRP stain a subset of CNS neurons in brainstem and forebrain circuits involved in a discrete set of functions including autonomic regulation. In particular, CGRP-like immunoreactivity is prominent in the nucleus of the solitary tract, trigeminal nucleus and nucleus parabrachialis - all structures implicated in neural regulation of cardiovascular function (Rosenfeld et al Nature, 1983). It was of interest, therefore, to determine whether this novel peptide is associated with cerebral vascular structures. Here we report that cerebral arteries are abundantly supplied by CGRP containing fibers and that these fibers are distributed in an intriguingly regular grid-like pattern.

Immunocytochemical staining with CGRP antisera (generously supplied by W. Vale - Salk Institute) was performed on whole mounts of the internal carotid, vertebral, basalar, posterior communicating, middle cerebral and anterior cerebral arteries. CGRP fibers were found on all arteries but the density of axons was highest on the posterior communicating, middle and anterior cerebral arteries. The distribution pattern of CGRP fibers is distinctly different from that of noradrenergic, cholinergic and other peptidergic fiber. In many instances CGRP fibers form a strikingly regular gridwork of longitudinal and circumferential fibers. These fibers are studded with numerous varicose swellings. The origin of the cerebrovascular CGRP fibers is currently under investigation. One possible source is the trigeminal nerve since we find that the trigeminal ganglion, especially the opthalamic division, is extremely rich in CGRP reactive neurons.

Given the intriguing localization of CGRP in brain sites involved in cardiovascular regulation and the present findings that cerebral arteries are richly supplied by CGRP axons, it will be of interest to study the potential physiological actions of CGRP on cerebral arteries and to investigate possible changes in the distribution of fibers following vasospasm. Studies of this kind are in progress.

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MOLECULAR BIOLOGY OF GENE EXPRESSION AND NUCLEIC ACIDS II

108.1 IDENTIFICATION OF GENES USING LIGAND PROBES: CALMODULIN-BINDING PROTEINS IN λgt11 EXPRESSION LIBRARIES. J. M. Sikela* and W. E. Hahn. Dept. of Anatomy, Univ. of Colorado Sch. Med., Denver, CO 80262.

Screening of expression libraries with antibodies to proteins of interest has become a powerful approach to the isolation of specific genes. Moreover, fusion proteins produced by recombinant DNA systems often display appropriate biological activity. Therefore we considered the use of various ligands as direct probes to identify the genes of the respective binding proteins (receptors).

Because calmodulin is known to bind several specific brain proteins (even as dissociated polypeptides) in the presence of Ca^{++} and because of calmodulin's major role in modulating the effects of Ca^{++} in the brain and other tissues, we chose to use calmodulin as a "model" probe to screen an expression library.

An adult mouse brain cDNA expression library was constructed in phage λgt11 and directly screened with ^{125}I calmodulin. Three clones were isolated that bound the probe in a Ca^{++} dependent manner. Lysogens were prepared from each phage clone and proteins produced by the lysogens used in dot blot analyses. Binding of ^{125}I calmodulin to the protein extracts was reduced in a competitive manner by addition of unlabeled calmodulin, was Ca^{++} dependent (EGTA sensitive), and only occurred in clones that had been induced to produce fusion proteins. SDS electrophoresis of the protein extracts from the induced clones yielded a band of the expected size for the fusion protein, whereas no similar sized band appeared using protein from uninduced lysogens.

When the proteins were electro-blotted onto nitrocellulose after SDS electrophoresis and the filter probed by ^{125}I calmodulin, only the band corresponding to the fusion protein bound calmodulin.

Characterization of the insert sizes of the clones indicates that they have a coding capacity of no more than 200 amino acids and, because of the cloning strategy used, represent the C-terminal end of the encoded proteins.

108.2 NERVOUS TISSUE SPECIFIC EXPRESSION OF CALMODULIN mRNA IN APLYSIA. K. Kruger*, M. Swanson, M. Palazzolo, S. Sturner*, J.H. Schwartz, (SPON: E. Shapiro). Howard Hughes Medical Institute, Columbia Univ. Coll. Physicians & Surgeons, New York, NY 10032.

Calmodulin is the common Ca^{2+}-binding protein that mediates ubiquitous cellular responses to increases in intracellular Ca^{2+}. In nervous tissue, Ca^{2+} is important for nerve cell transmission and synaptic plasticity. We have used the techniques of recombinant DNA to study the gene structure of this important regulatory molecule in the marine mollusc Aplysia, and to determine how its expression differs in nervous tissue from that in other parts of the body.

Calmodulin cDNA clones from electric eel and Torpedo were used as hybridization probes to isolate a partial calmodulin clone from an Aplysia abdominal ganglion cDNA library. This clone codes for the sequence of amino acids at the C-terminal third of Aplysia calmodulin which is identical to the amino acid sequence of vertebrate calmodulin. The Aplysia calmodulin cDNA was used as a hybridization probe to examine its gene organization in Aplysia. Southern blotting analysis with DNA isolated from the sperm of several individual specimens suggests that Aplysia calmodulin is encoded by a single gene locus, but with at least three polymorphic alleles. Thus, to characterize the nature of these genomic DNA sequences further, four unique genomic clones were isolated from an Aplysia sperm genomic library (provided by R.H. Scheller, Stanford University) using the Aplysia cDNA clone as hybridization probe. Restriction site analysis indicates that three of the four clones were derived from a larger contiguous 40Kb segment of the Aplysia genome. The fourth clone contained some restriction site variations when compared to this 40Kb genomic fragment and may be a second allele of this locus.

A mRNA specific to nervous tissue was identified by northern blot analysis of poly A^+ RNA from several Aplysia tissues, using the Aplysia calmodulin clone as hybridization probe. Abdominal ganglion and ring ganglia each contained three abundant mRNAs (2.5Kb, 3.0Kb, and 3.8Kb), while atrial gland, ovotestis, hepatopancreas, and buccal mass each contained only two mRNAs (2.5Kb and 3.0Kb). How is the larger mRNA generated and what is its function? Translation of hybrid-selected mRNA and mapping of the extra sequences in the 3.8 Kb mRNA should provide clues to the possible function of this specific neuronal mRNA.

108.3 HIPPOCAMPAL AND AMYGDALOID GENE EXPRESSION: NOVEL mRNAs IDENTIFIED BY SUBTRACTED cDNAs AND IN SITU HYBRIDIZATION. G.A. Higgins* and M.C. Wilson*. (SPON: K. Heidenreich) Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

We have used subtractive hybridization to isolate cDNAs which recognize mRNAs expressed by different neuronal cell types of the hippocampus and amygdala, but not other regions of the mammalian central nervous system. Single-stranded cDNA generated from poly(A^+) RNA from the hippocampus or amygdala was hybridized with a 20-fold excess of poly(A^+) RNA from cerebellum or cerebral cortex of the rabbit. Hydroxyapatite chromatography was used for isolation of cDNA sequences which did not hybridize to cerebellar or cortical RNAs, and thus are not expressed in, or are present in low abundance in these regions. Hippocampal- and amygdala-specific cDNA were rendered double-stranded and cloned into pBR322. Four cDNA libraries: hippocampus-cerebellum (H-Cb), hippocampus-cerebral cortex (H-Cx), amygdala-cerebellum (A-Cb) and amygdala-cortex (A-Cx) were screened by colony hybridization with a twice-subtracted cDNA probe and/or plus-minus screening with whole hippocampal and cerebellar cDNA. Regional specificity was assayed by hybridization of individual nick-translated cDNAs to blots containing fractionated RNA from several rabbit brain regions, whole mouse brain and liver.

Several different regionally distributed transcripts were identified. For example, H-CbE3 detects a 4.3 kb mRNA present at high abundance in the hippocampus, amygdala, and cerebral cortex, but expressed at lower levels in the cerebellum, and virtually absent from whole mouse brain and liver. Genomic blotting indicates that this cDNA hybridizes to multiple Eco RI and Bam HI restriction fragments in both mouse and rabbit. In contrast, A-CbE7 recognizes 1.1 and 0.6 kb mRNAs in all regions and tissues studied, but hybridizes to a 5.3 kb mRNA expressed only in the amygdala. We are currently mapping the cellular distribution of these hippocampal and amygdaloid mRNAs by in situ hybridization, and have begun sequencing to determine the identity of proteins encoded by these transcripts.

108.4 DETECTION OF mRNA BY IN SITU HYBRIDIZATION HISTOCHEMISTRY USING COMPLEMENTARY RNA (cRNA) PROBES. Ruth E. Siegel and W. Scott Young, III (SPON. G. David Lange), Lab. of Cell Biology., NIMH, Bethesda, Maryland 20205

Neuropeptide mRNAs in tissue sections have been detected by in situ hybridization histochemistry with labelled, complementary polynucleotide probes. Generally, nick-translated probes are used but suffer from random labelling, probe size variation and self-annealing. Single-stranded DNA or RNA probes of high specific activity are now easily made and avoid the above problems. The RNA:RNA hybrids formed also have higher melting temperatures than DNA:RNA hybrids.

We have made cRNA (and mRNA) probes using bacteriophage SP6 promoter and polymerase. We subcloned the mouse proopiomelanocortin (POMC) cDNA from MKSU16 (922bp; courtesy of Dr. E. Herbert) into pSP64 and pSP65. Two overlapping cholecystokinin (CCK) cDNA clones (courtesy of Dr. J. Dixon) were ligated at the XmaIII site and a 530bp fragment was inserted into pSP64 and pSP65. Drs. Yoshikawa and Sabol kindly supplied a 940bp preproenkephalin A (ENK) cDNA in pSP64 and pSP65. RNA probes were labelled with α-^{35}S- UTP to >10^8DPM/μg.

Mice and rats were perfused with 4% paraformaldehyde and 10% sucrose. Twelve micron frozen sections were hybridized according to the protocol of Harper (Science, 227, 177, 1985) with modifications. The sections were then apposed to emulsion-coated coverslips and exposed concurrently with ^{35}S-impregnated brain-paste standards.

We confirmed that POMC mRNA is located predominantly in the pituitary intermediate lobe and in scattered anterior lobe cells as well as arcuate nucleus neurons. We estimate that the most heavily labelled arcuate cells contain about 250 copies of POMC mRNA each. In these regions, there is an exact correspondance between ACTH immunostaining and hybridization signal. In addition to these regions positive for ACTH immunostaining, lateral hypothalamic neurons are stained for α-MSH, also believed to be encoded by the POMC gene. However, we do not detect any POMC mRNA in these cells at a sensitivity of less than 10 copies per cell. Therefore, we suspect that these neurons contain a non-POMC gene product. Still, a lower level of POMC mRNA synthesis may occur in these cells. Longer autoradiographic exposures, lower hybridization stringency, and synthetic oligonucleotide probes (Young et al., this volume) may resolve this issue.

Other neuropeptide mRNAs have been detected using cRNA probes. Using a CCK probe, we have observed specifically labelled neurons in the rat neocortex and pyriform cortex, for example. We have also observed ENK mRNA in neocortex, amygdala and hypothalamus. Studies to extend these observations are in progress.

108.5 MOLECULAR CLONING OF cDNAs FROM DIFFERENTIALLY-EXPRESSED HIGH AND LOW ABUNDANCE mRNAs OF DIFFERENTIATED AND UNDIFFERENTIATED MOUSE NEUROBLASTOMA CELLS. B.K. Schrier, Molecular Neurobiology Unit, Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD. 20205

In our previous work (Grouse et al., J. Biol. Chem. (1980)255: 3871-3877) we examined, by mRNA-cDNA hybridizations, changes in polysomal poly(A)$^+$ mRNA sequences during a Bu$_2$cAMP-induced differentiation step in mouse NS20Y neuroblastoma cells. We found that differentiation resulted in the disappearance of approximately 250 sequences which had been expressed in the undifferentiated (S) cells and the appearance of approximately 320 new sequences in the differentiated (P) cells. We have now purified the cDNAs of sequences unique to S and P cells by three cascade hybridizations. These cDNAs were made double-stranded using random hexamers as primers and were cloned using the Pst I site of pBR322 and E. coli strains C-600 and RR1. Based on samplings of the two libraries, they consist of >2700 (S-unique) and >1700 (P-unique) tet^R amp^S clones. Among 175 amp^S S-clones examined so far, 77 (44%) contained inserts <100 bp, which were considered likely to be of limited value; the average insert length of the remaining 98 clones (66% of those examined) was 356 bp, and 58% of these had inserts >200 bp (avg. 555 bp). Among 14 amp^S P-clones examined so far, 5 clones (36%) had inserts >185 bp (avg. 453 bp). These 103 S- and P-clones were grown on filters and probed with cDNA preparations made from total poly(A)$^+$ mRNAs from either S or P cells. Thirty-four of the S-clones (avg. 219 bp) and 2 of the P-clones (avg. 483 bp) appeared to be from middle-high abundance (HA) mRNAs; the remaining 64 S-clones (avg. 429 bp, 65%) and 3 P-clones (avg. 457 bp, 60%) were presumed to be low abundance (LA). Some HA and LA S-clones were used to hybridize to S-cell and P-cell mRNAs blotted onto nitrocellulose filters out of saturated NaI. Among 14 clones for which the result was clear, 8 were S-specific (i.e., did not hybridize to P-cell mRNAs), 3 reacted with S>>P, one reacted with S>P, one with S = P, and one appeared to be P-specific. This indicated that the cascade purification of S-cDNAs was successful, albeit imperfect. Further analyses of these and other clones from these libraries are continuing.

108.6 CLONING OF STAGE-SPECIFIC DORSAL ROOT GANGLION GENES. S.C. Ng, K. Wood*, M.C. Fishman. Neuroscience Group of the Howard Hughes Medical Institute, and the Department of Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114.

In order to help identify molecular changes that characterize the presynaptic neuron during various stages of synaptogenesis, we have begun isolation of stage specific cDNA from rat dorsal root ganglia (DRG). Five to ten thousand DRG were utilized to generate poly(A) RNA from embryonic day 14 (E-14), embryonic day 17 (E-17), and newborn (NB) rats. The majority of neurons in E-14 DRG have finished their final mitosis, and some have extended fibers to the spinal cord (SC). By E-17 DRG neurons are actively engaged in synaptogenesis with target cells in the SC, and these contacts evidence continued morphological maturation through birth.

The complexity of poly(A)$^+$RNA from each stage was examined by in vitro translation and subsequent two-dimensional gel electrophoresis of the translational products. Comparison of the electrophoretic profiles of the translational products at E-14, E-17, and NB revealed an essentially uniform pattern throughout development, with a very restricted subset that appeared to be developmentally regulated. These differences in translatable RNA suggested that each stage might indeed be marked by expression of a set of specific mRNAs.

Thus, cDNA cloning was performed using poly(A) enriched RNA from the three stages. A modification of the RNAase H method of Okayama and Berg (1982) was utilized for generation of the second strand, followed by methylation, addition of Eco RI linkers, and ligation into the Eco RI site of λgt10 and λgt11 vectors. Cloning efficiency was about 10^6 recombinants per microgram of poly(A)$^+$ RNA. Those clones representing mRNA expressed in a stage-specific manner are being isolated by examining each library using: (1) total ^{32}P labelled cDNA probes from different ages, and, (2) subtracted ^{32}P-cDNA probes that have been enriched for sequences representing one specific stage.

108.7 NGF INDUCTION OF GLUTAMATE DECARBOXYLASE (GAD) MRNA IN PHEOCHROMOCYTOMA CELL LINES.
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We are using our recently isolated recombinant DNA probe to study the induction of GAD mRNA in two pheochromocytoma cell lines -- PC-12 and PCG2. PCG2 is a clonal cell line established by Goodman and Herschman (1978) from an experimentally induced rat pheochromocytoma. Unlike PC-12 cells, PCG2 cells continue to divide after NGF induction and produce increased levels of tyrosine hydroxylase (TH) and TH mRNA.

To analyze the mechanisms by which NGF induces changes in these cell lines, we are measuring GAD mRNA levels in cells after different times of exposure to 7S NGF. Total RNA was isolated by the guanidine thiocyanate-cesium chloride method, and GAD mRNA was measured by "slot blot" hybridization to ^{32}P-nick translated GAD cDNA. This technique allows the quantitative determination of the concentrations of individual mRNAs. We have measured the sensitivity of this method using purified RNA transcribed by sp6 polymerase from GAD cDNA subcloned in pSP65 and can detect less than 1 pg of GAD mRNA.

Total RNA (10ug) from untreated PC-12 and PCG2 cells contains no detectable GAD mRNA sequences; that is GAD mRNA must be less than 0.1 ppm of the total, or less than 10 ppm of the poly (A) RNA. NGF-treated PCG2 cells contain detectable GAD mRNA after 3 days. PC-12 cells also contain GAD mRNA sequences after exposure to NGF for 10-14 days. We are now in the process of examining the time course of induction of GAD mRNA by NGF in both cell lines.

We have employed electrophoresis in methyl mercury followed by RNA blot hybridization to examine the size distribution of GAD mRNA sequences in PC-12 cells exposed to 7S-NGF for 14 days. The GAD mRNA sequences appear as a single size component, 3.7 kb long. This is the same size as the GAD mRNA in the brains of humans, cats, mice, and rats.

We are now using these cell lines to examine the regulation of GAD gene expression.

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108.8 CATECHOLAMINERGIC MODULATION OF PROOPIOMELANOCORTIN mRNA IN RAT PITUITARY INTERMEDIATE LOBE.
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The intermediate lobe (IL) of the rat pituitary contains both dopaminergic and beta-adrenergic receptors on cells that synthesize and secrete proopiomelanocortin (POMC) peptides. Dopoaminergic agonists decrease release of POMC peptides from the IL, whereas beta-adrenergic agonists increase release. To investigate if these compounds also exert opposite effects on POMC mRNA levels in IL cells we treated rats with a variety of catecholaminergic agents and determined POMC mRNA levels with a dot blot assay. Isoproterenol, a beta-adrenergic agonist, (5mg/kg ip, 4 days) produced a 30-35% increase in POMC mRNA, this increase was not seen with a lower dose (2mg/kg) given for 4 days, but injection of 2mg/kg on days 1,2 and 4 with sacrifice on day 5 produced a 23% increase suggesting that repeated administration might have produced desensitization. Haloperidol (2mg/kg ip, 4 days) produced a 25-35% increase in POMC mRNA which was also obtained by alternating injections of haloperidol (2mg/kg) and isoproterenol (5mg/kg) for 4 days ie. haloperidol on days 1 and 3, isoproterenol on days 2 and 4. Combined injections of haloperidol (2mg/kg) and isoproterenol (5mg/kg) produced an additive increase. In vitro studies using NIL primary cultures showed apomorphine (1 uM, 4 days) to produce a reversible decrease of approxiamately 50% in POMC mRNA levels. Isoproterenol (1 uM, 4 days) showed only a slight increase, due perhaps to either desensitization of the response as seen in vivo, or the removal of the tonic dopaminergic inhibition also found in vivo. These studies suggest that dopaminergic and beta-adrenergic compounds exert opposite effects on POMC mRNA levels. This might be modulated via cAMP formation as the two receptors are oppositely coupled to adenylate cyclase.
This work was supported by NIMH Grant #MH09059 (JK), NIDA Grant #DA02265 (SW and HA) and the T. Raphael Research Fund.

108.9 STUDIES ON PROENKEPHALIN GENE EXPRESSION IN PC12 CELLS.
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PC12 cells are widely used as a model to study neuronal differentiation. Unlike the adrenal medulla from which they are derived, these pheochromocytoma cells contain exceedingly low levels of opioid peptides. By using butyric acid, a differentiation promotor, we have been able to induce in these cells a twenty-fold increase in the levels of met^5-enk-arg^6-gly^7-leu^8-immunoreactive (met^5-enk-RGL-IR) peptides in a time and dose dependent manner. This increase is preceded by the appearance of proenkephalin mRNA, which is not readily detectable in untreated cells. This increase does not appear to be due to a general stimulation of DNA transcription, since the content of other specific mRNA species is simultaneously decreased. This suggests a direct action of butyrate on transcription; however, a post-transcriptional effect cannot be completely excluded. The molecular weight profile of the met^5-enk-RGL-IR peptides was examined using Bio-Gel P-30 chromotography. The antiserum used was very sensitive (IC_{50} = 10 fmol) and directed against the C-terminal end of met^{5}enk-RGL, thereby permitting detection of high molecular weight, N-terminally extended precursors. Butyrate-treated PC12 cells contained predominately low molecular weight (approx. 1kDal) and intermediate weight (5-10 kDal) met^5-enk-RGL-IR peptides. This contrasts with the profile of the met^{5}enk-RGL-IR peptides in the adrenal medulla of the adult New England Deaconess (NED) rat -the source of the PC12 cell line-which contains approximately equal amounts of intermediate and high molecular weight (15-20 kDal) IR peptides. When PC12 cells are grown in vivo as a tumor in the NED rat, they express low levels of met^5-enk-RGL peptides which are exclusively of the high molecular weight class. In summary, these results suggest that butyrate elicits the expression of the proenkephalin gene. This tool may be useful for investigating the molecular mechanisms involved in the expression and processing of proenkephalin-derived peptides.

108.10 THE REGULATION OF PRO-OPIOMELANOCORTIN GENE EXPRESSION IN AtT-20/D16-16 ANTERIOR PITUITARY CELLS.
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The AtT-20/D16-16 cell line offers an excellent model for studying the regulation pro-opiomelanocortin (POMC) gene expression. POMC is the precursor of several pituitary peptides that include β-endorphin, the melanocyte-stimulating hormones (α- and γ-melanocyte-stimulating hormones), and adrenocorticotropin hormone (ACTH). In studying the regulation of POMC gene expression, AtT-20/D16-16 cells were incubated with various doses and for various periods of time with a known stimulus of ACTH secretion, corticotropin-releasing factor (CRF). POMC mRNA levels were determined with a dot blot hybridization assay using a [^{32}P]-POMC cDNA probe labeled by nick translation. POMC mRNA was bound to nitrocellulose filters, incubated with labeled POMC cDNA probe, exposed to x-ray film and the mRNA levels were quanitated by densitometry. There was a gradual increase in POMC message levels up to 24 hours after treatment with 0.1μM CRF. It was also found that a maximum increase in POMC message was obtained after an incubation period of 24 hours with CRF. α-aminatin, a known transcription blocking agent, was shown to have an significant inhibition effect on POMC mRNA levels at a concentration of 1.0 μg/ml after 48 hours of exposure with and without CRF stimulation. This data indicated that CRF increased mRNA by inducing transcription. To directly determine if CRF affected the transcription rate of the POMC gene, nuclear run-off transcription assays were preformed. After treatment with 0.1 μM CRF, extracted nuclei were then incubated with [^{32}P]UTP. Nuclear RNA was then purified and hybridized to POMC cDNA probes bond to nitrocellulose filters with PBr322 plasmids as controls. A significant and maximal increase in POMC gene transcription was observed after 30 minutes of CRF stimulation. The results of these studies indicate that CRF induction of POMC gene expression is regulated at the transcriptional level.

108.11 GLUCOCORTICOIDS AND CYCLIC AMP SYNERGISTICALLY ELEVATE ENKEPHALIN-PRECURSOR mRNA IN NG108-15 HYBRID CELLS AND C6 GLIOMA CELLS. S. L. Sabol and K. Yoshikawa, Lab. Biochem. Genetics, NHLBI, National Institutes of Health, Bethesda, MD 20205.

NG108-15 mouse neuroblastoma x rat glioma hybrid cells contain very low amounts of Met- and Leu-enkephalin that are increased 2-3 fold by glucocorticoids such as dexamethasone (Dex) or by cyclic AMP (cAMP) derivatives (Glaser, et al. (1982) J. Neurochem. 39, 59; Braas et al. (1983) J. Neurosci. 3, 1713). This cell line was chosen by us as a model system to study mechanisms that regulate preproenkephalin (ppEnk) gene expression in neuronal cells.

NG108-15 cells were cultured in medium containing 10% steroid-depleted serum or in serum-free steroid-free medium and treated with Dex (varied concentrations) and/or 8-Br-cAMP (1 mM) or an adenylate cyclase (AC) activator (forskolin (10 μM) or prostaglandin E1 (10 μM)). Total RNA was electrophoresed, blotted, and hybridized with ^{32}P-labeled rat ppEnk cDNA (Yoshikawa, et al. (1984) J. Biol. Chem. 259 14301). PpEnk mRNA (1500 bases) was quantitated by densitometry of autoradiograms. To estimate absolute abundances of ppEnk mRNA, known amounts of RNA identical to the coding portion of ppEnk mRNA, prepared by SP6-polymerase dependent transcription of a pSP65-derived plasmid, were blot-hybridized as internal standards. The abundance of ppEnk mRNA in untreated NG108-15 cells was 50-100 fg/μg total RNA. Treatment with Dex alone (1 μM) for 48 hr elevated this level to 3 X the control; the half-maximally effective Dex concentration was 0.01 μM. Other glucocorticoid hormones were fully active while other classes of steroid hormones were inactive. 8-Br-cAMP or AC activators alone weakly elevated ppEnk mRNA levels to 1.4-1.6 x the control in serum-supplemented medium and had no effect in serum-free medium. In contrast, the combination of Dex + 8-Br-cAMP or AC activator elevated the ppEnk mRNA level to 6-8 X the control at 24 hr and 13 X the control at 5 days of culture in either medium. Similar stimulations of the ppEnk mRNA abundance were found when equal amounts of poly A+ RNA were analyzed. Actinomycin D, an inhibitor of transcription, almost totally suppressed the responses to Dex + 8-Br-cAMP. The ppEnk gene expressed in the hybrid cells is the rat rather than the mouse gene, as determined by hybridization with a rat-preferring probe of the 3' untranslated region of the mRNA.

C6 and C6BU-1 rat glioma cells (the latter a parent of NG108-15) were found unexpectedly to contain 2-3 pg ppEnk mRNA per μg total RNA, i.e. 20-60 times as much as NG108-15 cells, although the enkephalin content of C6BU-1 cells was reported to be generally lower than that of NG108-15 cells (Glaser et al.). The ppEnk mRNA levels in C6 cells were elevated to 6 X the control by Dex + 8-Br-cAMP, while treatment with Dex or 8-Br-cAMP alone had little or no effect. Beta-adrenergic receptor agonists, which stimulate C6 AC, elevated the ppEnk mRNA in the presence but not absence of Dex.

These findings demonstrate that the rat ppEnk gene can be transcriptionally activated by the synergistic action of glucocorticoids and elevated cAMP. This activation may be important in neuronal development as well as in certain types of stress. The results with C6 cells also suggest that the ppEnk gene may be expressed, at least transcriptionally, during the development of glial cells.

108.12 CLONED cDNA PROBES FOR SPECIES OF mRNA THAT ARE POSITIVELY OR NEGATIVELY REGULATED BY DIBUTYRYL cAMP IN NEUROBLASTOMA AND HYBRID CELLS. M.Y. Giovanni*, B. Raj*, B.S. Schrier, and M. Nirenberg. (SPON: M. P. Daniels.) NIH, Bethesda, MD, 20205.

Elevation of cellular cAMP levels of NG108-15 neuroblastoma-glioma hybrid cells or NS20-Y neuroblastoma cells for 5 or more days shifts relatively undifferentiated cells to a differentiated state with respect to some neural properties and increases the ability of the cells to form synapses. Cytoplasmic poly A$^+$ RNA was prepared from undifferentiated (D$^-$) logarithmically dividing NG108-15 and NS20-Y cells and from differentiated (D$^+$) cells that had been treated with 1 mM dibutyryl cAMP for 5-7 days. cDNA was synthesized from D$^+$ poly A$^+$ RNA and cloned into pBR322 by G-C tailing. Approximately 400,000 and 800,000 recombinants were obtained in the NG108-15 and NS20-Y cDNA libraries, respectively.

Some recombinants from each library were screened with D$^-$ and D$^+$ ^{32}P-cDNA probes prepared from cytoplasmic poly A$^+$ RNA of D$^-$ and D$^+$ cells by colony hybridization on replica filters after amplification of plasmid DNA. Four classes of recombinants were found: (1) colonies with more D$^+$ ^{32}P-cDNA hybridized than D$^-$ ^{32}P-cDNA, (2) colonies with less D$^+$ ^{32}P-cDNA hybridized than D$^-$ ^{32}P-cDNA, (3) colonies with approximately equal amounts of D$^+$ and D$^-$ ^{32}P-cDNA hybridized, and (4) colonies with little or no D$^+$ or D$^-$ ^{32}P-cDNA hybridized, i.e., either cloned cDNA for low abundance species of mRNA or plasmids that lack DNA inserts. In addition, dot blots of cytoplasmic poly A$^+$ RNA from D$^+$ or D$^-$ cells were hybridized with ^{32}P-DNA from different recombinant plasmids labeled by nick translation. The results show that prolonged treatment of NG108-15 or NS20-Y cells with dibutyryl cAMP results in increases in the abundance of some species of poly A$^+$ RNA, decreases in the abundance of other species of poly A$^+$ RNA, but has no effect on the abundance of most species of mRNA studied. The cloned DNA can be used as probes to study the mechanisms of regulation of mRNA abundance.

108.13 PROTO-ONCOGENE EXPRESSION IS INDUCED IN PC12 CELLS BY NERVE GROWTH FACTOR. T. Curran* and J. Morgan* (SPON: R. Wurzburger). Dept. of Experimental Oncology and Roche Institute of Molec. Biol., Roche Research Center, Nutley, NJ 07110.

Several retroviral genes, termed oncogenes (v-onc), are capable of subverting normal growth regulation thus resulting in malignant transformation. The viral genes have normal progenitor counterparts or proto-oncogenes (c-onc) in chromosomal DNA. The proto-oncogenes are presumed to function as critical modulators of cell proliferation and differentiation. Further, a number of polypeptide growth factors are known to induce proto-oncogenes in a variety of cell types.

This study has addressed the question of whether nerve growth factor (NGF) induces any c-onc in PC12 cells. Such information may then provide clues as to the mode of action of this trophic agent. A very specific stimulation of the c-fos oncogene has been detected in PC12 cells 30 minutes following addition of NGF. This stimulation can be detected by an increase in the c-fos protein after immunoprecipitation of pulse labeled proteins from PC12 cells as well as by an increase in total c-fos mRNA resolved by Northern blotting. Changes in the levels of mRNA for other oncogenes (c-ras^{Ha}, c-ras^{Ki}, c-myc and N-myc) were small, never exceeding 3-fold stimulation, and occurred at later times after addition of NGF. It has been established that certain peripheral type benzodiazepines (BZDs) can modify the response of PC12 cells to NGF. These actions include effects on neurite outgrowth and induction of ornithine decarboxylase. When these agents are present in the culture with NGF there ensues the superinduction of c-fos to at least 100-fold over basal expression. It should be noted that dose-response curves for both NGF and BZDs in inducing c-fos are indistinguishable from those for the induction of neurite growth and the ability of the latter to modify this action of NGF. Furthermore, the structure-activity relationship for the BZDs in modifying NGF-induced neurite outgrowth is identical to that for the superinduction of c-fos. Since the induction of c-fos is one of the most rapid genomic actions of NGF it may be that this gene plays a key role in mediating later transcription-dependent events.

In addition to the above results, BZDs may be used to begin to dissect the events that link NGF receptor binding with the induction of genes such as c-fos or ornithine decarboxylase.

108.14 ABUNDANT RAS IMMUNOREACTIVITY IN APLYSIA NEURONS. M. Swanson, T. Aldrich,* A. Elste,* M. Furth,* S.M. Greenberg and J.H. Schwartz. Howard Hughes Med. Inst., Columbia Univ. Coll. Physicians & Surgeons, New York, NY 10032; and Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Cellular homologs of the ras oncogenes of Harvey and Kirsten rat sarcoma virus have been isolated in phylogenetically diverse organisms including human, rodent, Drosophila, and yeast. Although the physiological functions of cellular ras proteins are not yet known, mammalian ras genes have been shown to code for guanine nucleotide-binding proteins with M_r 21,000. These are homologous to two higher molecular weight proteins in yeast that are required for GTP-stimulated adenylate cyclase activity; the ras p21 proteins also are related to the G proteins and to transducin.

Because cAMP mediates the presynaptic facilitation that underlies simple forms of learning in Aplysia and because of the possibility that ras genes are also important for cAMP metabolism in higher organisms, we have used a monoclonal antibody to p21-transformed protein encoded by Harvey murine sarcoma virus to identify molecules that might modulate the adenylate cyclase of Aplysia. Ras-immunoreactive antigen was detected by indirect rhodamine immunofluorescence in 16-um sections of nervous tissue. Strong immunofluorescence was detected in cell bodies, neuropil and axons of abdominal and cerebral ganglia, but not in the connective tissue sheath. Bright immunofluorescence was also detected in dissected sensory cells of the pleural ganglion. Some immunofluorescence was also seen in ovotestis, sperm, and eggs. Salivary gland was only weakly immunofluorescent and buccal muscle showed little or no fluorescence. Four of 8 monoclonal antibodies raised against viral H-ras p21 protein precipitated ^{35}S-methionine-labeled protein from Aplysia nervous tissue. These 4 antibodies cross-react with both cellular K-ras and H-ras proteins in all mammalian cells so far examined. Gel electrophoresis of immune precipitates revealed major labeled proteins of M_r 21,000, which are similar to mammalian ras proteins on two-dimensional gels, and a minor M_r 26,000 protein also seen in mammals. One of the 4 antibodies also precipitated labeled Aplysia proteins with M_r of 36,000 and 86,000. Similar cross-reactive proteins also were seen in many mammalian cell lines. Using the technique of genomic Southern blotting, we have identified two distinct Aplysia DNA sequences, one homologous to Harvey sarcoma virus ras DNA and the other to Drosophila ras DNA. We are currently cloning these sequences and shall determine their relationship to the conserved ras gene family.

108.15 DIFFERENTIAL EXPRESSION OF BETA-TUBULIN GENES WITH NEURITE GROWTH INDUCED BY NERVE GROWTH FACTOR.
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Microtubules consisting of tubulin proteins and microtubule-associated proteins are a major component of the neuritic cytoskeleton. They are important both for the structural integrity of the neurite and for axoplasmic transport. Tubulin proteins are encoded by several closely related genes that form a multigene family. It appears likely that individual members of this family are expressed in tissue- and function-specific manners. We have been studying the patterns of expression of tubulin mRNA before and during the period of neurite growth induced in PC-12 pheochromocytoma cells by nerve growth factor (NGF) with the use of cDNA probes isolated from a cDNA library produced from NGF-treated PC-12 cell mRNA.

We have found that RNA of untreated PC-12 cells contains two size classes that hybridize with a chicken beta-tubulin (pT2) cDNA probe in RNA filter blot hybridizations while NGF-treated cells contain only one size class (Huttner et al, 1984, Dev Biol Abstr 57). As a first step towards determining whether this changing pattern is due to differential gene expression within the tubulin gene family a cDNA library was created in lambda-GT 11 using mRNA of PC-12 cells treated 14 days with NGF. Several beta-tubulin encoding clones have been selected from this library by plaque hybridization screening with the chicken pT2 probe. The inserts of these clones have been partially characterized by restriction mapping and their 3' and 5' untranslated regions identified.

The gene-specific untranslated region fragments of these inserts are being used as hybridization probes with RNA filter blots containing RNA of NGF-treated and untreated PC-12 cells. Preliminary observations indicate that one of these contains cDNA for a beta-tubulin gene that is expressed only in NGF-treated cells. We are continuing to characterize the patterns of expression of the other clones, as well.

This work was supported by grants from NIH (#NS20356 and NS22256 to AJT), UCLA BRSG, and Dysautonomia. SLH was supported by an NIH Training Grant.

REGULATION OF PITUITARY FUNCTIONS II

109.1 CHARACTERIZATION AND REGULATION OF ION TRANSPORT IN CULTURED MONOLAYERS OF BOVINE PITUITARY FOLLICULAR CELLS.
N. Ferrara*, M. Moss, J. Widdicombe*[1] and R. Weiner. Dept. of OB/GYN and Repro. Sci. and CVRI[1], UCSF, San Francisco, CA 94143.

We have reported a method for the culture of follicular cells (FC) of the bovine anterior pituitary (AP) or pars tuberalis. FC grown on plastic or on extracellular matrix form homogeneous, contact-inhibited monolayers which rapidly develop domes. It is well established that domes are an expression of active transepithelial ion and fluid transport observed in morphologically and functionally polarized cells. The ultrastructure of FC, i.e. tight junctions and apical microvilli, is also consistent with such a function. We investigated the ion transport properties of FC monolayers mounted in Ussing chambers. Primary cultures of FC were grown in DMEM 0.1% glucose supplemented with 15% fetal bovine serum, glutamine and antibiotics. At confluency, cells were passaged and cultured onto Nucleopore filters coated with human placental collagen. A detectable potential difference (PD) and resistance developed 2-3 days after plating and rapidly reached a steady-state of 2-4 mV and 80-400 ohms x cm^2, respectively. Amiloride (10^{-4}M) dramatically decreased PD and short circuit current (I_{sc}) when applied on the luminal side of the chamber but was ineffective on the serosal side. The addition of serosal but not luminal ouabain (10^{-4}M) almost completely abolished the residual PD and I_{sc}. Serosal application of isoproterenol or epinephrine induced an immediate dose-dependent increase in PD and I_{sc} followed by a decline to levels higher than the original. The complete prevention of these effects by propranolol (10^{-5}M) was consistent with an action mediated by β-adrenergic receptors. The stimulatory action of β-adrenergic agonists on I_{sc} was not prevented by amiloride, but was significantly decreased in the absence of Cl, suggesting that at least some of the increase in I_{sc} was due to stimulation of active Cl^- secretion. Receptor binding studies on FC membrane preparations, using ^{125}I-iodocyanopindolol as a ligand, demonstrated the presence of a high affinity saturable β-adrenergic binding site. These studies demonstrated the ion transport properties of FC and the regulation of ion transport by β-adrenergic agonists. It is tempting to speculate that in vivo FC are involved in the regulation of the ion concentration and osmolarity of the interstitial fluid of the AP. This work supported by NIH grant HD08924 and NIH PPC-HL-24136.

109.2 PRESENCE OF ARTERIOGENESIS IN SPONTANEOUS ANTERIOR PITUITARY TUMORS OF OLD AGE MICE.
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We have reported that the development of estrogen-induced prolactin-secreting anterior pituitary gland (AP) tumors in rats is accompanied by the development of a direct arterial blood supply (arteriogenesis) to the AP. Since systemic arterial blood contains subphysiological concentrations of the prolactin inhibitory hormone, dopamine, regions supplied by the artery escape tonic dopaminergic inhibition. Removal of dopamine inhibition was hypothesized to be involved in the etiology of tumor formation. In the present study, we examined the development of arteriogenesis coincident with the presence of spontaneous tumors in old age mice. Approximately 50% of this strain of mouse develops prolactin secreting tumors by 24 months. Fifteen- and 30-month-old mice C57B6/6J (USC colony) were anesthetized and 0.2 ml saline containing 300,000 microspheres (15 μm in diameter) were injected into the left ventricle of the heart. The pituitary was removed, fixed in paraformaldehyde, embedded in JB-4 plastic, 5 μm sections cut, and every other section microscopically examined for the presence of microspheres. Because of their size, microspheres are trapped in the primary portal capillaries and normally do not reach the AP. If a direct arterial supply has developed, microspheres can reach the gland. In nine 15-month-old mice (pit. wt $\bar{X}$ = 2.5 ± 0.1 mg), no AP tumors were observed. Eight of 12 AP from 30-month-old mice contained grossly identifiable tumors. Histologically, the tumors were composed of cords of secretory cells surrounded by vascular lakes consisting of extravasated red blood cells. The four AP from 30-month-old mice (pit. wt $\bar{X}$ = 2.2 ± 0.1 mg) without identifiable tumors and the nine AP from the 15-month-old mice contained no microspheres. Three AP from 30-month-old mice with tumors contained 15, 65, or 76 microspheres. The total pituitary weight of these pituitaries was 6.6, 43.6, and 5.7 mg, respectively. The additional five AP with tumors (pit. wt $\bar{X}$ = 3.3 ± 1.5 mg) contained 3 or less microspheres. These data demonstrate the occurrence of arteriogenesis in some spontaneously formed AP tumors in old aged mice, and are consistent with our previous observations in estrogen-induced prolactin-secreting tumors in rats. The findings further support a role for vascular changes in the etiology of AP tumors. Supported by NIH Grants HD 08035, HD 06243, and AG 00117.

109.3 POTASSIUM STIMULATED CALCIUM 45 UPTAKE INTO GH_3 PITUITARY CELLS: EFFECTS OF INHIBITORS OF LIPOXYGENASE AND PHOSPHOLIPASE A_2. L. Baird* and L. Grandison (SPON: A. Sinha) UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854.

The intracellular processes regulating secretion of prolactin from pituitary cells involve several second messenger systems. Recently we and others have suggested that arachidonic acid or its metabolites may participate in the intracellular processes modulating secretion. Part of the evidence supporting involvement are the observations that pharmacological blockade of arachidonic acid release or metabolism potently inhibits prolactin release. Phospholipase A_2 (PLA2) hydrolyzes phospholipids thereby releasing arachidonic acid. Lipoxygenase (LPO) catalyzes the conversion of arachidonic acid to leukotriene (LT) products. In order to characterize the processes affected by PLA2 and LPO inhibitors we examined their action on potassium stimulated calcium uptake. The PLA2 inhibitors quinacrine, dibromacetophenone and U10029A blocked potassium induced calcium 45 uptake. The LPO inhibitors nordihydroguaiaretic acid (NDGA), BW 755C, ETYA and 15 HPETE also blocked induced uptake. None of the agents tested prevented potassium induced depolarization of the membrane. In further studies using cells preloaded with calcium 45 NDGA was found to block efflux induced by arachidonic acid. The actions of the PLA2 and LPO inhibitors on prolactin release occurred within the dose range found in other cell types to block phospholipase A_2 and lipoxygenase activity. The ability of PLA2 and LPO inhibitors to block calcium uptake can account for their ability to reduce prolactin release, a process partially dependent on calcium influx. Still unclarified, however, is the relationship between the ability of these agents to affect calcium influx and to inhibit PLA2 and LPO activity. If the activities are unrelated, these agents are inappropriate for investigating the involvement of arachidonic acid in the functioning of intact cells. However, these agents could be blocking the formation of arachidonic acid products that modulate calcium mobilization. (Supported in part by NIH Grant AM 32827)

109.4 INVESTIGATION INTO THE INVOLVEMENT OF DIACYLGLYCEROL LIPASE IN PROLACTIN SECRETION FROM GH_3 PITUITARY CELLS. A.M. Camoratto and L. Grandison. UMDNJ-Rutgers Medical School, Piscataway, N.J. 08854

Recent evidence suggests that arachidonic acid may modulate secretion of prolactin from the anterior pituitary. Arachidonic acid can be released from membrane phospholipids by the action of phospholipase A_2; alternatively, arachidonic acid can be derived from 1,2-diacylglycerol by the sequential actions of diacylglycerol lipase (DG lipase) and monoacylglycerol lipase (MG lipase). In this study we examined the involvement of DG and MG lipases in prolactin secretion from GH_3 cells using RHC80267, an inhibitor of DG and MG lipases. Incubation of the cells with RHC80267, at concentrations ranging from 10-100uM, resulted in a dose-related inhibition of basal prolactin secretion. Inhibition persisted for up to four hours, the longest time period examined. We next examined the effects of RHC80267 on secretagogue-induced prolactin release. The stimulatin of prolactin secretion produced by thyrotropin releasing hormone (TRH) could be reduced by a dose of RHC80267 which did not effect spontaneous prolactin release. The mechanism of TRH action is believed to include the activation of an inositol lipid-specific phospholipase C. Treatment of the cells with exogenous phospholipase C resulted in an increase in prolactin secretion; this increase could be blocked by a low dose of RHC80267. Both TRH and phospholipase C increase the levels of diacylglycerol in GH_3 cells. In contrast RHC80267 did not block stimulation of prolactin release produced by phorbol myristate acetate (PMA) or by melittin. The actions of PMA and of melittin occur by pathways which do not result in generation of diacylglycerol. In order to further characterize the mechanisms involved in the inhibition of basal and stimulated prolactin release by RHC80267 we examined the effect of this compound on potassium-stimulated 45-calcium uptake. RHC80267 blocked depolarization-induced calcium uptake into GH_3 cells at the same doses which had been effective in reducing prolactin secretion. The ability of RHC80267 to block TRH- and phospholipase C-stimulated prolactin release may be related to its effect on calcium influx. This action could be due to a direct effect of RHC80267 on calcium uptake which is not related to its ability to inhibit DG and MG lipase activity; however, inhibition would also occur if the products of DG and MG lipases were able to modulate calcium influx. (Supported in part by NIH Grant AM 32827).

109.5 GNRH RAPIDLY STIMULATES POLYPHOSPHOINOSITIDE BREAKDOWN IN ANTERIOR PITUITARY CELLS IN CULTURE. M.A.Sortino*, P.L. Canonico, C. Speciale*, R.M.MacLeod and W.S.Evans* (SPON: R.J.Krieg). Department of Pharmacology (MAS, PLC, CS), University of Catania, Catania, Italy and Department of Internal Medicine (RMM, WSE), University of Virginia, Charlottesville, VA 22908.

The mechanism of action of gonadotropin-releasing hormone (GnRH) seems to involve membrane phospholipids. An increase in [^{32}P] incorporation into phosphatidylinositol and phosphatidic acid by the peptide in cultured rat pituitary cells has been reported. It is now generally agreed that the initial reaction following receptor activation is the breakdown of inositol lipids, while phospholipid labeling may represent only a secondary effect. Therefore, to investigate the direct effect of GnRH on phosphoinositide breakdown we measured water soluble products (inositol phosphates) released during the hydrolysis of phosphatidylinositol by phospholipase C. During a 60 minute incubation, GnRH in a concentration dependent manner (and in the presence of 10 mM LiCl), caused a progressive increase in total [^{3}H]-inositol phosphate content in anterior pituitary cells from female rats in primary culture. The effect was significant at 1 pM ($p < 0.01$) and maximal (4-5-fold increase) at 100 nM. This effect was completely abolished by a 10 min preincubation with the potent antagonist [Nac-L-Ala^1,pCl-D-Phe^2,D-Trp^{3,6}] GnRH. Ten nM GnRH significantly stimulated total [^{3}H]-phosphate accumulation within 1 min. The fractionated measurement of inositol phosphates showed that inositol triphosphate (IP_3) and inositol bisphosphate appearance preceded that of inositol monophosphate. Ten nM GnRH also significantly reduced the level of [^{3}H]-phosphatidylinositol-4,5-biphosphate and [^{3}H]-phosphatidylinositol-4-phosphate, an effect that was already present within 20 sec. These results indicate that GnRH may act at the gonadotroph through a stimulation of polyphosphoinositide breakdown and a consequent increase of IP_3 and diacylglycerol responsible for gonadotropin secretion.

109.6 TRANSMEMBRANE SIGNALS MEDIATING NEURAL PEPTIDE SECRETION: ROLE OF PROTEIN KINASE C ACTIVATORS IN LHRH RELEASE. M. M. Valenca* and A. Negro-Vilar. Reproductive Neuroendocrinology Section, Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709

A variety of neurotransmitters and several intracellular messengers (Ca^{2+}, cAMP, PGE_2) have been shown to be involved in the secretion of LHRH from hypothalamic neurons. In few cases, however, activation of a given receptor has been linked with a specific intracellular messenger system to elicit release of this peptide. Many recent reports suggest that a variety of secretagogues act in different cells by enhancing metabolism of inositol phospholipids. This leads to the formation of a series of compounds which result in Ca^{2+} mobilization, activation of protein kinase C and also release of arachidonic acid. The latter can be metabolized to different products, including PGE_2, which we have shown to be a potent secretagogue of LHRH (Endocrinology 104:617, 1979). The present experiments were designed to determine the effects of a diacylglycerol and a phorbol ester, two specific activators of protein kinase C, on the secretion of LHRH in vitro. Phorbol 12,13-dibutyrate (PDBu) and a synthetic diacylglycerol, 1,2-didecanoylglycerol (DiC_{10}), were used to evaluate their effects on release of LHRH from median eminence (ME) fragments incubated in vitro. Adult intact male rats were used as tissue donors and all incubations were run at 37C in Krebs Ringer bicarbonate buffer. PDBu significantly increased LHRH secretion at concentrations of 50-200 nM. This stimulatory effect was also observed when tissues were incubated in Ca^{2+}-free, EGTA-containing media. The stimulation of LHRH by PDBu was apparently not dependent on an enhanced formation of arachidonic acid metabolites, since PDBu stimulated LHRH release in the presence of cyclooxygenase and lipoxygenase blockers. DiC_{10} produced a small increase in LHRH release at a concentration of 100 uM, while markedly enhancing PGE_2 formation. Blockade of lipoxygenase (NDGA,10-30 uM) greatly enhanced the LHRH release induced by DiC_{10}, an effect also observed in the absence of extracellular calcium. In conclusion, our results indicate that these well-known activators of protein kinase C can stimulate secretion of a neuropeptide, LHRH, from nerve terminals in vitro and, further, that endogenous 1,2-diacylglycerol may represent an important intracellular messenger participating in the events leading to LHRH release in response to a given secretagogue.

109.7 Leukotriene C_4 plays a role in prolactin release from 7315a tumor cells. A. M. Judd*, K. Koike*, I. S. Login*, and R. M. MacLeod. Dept. of Internal Medicine and Neurology, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

Arachidonate metabolism and hormone release appear to be closely linked processes in several endocrine organs. We have studied the effects of maitotoxin, a calcium channel activator, on arachidonate metabolism and on prolactin release from 7315a tumor cells. Primary cultures of 7315a cells were labeled for 90 minutes with [^{3}H] arachidonate (1 uCi/ml) to incorporate the fatty acid into esterified lipids. The cells were then washed with buffer without [^{3}H] arachidonate, incubated 30 minutes with vehicle or an inhibitor of arachidonate metabolism, and then exposed for an additional 30 minutes to vehicle or an inhibition of arachidonate metabolism and/or maitotoxin (5 ng/ml). The incubation medium was analyzed for prolactin by RIA; arachidonate and prostaglandins by TLC, and leukotrienes by HPLC. In 7315a cells maitotoxin significantly increased release of prolactin (300%), arachidonate (400%), prostaglandins E_2 and F_2 (PGE_2, PGF_2 , 300%), and leukotriene C_4 (LTC_4, 500%). We were unable to detect any production of leukotrienes B_4 or D_4 by the tumor cells. The increase in arachidonate metabolism produced by maitotoxin was related to calcium mobilization because maitotoxin had no effect on prolactin or arachidonate release in low calcium incubation medium. In order to assess the role of arachidonate metabolites on prolactin release, 7315a cells were exposed to inhibitors of arachidonate metabolism. Quinacrine, an inhibitor of arachidonate liberation from phospholipids decreased LTC_4 and prolactin release. BW755c, an inhibitor of conversion of arachidonate to its metabolites, inhibits maitotoxin-induced prolactin, PGE_2, PGF_2 , and LTC_4 release but did not alter arachidonate release. Indomethacin, an inhibitor of PG systhesis, decreased PGE_2 and PGF_2 release but did not modify prolactin, LTC_4, or arachidonate release. In contrast nafazatrom and NDGA inhibitors of leukotriene production decreased LTC_4 and prolactin release but did not alter PGE_2, PGF_2 or arachidonate release. In summary, calcium mobilization, arachidonate release and prolactin release are closely coupled processes in 7315a tumor cells. Furthermore LTC_4 production and prolactin release are closely related events. It is hypothesized that LTC_4, a potent activator of intracellular calcium, may have a major role to augment prolactin release from 7315a tumor cells. (Supported by NIH Grant CA 07535).

109.8 PROPERTIES OF WHOLE-CELL CALCIUM AND POTASSIUM CURRENTS IN IDENTIFIED LACTOTROPHS FROM DISSOCIATED RAT ANTERIOR PITUITARY (AP). C.J.Lingle, S. Sombati*, M. Truman* and M.E. Freeman*. Dept. Biol.Sci.,Florida State University, Tallahassee, FL 32306.

The study of the electrophysiological properties of cultured primary cells is frequently compromised by the inability to identify specific cell types in a heterogeneous population. In our studies of the properties of rat AP cells, we have overcome this problem by using the reverse hemolytic plaque assay (Neill & Frawley, Endocr. 112:1135, 1983) to identify lactotrophs for concurrent electrophysiological examination with standard patch-clamp techniques (Lingle, et.al. Biophys. J.47:136a,1985).

Inward currents of plaque-identified lactotrophs from long-term ovariectomized rats were examined following rupture of cell-attached patches (inside solution: 130 mM CsCl, 20 mM EGTA, 10 HEPES; outside: 0.5-1 uM TTX, TRIS and 10 or 50 mM $CaCl_2$). Inward currents following subtraction of leak and capacitative currents appears to consist of two components: a transient (3-10 ms to peak) component activated at low voltages (-30 to 0 mV) which inactivates almost completely over 100 ms and a sustained inward current. 0.5 mM Cd^{++} exerts a differential effect on the two components, blocking the sustained current completely, while the transient component is somewhat resistant particularly at depolarizations to less than 0 mV. For over 10 cells, peak inward current in 10 mM Ca^{++} was approximately 50 pA, and, in 50 mM Ca^{++}, near 100 pA. The magnitude of Ca^{++} currents from AP cells of untreated ovariectomized rats appears to be substantially smaller than observed for clonal AP lines.

Three outward current components have been resolved (in: 115 KCl, 10 EGTA, 10 HEPES; out: TTX, 1.6 Ca^{++}). On depolarizing steps from -60 mV, outward currents contain a transient shoulder which decays to a steady-state current. 30 mM TEA or 0 Ca^{++} blocks most of the late-sustained current, revealing an almost isolated transient current. Following inactivation of the transient current by conditioning steps to -20 mV, an additional small current was observed that activated with a sigmoidal time course over 20-50 ms and did not inactivate over 200 ms. The transient current appears to be an A current, the TEA- and Ca^{++}-sensitive current is a BK calcium-activated K^+-channel, and the sigmodal current appears to be a delayed rectifier current. Candidates for the unitary channels underlying each of these outward currents have been identified in cell-attached patch recordings. (Supported by NS-19139 to CJL and HD-11669 to MEF)

109.9 SEROTONIN AND DOPAMINE RECEPTORS IN THE RAT PITUITARY GLAND: AUTORADIOGRAPHIC LOCALIZATION. Errol B. De Souza and Theresa A. Kopajtic*. Laboratory of Neuroscience, Addiction Research Center, NIDA, Baltimore, MD 21224, and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Serotonin and dopamine have been shown to regulate pituitary hormone secretion both through effects in brain and by direct actions on the pituitary gland. In addition to the primary role for dopamine in the portal blood as an inhibitor of prolactin secretion from the anterior pituitary gland, dopamine-containing fibers that terminate on intermediate lobe cells of the rat pituitary have been shown to inhibit the release of proopio-melanocortin derived-peptides. High serotonin concentrations have been detected and serotonergic nerve fibers have been localized in the intertermediate lobe of the rat pituitary. Radioligand binding studies in homogenates have identified and characterized dopamine receptors in anterior, intermediate and posterior lobes of the pituitary gland; however, serotonin receptors remain to be identified in the pituitary. In the present study, we used ^{3}H-spiperone to identify and localize dopamine D-2 and serotonin 5HT-2 receptors in rat pituitary by in vitro labeling light microscopic autoradiography. Differential drug sensitivity allowed the selective displacement of ^{3}H-spiperone from D-2 receptors by the dopamine agonist ADTN (1 uM) and from 5HT-2 receptors by the serotonin receptor antagonist cinanserin (0.3 uM). A very high concentration (fmoles/mg protein; mean + SEM) of dopamine D-2 receptors was found in the intermediate lobe (985 + 28) with much lower concentrations present in the anterior lobe (89 + 7); ^{3}H-spiperone binding in the posterior lobe was variable. A significant concentration of cinanserin-displaceable ^{3}H-spiperone binding sites was present only in the intermediate pituitary (290 + 28). There was a fairly uniform pattern of distribution of ^{3}H-spiperone binding sites within each lobe of the rat pituitary. To selectively visualize 5HT-2 receptors in the intermediate lobe of the pituitary gland, we examined the autoradiographic distribution of ^{125}I-LSD which binds with a high affinity to 5HT-2 receptors. The specific serotonin 5HT-2 receptor agonist ketanserin (100nM) was used to define ^{125}I-LSD binding to 5HT-2 receptors. Ketanserin-displaceable ^{125}I-LSD binding sites were present in significant quantities and were uniformly distributed in the intermediate lobe of the rat pituitary. In summary, our results provide the first identification of serotonin 5HT-2 receptors in the pituitary gland and confirm the heterogeneous distribution of dopamine D-2 receptors within the rat pituitary. These data provide further evidence for the functional importance of dopamine in regulating pituitary function and suggest a physiological role for serotonin in regulating intermediate lobe hormone secretion. Supported by DA00266, NS15080 and MH00053 and the McKnight Foundation.

109.10 THE ROLE OF SEROTONIN RECEPTOR SUBTYPES IN THE REGULATION OF CORTICOSTERONE AND BETA-ENDORPHIN SECRETION IN THE RAT. J.I. Koenig, G.A. Gudelsky and H.Y. Meltzer. Dept. of Psychiatry, Univ. of Chicago and Ill. State Psychiatric Inst., Chicago, IL. 60637.

The activation of serotonin (5-HT) receptors by quipazine has been found to stimulate the secretion of corticosterone (B) in the rat. Ketanserin, a highly selective 5-HT_2 antagonist, potently inhibited the effect of quipazine, suggesting that 5-HT_2 receptor activation mediated the B-releasing effect of quipazine (Res. Comm. Chem. Path. Pharm. 46:151). Quipazine and the serotonin precursor, 5-hydroxytryptophan, have also been shown to elevate plasma levels of beta-endorphin (END) in the rat (Endocrinology 109:421). The goal of the present study was to ascertain the role of the 5-HT_1 receptor and further clarify the role of 5-HT_2 receptors in the regulation of the secretion of B and END in the rat. Adult male Sprague-Dawley rats were used in all studies. Thirty min after the administration of the 5-HT_1 agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) or the 5-HT_2 agonist 6-chloro-2-(1-pipraziny1)pyrazine (MK-212) or their respective vehicles, the animals were sacrificed by decapitation. When antagonists were employed, these drugs were injected 30-60 min before the agonists. Trunk blood was collected into chilled plastic tubes containing EDTA and plasma was collected following centrifugation. Plasma concentrations of B and END were determined by RIA. The administration of the 5-HT agonists 8-OH-DPAT (0.03-0.3 mg/kg) or MK-212 (0.3 mg/kg-3.0 mg/kg) caused dose-related elevations in plasma levels of both B and END. The injection of the vehicle did not change the concentration of either B or END in the plasma. The B- and END-releasing effects of MK-212 were potently blocked by the selective 5-HT_2 antagonists ketanserin (1 mg/kg) , mianserin (10 mg/kg) and altanserin (1 mg/kg). Activation of B and END secretion by 8-OH-DPAT (0.1 mg/kg) which selectively activates 5-HT_1 receptors was not antagonized by ketanserin, mianserin, altanserin or ritanserin. However, the B-releasing activity of 8-OH-DPAT was blocked by spiroperidol(1.0 mg/kg),which has substantial activity at the 5-HT_1 receptor subclass. Although 8-OH-DPAT also has dopaminergic properties, its effects on B and END were not blocked by haloperidol. Therefore, our data would suggest that B and END secretion can be stimulated by the activation of the 5-HT_1 or 5-HT_2 receptors. The effects of 5-HT_2 agonists may be blocked selectively by 5-HT_2 antagonists whereas activation of 5-HT_1 receptors is only antagonized by compounds possessing affinity for the 5-HT_1 receptors.
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109.11 EFFECTS OF FLUOXETINE, A SEROTONIN - UPTAKE INHIBITOR, ON SEROTONIN-IMMUNOREACTIVE INNERVATION OF THE PITUITARY NEUROINTERMEDIATE LOBE. L.C. Saland, J.A. Wallace and F. Comunas*. Dept. of Anatomy, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

We have previously demonstrated that serotonin-immunoreactive (5-HT-IR) nerve fibers to the pituitary gland are differentially affected by anti-serotonin and anti-catecholamine (CA) drugs (Saland et al, 1984, Soc. Neurosci. Abst. 10:87 and Manuscript submitted). P-chlorophenylalanine eliminated 5-HT-IR in the entire neurointermediate lobe, while 6-hydroxydopamine only eliminated intermediate lobe fiber staining. These results suggest that nerves that sequester the CA neurotoxin, might also take up and/or synthesize 5-HT. To further examine possible 5-HT uptake by pituitary innervation, we administered the 5-HT uptake inhibitor, fluoxetine hydrochloride, and studied 5-HT-IR in the rat neurointermediate lobes. Adult male Sprague-Dawley rats received 10 mg/kg fluoxetine hydrochloride (a gift of Eli Lilly and Co.) by intraperitoneal injection, while controls received saline vehicle. Two to three hours post-injection, animals were ether-anesthetized and perfused via the heart with cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Pituitary glands were routinely dehydrated and paraffin embedded. Sections were incubated with 1:1000 or 1:2000 dilution of anti-5-HT primary antibody, and immunoreactive sites were visualized using an avidin-biotin peroxidase technique. Sections from controls contained a fine varicose pattern of pars intermedia fiber staining, with heavier staining in the pars nervosa. Fluoxetine treatment induced a diminution or elimination of fiber staining in the pars intermedia, but had little or no effect on the pattern or intensity of pars nervosa staining. We interpret the effects of fluoxetine to indicate that 5-HT-stained fibers of the pars nervosa derive from indoleamine neurons of the CNS. However, a significant portion of pars intermedia 5-HT-IR fibers may be present as a result of uptake of 5-HT. We are presently comparing 5-HT and tyrosine hydroxylase staining patterns in the pituitary to determine the possibility that 5-HT and CA co-exist in fibers innervating the pars intermedia. Supported by NIHRR08139 (LCS), NIH20039 and NSF BNS 82-08433 (JAW)

109.12 RADIOAUTOGRAPHIC LOCALIZATION OF β-ADRENERGIC RECEPTORS IN THE RAT AND RABBIT PITUITARY. S. Schimchowitsch*, L. Williams*, P. Poyet* and G. Pelletier, MRC Group in Molecular Endocrinology, CHUL, Quebec G1V 4G2, Canada.

It is well documented that catecholamines can stimulate the release of POMC-related peptides from the intermediate lobe of the pituitary via interaction with β-adrenergic receptors (Meunier et al., Eur. J. Pharmacol. 81: 411, 1982). This mechanism which has been extensively studied in the rat has recently been demonstrated in the intermediate lobe of the rabbit pituitary. In order to identify the sites of β-adrenergic receptors in the pituitary of both the rat and rabbit, we used a technique which allows the localization and characterization of receptors on slide mounted cryostat sections (Herkenham, M. and Pert, C.B., J. Neurosci. 2: 1129, 1982). Cryostat sections (15 to 20 μm thick) from rat and rabbit pituitaries were incubated with [^{125}I]cyanopindolol, a high affinity ligand which binds equally to both β_1 and β_2 subtypes. Labelling was localized by radioautography. Characterization of receptor subtypes was achieved by measuring in scrapped off sections or in radioautographs (densitometry) the inhibition of [^{125}I]cyanopindolol binding by varying concentrations of two drugs, practolol which has a greater affinity for β_1 subtype and zynterol which has a greater affinity for β_2 subtype. In the rat pituitary receptors which appeared to be of the β_2 subtype were almost exclusively located in the intermediate lobe although light labelling could also be detected in the anterior and posterior lobes. In the rabbit pituitary the IC_{50} values for zinterol and practolol for inhibiting [^{125}I]cyanopindolol binding were 60.7 ± 4.8 nM and 2.35 ± 0.47 μM, respectively, thus indicating that the receptors were mostly of the β_2 subtype. As observed in the rat pituitary, the receptors were mainly concentrated in the rabbit intermediate lobe. However, radioautographic data showed that a moderate concentration of receptor sites was homogenously located in the anterior lobe. The demonstration of high concentration of β_2 receptors in the intermediate lobe of the two species are in agreement with previous physiological and pharmacological studies. The exact role of these receptors in the anterior and posterior lobes however remains to be clarified.

109.13 DOPAMINERGIC MODULATION OF PEPTIDE SECRETION AND ANATOMICAL FEATURES OF THE INTERMEDIATE LOBE OF THE RAT PITUITARY GLAND. B.M. Chronwall, W.R. Millington, G.P. Mueller, W.S.T. Griffin, J.L. Roberts, T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205; Dept. of Physiology, USUHS, Bethesda, MD 20014; Dept. of Cell Biology, Univ. Texas Southwestern Med. School, Dallas, TX 75235 and Dept. of Biochemistry and Center for Reproductive Sciences, Columbia Univ. Medical School, New York, NY 10032.

The secretion of α-MSH and β-endorphin (α-END) by the intermediate lobe (IL) is regulated predominantly by dopamine releasing neurons projecting from the arcuate nucleus, which inhibit the spontaneous depolarization of melanotrophs. Chronic treatment of rats with haloperidol (2 mg/kg/d; 21 d), a dopamine antagonist, stimulated the release of α-MSH and β-END from the IL and increased IL peptide content. Chronic administration of bromocriptine (4 mg/kg/d; 21 d) had the opposite effect. Similar to the changes in pro-opiomelanocortin (POMC) derived peptides, the POMC mRNA level was increased by haloperidol and decreased by bromocriptine.

To determine the cellular basis for dopamine regulation of melanotrophs, their ultrastructure was investigated after haloperidol and bromocriptine treatment. In control rat IL, approximately 15% of the melanotrophs stain lighter than the rest with toluidine blue on plastic semithin sections. In EM preparations, the light staining cells contain fewer metabolically active organelles (RER 50%, mitochondria 33%, Golgi complex 25%) and have less dense cytosol than the dark staining cells. Short term (2 d) haloperidol treatment increased both the total number of cells and the proportion of dark staining cells in the IL. Long term treatment (21 d) resulted in a great increase in the cell number in the IL. These cells are predominantly dark staining. Short term bromocriptine treatment had the opposite effect; it decreases the cell number in the lobe and increased the proportion of light staining cells. With long term treatment, the IL is reduced to a thin layer of cells containing a considerable amount of heterochromatin.

Dopamine seems to modulate the IL peptide secretion at three different levels. Acutely there is a change in POMC mRNA synthesis; with subchronic treatment the biosynthetic capacity is altered by increase or decrease of the metabolic rate of the melanotrophs and with chronic treatment the mitotic rate of the IL cells is changed. Current studies are using in situ hybridization employing a POMC cDNA probe and a POMC riboprobe to compare POMC mRNA levels in dark and light staining cells and to study changes in message levels during pharmacological manipulation.

109.14 PEPTIDE AMIDATION IN INTERMEDIATE PITUITARY CELL CULTURES. V. May and B. A. Eipper*. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The rat intermediate pituitary contains high levels of two pro-ACTH/endorphin-derived α-amidated product peptides, αMSH and joining peptide, and high levels of a copper and ascorbate dependent peptidyl glycine α-amidating monooxygenase (PAM) capable of converting glycine-extended peptide precursors (-X-Gly) into amidated products (-X-NH_2). The present studies investigate the relationship of cellular ascorbate content to both PAM activity and ability of cultured pituitary cells to produce α-amidated peptide products (αMSH and joining peptide). Intermediate pituitary tissue was enzymatically dispersed and cultured in complete serum-free medium. After various times in culture, the cells were harvested in 20 mM NaTes buffer, pH 7.0, for PAM assays and in 50 mM perchloric acid, containing 1 mM EDTA and 2 mM thiourea for ascorbate determinations by HPLC/electrochemical detection. The ability of cultured cells to accumulate ascorbate was measured using [^{14}C]-ascorbic acid. The ability of the cultured cells to α-amidate newly-synthesized peptides was determined by analyzing αMSH-related molecules biosynthetically labeled with [^{3}H]Trp and joining peptide-related molecules biosynthetically labeled with [^{3}H]Pro. Primary intermediate pituitary cells in culture rapidly lose their ability to produce both the Val-NH_2 at the C-terminus of αMSH and the Glu-NH_2 at the C-terminus of joining peptide. A 2-fold decline in intermediate pituitary culture PAM activity assayed in vitro under optimized conditions failed to account for the lack of α-amidated αMSH production while a simultaneous 100-fold decline in cellular levels of ascorbate could account for the lack of production of α-amidated product. Incubation of cultured cells with ascorbate partially restored the ability of the cells to produce α-amidated αMSH and joining peptide without increasing PAM activity levels. In intermediate pituitary cultures that were made competent to α-amidate 75% of the newly-synthesized αMSH by the addition of 500 μM ascorbate to the culture medium, the effect of various secretagogues on the extent of amidation was determined. Chronic (72h) treatment with the dopaminergic agonist, bromocriptine, consistently decreased the ability of cells to α-amidate newly-synthesized αMSH to 55-60%. Acute or chronic treatment with CRF resulted in an increased synthesis of α-MSH-related material but a significant decline in the percent of newly-synthesized material that was α-amidated. Incubation with cAMP, GABA, isoproteronol, or serotonin failed to alter the extent of amidation. Production of amidated products therefore appears to be regulated by cellular levels of ascorbate, cellular levels of PAM activity and by the specific secretagogues to which the cells are exposed. Supported by AM-32949, ES-07094, DA-00266 and DA-00098.

109.15 LESIONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS PREVENT STRESS-INDUCED INCREASES IN PITUITARY CYCLIC AMP. J.L. Meyerhoff, E.H. Mougey*, C.B. Wormley*, L.L. Pennington*, and G.J. Kant. Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307-5100.

We have previously shown that stress elevates pituitary cyclic AMP in vivo. These increases were associated with increased plasma levels of pituitary hormones (ACTH, β-endorphin and β-LPH) thought to be chiefly regulated by the hypothalamic peptide, corticotropin-releasing factor (CRF). CRF is one of several substances reported to increase pituitary cyclic AMP in vitro. The paraventricular nucleus (PVN) of the hypothalamus has been identified as a major center involved in the regulation of ACTH secretion from the anterior pituitary gland, and it has been recently shown that CRF is highly concentrated in the PVN. To determine the role of the PVN in the stress-induced increase of pituitary cyclic AMP in vivo, we lesioned the PVN bilaterally in male Sprague-Dawley rats.

Lesioned and sham-operated rats were killed by decapitation immediately after being subjected to 15 minutes of unavoidable, variable-interval footshock at 1.6 mA intensity. A control group of lesioned and sham-operated rats was decapitated immediately upon removal from their home cages. Whole pituitaries were rapidly dissected and immediately placed in 90°C sodium acetate buffer to inactivate adenylate cyclase and phosphodiesterase. Following sonication and centrifugation, supernatants were stored at -70°C until cyclic AMP levels were measured by radioimmunoassay, using antibodies characterized in our laboratory. Brains were immersed in 10% formalin, sectioned and stained with cresylecht violet to permit evaluation of the lesions.

While footshock markedly increased levels of pituitary cyclic AMP in sham-operated rats, this effect did not occur in the rats with bilateral lesions of the paraventricular nucleus of the hypothalamus. The lesions had no effect on pituitary cyclic AMP in unshocked rats. The results of the present experiment provide support for the role of CRF as an endogenous mediator of the stress response, and indicate that an intact hypothalamic paraventricular nucleus is essential for the stress-induced increase of pituitary cyclic AMP in vivo.

109.16 PRELIMINARY OBSERVATIONS OF PULSATILE ACTH SECRETION IN CONSCIOUS UNRESTRAINED RATS. M. Carnes*, N.H. Kalin*, C. Barksdale*, and M.S. Brownfield*. (SPON: L. Hegstrand). Veterans Administration Medical Center, Depts. of Medicine and Psychiatry, and the School of Veterinary Medicine, University of Wisconsin, Madison, WI 53705.

The circadian rhythm of pituitary adrenocorticotropin (ACTH) secretion has been well characterized in a number of animal species and humans. The ultradian rhythms have been less thoroughly investigated and have been derived primarily from human and dog studies. Such work in the rat has been limited by the relative insensitivity of previous ACTH assays and the need for large sample volumes. We have been able to investigate pulsatile secretion of ACTH in the rat by using a direct RIA which allows measurement of ACTH in 10-20 µl of plasma. Male 275-300 g Sprague-Dawley rats housed in a sound attenuated, environmently controlled box were bled continuously with a peristaltic pump. Samples were collected at 5 (N=2) and one (N=4) min intervals for approximately 60 min per rat. Hemorrhagic induced ACTH secretion had a rapid onset which consistently occured at 2.5-3.0 ml blood volume loss. Pulse parameters were evaluated before this observed ACTH rise. A pulse was identified when the coefficient of variation (CV) of the peak and trough values was two times greater than the CV of the assay for 10 plasma replicates at similar ACTH levels.

Sampling Freq (min)	N	# Time Points	ACTH ($\bar{x}$ ±SEM) (pg/ml)	Pulse Interval (min)	Pulse Freq (#/ hr)	Amplitude ($\bar{x}$ ±SEM) (pg/ml)
5	2	27	45.7 ± 3.0	31.7 ± 12.0	2.2 ± 0.8	17.7 ± 2.3
1	4	186	93.0 ± 5.1	5.3 ± 0.5	11.6 ± 1.4	32.8 ± 3.3

Analysis of our data indicates that ACTH is episodically secreted in rats. The detection of pulse interval and frequency varies greatly depending on the sampling interval. The detection of 11.6 ±1.4 ($\bar{x}$ ±SEM) pulses per hour at one min intervals may indicate that previous studies in other species using longer sampling intervals have underestimated the true pulse frequency. We are currently investigating sampling frequencies of less than one min.

109.17 ROLE OF CORTICOTROPIN RELEASING FACTOR AND VASOPRESSIN IN THE STIMULATION OF ACTH RELEASE BY ANGIOTENSIN II. C.K.Klingbeil*, L.C.Keil*, D.Chang* and I.A.Reid. Dept. of Physiology, Univ. of California, San Francisco, CA. 94143; Peninsula Labs, Belmont, CA. 94002; Ames Res. Center, Moffett Field, CA. 94035.

Angiotensin II (AII) stimulates ACTH release in dogs but the mechanism is not known. Corticotropin releasing factor (CRF) and vasopressin (VP) are potent secretogogues of ACTH and may be involved. Evidence supporting this possibility includes the observation that AII stimulates VP release and potentiates the ACTH response to CRF in anesthetized rats and in cultured rat pituitary cells. The aim of the present study was to determine if the stimulation of ACTH by AII in conscious dogs results from potentiation of the action of CRF or VP by AII. Experiments were performed in five chronically prepared dogs: 1) i.v. saline infusion; 2) AII (10 ng/kg/min) alone; 3) VP(1ng/kg/min) alone; 4) CRF (0.001, 0.01, 0.1 ug/kg) i.v. bolus; 5) AII and VP together; 6) VP and CRF(0.1ug/kg) together; 7) CRF (0.001, 0.01, 0.1 ug/kg) and AII together. Blood samples for assay of ACTH and VP were collected at 15 min intervals. AII, VP and CRF alone increased plasma ACTH concentration (Table). AII and VP together only had an additive effect on plasma ACTH concentration as did AII together with three doses of CRF. Thus AII did not potentiate the ACTH responses to VP or CRF. In contrast, the ACTH response to CRF was potentiated by VP. As expected, AII increased plasma VP concentration (2.4±0.2 to 4.2±0.5 pg/ml, $p<0.01$). Unexpectedly, CRF also increased plasma VP concentration. The highest CRF dose increased plasma VP from 2.7±0.3 to 4.8±0.4 pg/ml($p<0.01$) and the intermediate dose increased VP from 2.2±0.6 to 4.1±0.9($p<0.01$). In summary: 1) AII, VP and CRF increase ACTH release in conscious dogs; 2)AII does not potentiate the ACTH response to VP or CRF; 3) VP potentiates the ACTH response to CRF; 4) CRF increases VP release. These results indicate that the stimulation of ACTH release by AII in conscious dogs does not result from potentiation of the action of CRF or VP by AII.

Plasma ACTH Concentration (pg/ml)(*$p<0.05$; **$p<0.01$)

	Control	15 min	30 min
Saline	48.4 ± 7.3	50.4 ± 7.3	52.0 ± 6.5
AII	59.6 ± 6.0	67.0 ± 7.2*	70.8 ± 7.8*
VP	48.7 ± 5.7	53.4 ± 5.7	55.6 ± 6.5*
AII + VP	48.7 ± 6.6	57.7 ± 8.5*	61.5 ± 8.5*
CRF (0.1)	56.1 ± 8.1	89.4 ±14.4**	80.7 ±14.0**
AII + CRF (0.1)	52.1 ± 7.1	90.6 ± 8.2**	90.4 ± 7.0**
CRF (0.01)	44.5 ± 6.7	68.7 ±10.6**	70.0 ± 9.7**
AII + CRF (0.01)	52.4 ±10.3	88.3 ±11.1**	86.9 ± 8.1**
CRF (0.001)	48.9 ±11.8	61.2 ±10.8*	60.7 ±14.6*
AII + CRF (0.001)	48.1 ± 6.6	59.1 ± 7.7*	62.6 ± 9.6*
VP + CRF (0.1)	48.2 ± 6.1	100.1 ±11.0**	99.0 ±11.6**

109.18 MODULATION OF ADRENOCORTICOTROPIN (ACTH) SECRETION: IMPLICATION OF THE AMYGDALOID CENTRAL NUCLEUS, THE NORADRENERGIC, DOPAMINERGIC AND SEROTONINERGIC SYSTEMS. S. Beaulieu, T. Di Paolo and N. Barden. MRC Group in Molecular Endocrinology, CHUL, Quebec, G1V 4G2, CANADA.

The high concentration of corticotropin-releasing factor (CRF) within the amygdaloid central nucleus (ACE) supports the hypothesis that the amygdala might be involved in the control of ACTH secretion. In order to verify this hypothesis, we have studied the effect of bilateral lesions of the ACE on ACTH secretion in response to immobilization stress. Moreover, we have assessed the possible implication of the noradrenergic (NA), dopaminergic (DA) and serotoninergic (5-HT) systems within specific hypothalamic and amygdaloid nuclei in the control of ACTH secretion in response to stress and/or lesion of the ACE.

Intact or ACE-lesioned rats were left undisturbed (controls) or immobilized (stressed) for a period of one hour during which time blood was periodically sampled by a chronically implanted jugular cannula. Plasma and pituitary ACTH levels were measured by radioimmunoassay. The concentration, in discrete brain nuclei, of norepinephrine, dopamine, serotonin and their metabolites, was measured by HPLC coupled with electrochemical detection and turnover rates determined.

Bilateral lesions of the ACE diminished the secretion of ACTH in response to immobilization stress and increased the pituitary ACTH concentration in both control and stressed animals. Stress increased the NA activity within the ACE, the anterior and lateral hypothalamic areas , while it decreased the DA activity in the ACE, the amygdaloid cortical nucleus, the dorsomedial and ventromedial nuclei of the hypothalamus and the ventral tegmental area. The 5-HT activity was not modified by one hour of stress in any of the structures studied. When compared to intact stressed animals, the NA activity in stressed ACE-lesioned animals was decreased in the bed nucleus of the stria terminalis, the paraventricular and arcuate nuclei of the hypothalamus and in the anterior and lateral hypothalamic areas, while the 5-HT activity was increased in the glucocorticoid receptor-rich medial, basolateral and cortical amygdaloid nuclei.

These results support the hypothesis of a stimulatory role of the ACE in the control of ACTH secretion. They also substantiate the hypothesis that the NA system stimulates and the DA system inhibits ACTH secretion by acting in the ACE and other amygdaloid and hypothalamic nuclei, while the 5-HT system is possibly involved in the mediation of the negative feedback action of glucocorticoids at the level of the amygdaloid complex.
(Supported by the MRC)

109.19 PEPTIDE REGULATION OF ACTH SECRETION IN NORMAL HUMANS AND PATIENTS WITH MAJOR DEPRESSION. R.S. Jaeckle, J.F. Lopez*, and R.C. Kathol*. Department of Psychiatry and Neuroscience Program, The University of Iowa, Iowa City, Iowa, 52242.

Corticotropin releasing hormone (CRH) is the principal brain peptide involved in stimulating ACTH secretion from pituitary corticotrophs, and this effect of CRH is potentiated by several other hormones, including arginine-vasopressin (AVP) and catecholamines. Insulin-induced hypoglycemia (IH) is another potent stimulus for ACTH secretion which is thought to be mediated by CRH, AVP, and catecholamines (Federation Proceedings, 44: 207-213, 1985). Major depressive disorder (MDD) is characterized by abnormalities of pituitary-adrenal function, including increased ACTH and cortisol secretion. There have been no previous studies comparing ACTH secretory responses to exogenous CRH, exogenous AVP, and IH in normal human controls and patients with MDD. We studied ACTH secretory responses to these 3 stimuli in 18 healthy controls and in 15 MDD patients (DSM-III diagnoses). Subjects received ovine CRH (1 ug/kg IV), AVP (0.18 pressor units/kg IM), or regular insulin (0.15 u/kg IV) at 1600 hours on separate days, and serum ACTH immunoreactivity was determined at several times before and after giving each stimulus. In the controls, CRH was a more potent stimulus for ACTH than was AVP. In the controls, mean serum ACTH rose from a baseline of 24 pg/ml to a peak of 58 pg/ml 30-45 minutes after giving CRH, and serum ACTH rose from a baseline of 32 pg/ml to a peak of 42 pg/ml 15 minutes after giving AVP. IH was a significantly more potent stimulus for ACTH than either CRH or AVP in both groups. In controls, serum ACTH rose from a baseline of 22 pg/ml to a peak of 324 pg/ml 45 minutes after giving insulin. Baseline ACTH levels prior to CRH, AVP, or IH were similar in the MDD and control groups. However, the MDD group had significantly reduced peak ACTH responses to IH (157 pg/ml, $p<0.02$ vs. controls) and to CRH (34 pg/ml, $p<0.05$ vs. controls). Peak ACTH responses to AVP were similar in the two groups, although 9 of 15 MDD patients and only 3 of 18 controls had maximal ACTH increments >14 pg/ml after AVP ($p<0.02$).

To summarize, we have shown that in normal humans, the order of potency for stimulating ACTH secretion is IH$>>$ CRH$>$ AVP. Since the ACTH secretory response to IH was much greater than could be predicted from the sum of the ACTH responses to CRH or AVP in the same subjects, the findings suggest that IH stimulates ACTH secretion by releasing several hormones (CRH, AVP, and perhaps peripheral catecholamines) which have synergistic effects on ACTH secretion at corticotrophs. The ACTH secretory responses to IH and to exogenous CRH were attenuated in MDD, and the MDD group had increased ACTH increments after exogenous AVP compared with controls. These findings suggest that the increase in ACTH and cortisol secretion observed in MDD may be the result of dysregulation of corticotroph function by hypothalamic peptides, including CRH and AVP. A possible explanation could be increased secretion of these peptides.

109.20 THE ACTH RESPONSE TO HYPOTENSIVE HEMORRHAGE IS MEDIATED BY THE PARAVENTRICULAR NUCLEUS AND NOT BY CIRCULATING EPINEPHRINE IN THE ANESTHETIZED RAT. D.N. Darlington,* J. Shinsako,* and M.F. Dallman* (SPON: D.G. Ward). Dept. of Physiology, University of California, San Francisco, CA 94143 USA.

Hypotensive hemorrhage leads to increases in plasma ACTH and epinephrine. The rise in plasma ACTH is thought to be mediated by neural structures that include the peripheral cardiovascular receptors, the nuclei of the tractus solitarius and the paraventricular nuclei. Recent evidence suggests that high circulating epinephrine levels may play a role in hypoglycemia induced release of ACTH. It is not known whether high circulating levels of epinephrine, that are normally seen after hemorrhage, or whether neural structures such as the paraventricular nuclei mediate ACTH responses to hypotensive hemorrhage.

Pentobarbital anesthetized (45mg/kg, ip) male Sprague-Dawley rats had femoral artery and vein cannulas placed for measuring arterial blood pressure and for blood withdrawal respectively. Each rat was allowed one hour for stabilization from surgery and than received a hemorrhage of 15ml/kg*3 minute. Plasma ACTH and epinephrine were determined from the first 2ml of hemorrhaged blood and from blood taken 20 minutes later. The red blood cells from these samples were suspended in saline and reinfused into the rat. The paraventricular nuclei were then lesioned with a triangular knife that was lowered 1.6mm caudal to bregma on the midline and rotated. Sham lesions were made by lowering but not rotating the knife. Each rat received a 15ml/kg*3 minute hemorrhage after placement of the lesion and ACTH and epinephrine were determined as described above. Histology confirmed complete destruction of the paraventricular nucleus in the lesioned group and minimal damage of the paraventricular nucleus (PVN) in the sham group.

Mean arterial blood pressure (MABP) in the sham- (n=7) and PVN- (n=5) lesioned groups decreased during hemorrhage from 130±5 and 135±5 to 40±5 and 45±5 mmHg respectively before placement of lesion. After placement of lesion, the MABP response during hemorrhage was similar (115±5 and 120±5 to 35±5 and 40±5 mmHg respectively). Plasma ACTH rose significantly during hemorrhage before placement of lesion in both the sham- and PVN-lesioned groups (+191±36 pg/ml and +165±18 pg/ml respectively). However, only the sham-lesioned group demonstrated a significant rise in plasma ACTH (+144±23 pg/ml) postlesion whereas the PVN-lesioned group did not (+18±19 pg/ml). This strongly suggests that the paraventricular nuclei are involved in the ACTH response to hypotensive hemorrhage. Baseline ACTH levels were low (118±29 before PVN lesion and 76±15 after PVN lesion). Also, plasma epinephrine rose significantly during hemorrhage (from 385±151 to 11,713±4,093 pg/ml) after lesion of the PVN suggesting that the high circulating levels of epinephrine seen during hemorrhage are not involved in the ACTH response to hemorrhage. These data suggest that a neural pathway that includes the paraventricular nucleus is involved in the ACTH response to hypotensive hemorrhage and that circulating epinephrine is not. This study was supported by HL29714, AM28172 and NS07565.

BASAL GANGLIA: IMMUNOCYTOCHEMISTRY AND PHYSIOLOGY

110.1 GLUTAMIC ACID DECARBOXYLASE, SUBSTANCE P AND LEU-ENKEPHALIN-IMMUNOREACTIVE NEURONS IN THE RAT NEOSTRIATUM THAT PROJECT TO THE GLOBUS PALLIDUS AND SUBSTANTIA NIGRA.
S. Afsharpour, H. Kita, G.R. Penny, and S.T. Kitai. Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

We have previously reported the distribution and proportion of glutamic acid decarboxylase (GAD), substance P. (SP) and leu-enkephalin (L-Enk) immunoreactive neurons in the neostriatum of the rat and cat (Afsharpour et al., Neuro. Abs. '84). In the present study we have asked how these immunoreactive neurons are organized with regard to their projections to the major neostriatal efferent targets: the globus pallidus (GP) and the substantia nigra (SN).

The proportion of the GAD, SP, and L-Enk-immunoreactive neostriatal neurons projecting to GP, and SN were quantitatively analyzed using retrograde tracing combined with immunocytochemistry. WGA-HRP or HRP was injected in the SN or GP. A two color method was used to localize retrogradely labeled cells with a brown reaction product and, on the same section, cells immunocytochemically labeled by the ABC method with a green reaction product. From representative parasagittal sections, plots were made of every neuron only labeled for HRP transport and every neuron labeled for both HRP and a transimitter marker. These plots were used as samples to provide estimates of the proportion of neuroactive substance.

The samples sugguest that about 20% of the striatopallidal neurons are immunoreactive for GAD, 20% for SP, and 40% for L-Enk. In contrast, about 45% of the striatonigral neurons are immunoreactive for GAD, about 35% for SP and only about 0.2% for L-Enk. Of course, we know that these are not mutually exlusive populations; we know from our previous colocalization studies that there is partial overlap of these sets, that is many neurons contain more than one of these neuroactive substances.

From these result we infer (1) that the principal population of striatopallidal neurons is enkephalinergic, and that the populations of SP or GABAergic neurons are lesser, each only half as large as the enkephalinergic population; (2) that the principal population of striationigral neurons is GABAergic, a slightly smaller population contains SP, and an almost negligible population leu-enkephalinergic; (3) that GABAergic neurons form a relatively greater part of the striatonigral pathway than the striatopallidal pathway; (4) that the SP neurons form a relatively greater part of the striatonigral pathway than the striatopallidal pathway; and (5) that leu-enkephalinergic neurons are almost entirely confined to the striatopallidal pathway.

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110.2 EFFECTS OF [D-ALA^2]-METHIONINE ENKEPHALINAMIDE AND CARBACOL RECORDED INTRACELLULARLY FROM RAT NEOSTRIATUM IN SLICE PREPARATION. T. Kita, H. Kita and S.T. Kitai. Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Morphological studies have well documented that the neostriatum is rich with nerve terminals containing enkephalin and acetylcholine (ACh). What is sparse, however, is the information concerning neuropharmacological action of the above mentioned neuro-active substances in the neostriatum. We have therefore launched an investigation to examine the effects of carbacol (a stable ACh analogue) and [D-Ala^2] methionine enkephalinamide (a stable enkephaline analogue) on the synaptically induced responses (i.e., EPSPs) intracellularly recorded from rat striatal neurons in **in vitro** slice preparation.

Brains removed from the decapitated rats were blocked to contain the neostriatum and were sectioned using a Vibratome (400 um) in the parasagittal plane. Slices were placed in a recording chamber and were continuously superfused by the oxygenated Ringer solution (concentration in mM= NaCl 124, kCl 5.0, $MgSO_4$ 2.0 KH_2PO_4 1.25, $CaCl_2$ 2.0, $NaHCO_3$ 26 and glucose 10, pH 7.2-7.4). Local stimulation was applied through a pair of bipolar electrodes placed on the surface of a slice. Glass pipettes filled with 2M K-methylsulfate were used for recording. Drugs were applied to the superfusing Ringer solution with a concentration of 10 to 100 um.

Applications of carboral or enkephalin consistently led to hyperpolarization of the membrane by a few millivolts with a slight increase in the membrane conductance. It was also observed that the amplitude of depolarizing postsynaptic responses induced by local stimulation was clearly reduced by these drug applications. Carbacol effects were totally abolished by application of atropine but were only partially reduced by d-tubocurarine. The effects of enkephalin were clearly antagonized by naloxon but not by atropine. These results suggest that the action of ACh and enkephalin in the neostriatum is to hyperpolarize the neostriatal cell membrane and to reduce the efficacy of excitatory synaptic inputs. Even though the observed effects of these neuro-active substances are similar, they are likely to be mediated through different receptors; ACh through muscalinic receptors and enkephalin opioid receptors.

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110.3 ELECTROPHYSIOLOGY OF RAT SUBTHALAMIC (STH) NEURONS: INTRACELLULAR STUDY IN **IN VITRO** SLICE PREPARATIONS. H. Nakanishi*, H. Kita and S. T. Kitai (SPON: J. Whitaker), Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163.

Rat STH slices (350-450mm thick) in the parasagittal plane were prepared for intracellular recording. Slices were placed in a recording chamber and continuously superfused with the oxygenated Krebs-Ringer solution. Most of STH neurons showed spontaneous repetitive firing with the frequency of 5 to 40 Hz. The input resistance of the neurons estimated from the membrane potential shifts to hyperpolarizing current injection was 90-250 Meg Ohms. The resistance showed an anomalous rectification when large hyperpolarizing currents were applied. STH neurons generated two distinct patterns of firing to depolarizing current pulses depending on their resting membrane potentials. At zero current injection (membrane potential -50 to -70 mV), depolarizing current pulses generated fast repetitive firings with the maximum frequency of up to 500 Hz. When the same neuron was hyperpolarized by a continuous current injection (e.g., -0.2 to -0.5 nA), depolarizing current pulses triggered a burst response characterized by slow depolarizing potential with fast action potentials. Often the burst response was followed by long lasting depolarization (up to 500msec) with fast spikes which outlasted the current pulse. The burst response followed by long lasting depolarization was also observed at the offset of hyperpolarizing current pulse. The slow depolarizing potential and the prolonged depolarization were TTX resistant but were blocked by Co++ or replacement of Ca++ with Mg++ and augmented by Ba++ or TEA. Succession of repetitive action potentials or the burst response produced by direct stimulation was followed by hyperpolarization which was diminished by a replacement of Ca++ with Mg++.

Electrical stimulation of the internal capsule (IC) at 0.5-1 mm rostral to STH induced monosynaptic IPSPs in STH neurons with the latency of 1 to 3 msec. The polarity of IPSPs was reversed in the depolarizing direction after intracellular injection of Cl^-. Bath application of either bicuculline methiodide or strychnine (50-100 uM) markedly or completely suppressed IPSPs. With the suppression of IPSPs, IC stimulation evoked monosynaptic EPSPs with the latency of 3.5 to 4 msec. If a neuron is hyperpolarized by a continuous current injection, these EPSPs triggered the burst response or depolarization which may last up to 700 msec. These results demonstrated that STH neurons in **in vitro** preparation have spontaneous discharges, very high input resistance, capability to generate high frequency firings, and Ca++dependent conductances. The pattern of responses of the STH neuron to synaptic inputs is also very much dependent on its membrane potential. (Supported by NIH Grant NS20702 to STK)

110.4 INTRACELLULAR STUDY OF RAT SUBSTANTIA NIGRA PARS RETICULATA NEURONS IN **IN VITRO** SLICE PREPARATION: ELECTRICAL MEMBRANE PROPERTIES AND RESPONSE CHARACTERISTICS TO SUBTHALAMIC STIMULATION. S. T. Kitai, H. Nakanishi* and H. Kita. Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Rat brain slices containing substantia nigra pars reticulata (SNr) and subthalamus (STH) were sectioned (350-450 um thick) in the parasagittal plane using a Vibratome. Brain slices were placed in a recording chamber and continuously superfused with the oxygenated Krebs-Ringer solution. Two populations of SNr neurons were identified on the basis of their membrane properties. One population demonstrated spontaneous repetitive firings with the frequency of 1 to 6 Hz. Injection of depolarizing current pulses generated high frequency repetitive firings in these neurons. Termination of the depolarizing current pulse was followed by a long duration hyperpolarization. After bath application of TTX and TEA, depolarizing current pulses generated a slow spike potential accompanied by a long duration depolarization (up to 2 sec) which outlasted the current pulses. These responses were considered to be mediated by Ca++conductances. The other population of neurons had no spontaneous activity. Injection of depolarizing current pulses generated slow depolarization from which action potentials were triggered. Membrane responses to hyperpolarizing current pulses showed an existence of strong anomalous rectification. The membrane potential after the offset of the current often showed a slow ramp-shaped return to the base line (probably early K+conductance). The membrane properties of the second population of the neurons were very similar to those observed from the neurons of the substantia nigra pars compacta.

Electrical stimulation of STH evoked monosynaptic EPSP-IPSP sequence of responses in both populations. The latency of EPSPs ranged from 1 to 2 msec. The polarity of IPSPs was reversed in the depolarizing direction after intracellular injection of Cl^-. Recordings from SNr neurons in slices, incubated in the solution containing bicuculline methiodide (100 uM) or in slices obtained from the rats with chronic internal capsule transection at the level immediately rostral to STH, revealed only monosynaptic EPSPs to stimulation of STH. These results suggest that: (1) SNr contains two populations of neurons having different membrane properties and (2) both populations of neurons receive monosynaptic excitation probably originating from STH and inhibition originating from the sites rostral to STH.

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110.5 SUBTHALAMIC AFFERENTS TO THE GLOBUS PALLIDUS AND THE SUBSTANTIA NIGRA IN THE RAT; LIGHT AND ELECTRON MICROSCOPIC STUDIES USING PHA-L METHOD. H. Kita and S. T. Kitai. Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Anatomical and electrophysiological studies have shown that single subthalamic neurons send their branching axons to the globus pallidus (GP) and the substantia nigra (SN). However, the function of these projections is still a matter of controversy. We have conducted light microscopic (LM), and electron microscopic (EM) analysis of rat STH branching axons labeled by anterograde nerve tracer, phaseolus vulgaris leucoagglutinin (PHA-L). PHA-L injection and immunocytochemical procedures were slightly modified from the procedures originally described by Gerfen and Sawchenko (Brain Res. 290:219-238, 1984). Fixatives for perfusion of animals consisted of 0.15M acetate and borate buffers with 4% paraformaldehyde and 0.15% glutaraldehyde. Brains were sectioned (40mm thick) on a Vibratome. Some of the sections prepared for EM were processed for PHA-L immunohistochemistry without treating them with Triton-X. They were postfixed by osmium tetroxide, infiltrated with Spurr's plastic, and mounted on Teflon-coated glass slides. Sections for light microscopy were prepared in accordance with Gerfen and Sawchenko's protocol (Brain Res. 290:219-238, 1984).

PHA-L labeled STH afferent plexus in GP and SN were thin fibers with both boutons enpassage and boutons terminaux. The sites of the labeled terminal axonal plexus in their target nuclei differed with different PHA-L injection sites in STH, suggesting the existence of some topographically organized STH projections to GP and SN. Electron microscopic analysis revealed that the morphological features of the labeled processes were very similar in both EP and SN. They were both unmyelinated and relatively large myelinated labeled axons. The labeled terminals were 1-2 um in size and contained many small (20-50mm) spherical or ellipsoidal shaped synaptic vesicles. They were making contact with thin (less than 2 um) dendritic shafts with asymmetrical postsynaptic specialization. Based on these morphological data, we suggest that the subthalamic afferents to GP and SN will be likely to have similar physiological action on their target neurons. (Supported by NIH Grant NS20702 to STK).

110.6 ULTRASTRUCTURAL MORPHOLOGY OF VENTRAL PALLIDAL NEURONS INTRACELLULARLY LABELED WITH HRP. H.T. Chang and S.T. Kitai, Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN. 38163.

In our previous light microscopic analysis of ventral pallidal (VP) neurons intracellularly labeled with HRP (Teng et al., 1984), we have demonstrated that VP neurons have long dendrites which could radiate up to 1 mm away from the somata, but these dendrites remained bounded by the borders of the nucleus. Additionally, we have shown that VP neurons have extensive local collateral arborizations which also terminated mainly within the nuclear boundaries of VP. In order to determine the morphology and the distribution of different types of axon terminals which form synapses on individual VP neurons, we analyzed the morphology of several labeled VP neurons at the electron microscopic level. In addition, targets contacted by labeled axon collaterals of individual VP neurons were also examined.

The somata of all labeled VP neurons are characterized by large amount of rough endoplasmic reticulum and nuclei with deeply invaginated nuclear envelope. The somatic surfaces as well as the surfaces of all dendrites are ensheathed with numerous unlabeled axon terminals forming mainly symmetrical, and occasionally asymmetrical synapses. The appearance of such dense innervation resembles very closely the neurons of the globus pallidus and the substantia nigra pars reticulata reported previously. The intrinsic collaterals of labeled VP neurons are myelinated. However, these collateral fibers usually become unmyelinated a short distance prior to giving rise to either boutons en passant or boutons terminaux. These boutons, densely packed with large pleomorphic vesicles and many mitochondria, form symmetrical synapses with somata and dendrites of unlabeled VP neurons.

These findings suggest that VP neurons receive converging inhibitory inputs, probably from medium spiny neurons of the ventral striatum, and that they form recurrent inhibitory synapses with neighboring VP neurons.

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110.7 ELECTROPHYSIOLOGY AND MORPHOLOGY OF RAT THALAMO-CORTICAL RELAY NEURONS: AN **IN VIVO** INTRACELLULAR RECORDING AND LABELING STUDY. K. Yamabe*, H. Kita and S. T. Kitai (SPON: G. R. Penny) Div. of Neurosci., Dept. of Anatomy, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163

Intracellular recordings and HRP-labelings of thalamo-cortical neurons were performed on anesthetized male hooded rats (250-400g). In some rats, acute decortication was made by subcortical cuts rostral, dorsal and lateral to the neostriatum using a Halaz's knife. Bipolar electrodes consisting of a pair of insect pins (No.00) were used for stimulation of the frontal cortex (frontal pole) and the neostriatum. In the decorticated rats, stimulation electrodes were placed in the neostriatum to stimulate cortico-thalamic and thalamocortical fibers. Glass micropipettes filled with 3-4% HRP in 0.05 M Tris buffer (pH 7.6) and 0.5 M K-methylsulfate, and having DC-resistances of 50-80 Meg Ohms were used for intracellular recording and labeling. At the end of the experiment, the rat was perfused with a solution of 1% paraformaldehyde and 2% glutalaldehyde in 0.15 M phosphate buffer (pH 7.4). Serial parasagittal 50 um sections were made on a Vibratome and reacted with diaminobenzidine. Sections containing labeled neurons were postfixed in 1% osmium tetroxide in phosphate buffer, embedded in Spurr's plastic, and examined under light microscope.

Results were obtained from the neurons located in either the mediodorsal, paracentral, ventromedial or centrolateral nucleus. Stimulation of the frontal cortex evoked a sequence of responses; antidromic spike, initial depolarization, long lasting hyperpolarization and short depolarization. The initial depolarization was considered to be a monosynaptic EPSP which overlapped with an IPSP. Major constituents of the long lasting hyperpolarization were considered to be short duration IPSP and long duration disfacilitation of cortical inputs. These neurons also generated membrane responses corresponding to the low-threshold Ca++spike, Ca++dependent K+ and early K+ conductances that have been previously reported in **in vitro** studies of thalamic neurons. Somata of the HRP-labeled neurons were polygonal in shape with their diameters ranging from 20 to 30μm. Three to six thick primary dendrites arose from the soma. They soon formed thinner secondary and tertiary branches, some of which arborized further. The axon emerged either from the soma or from a primary dendrite. The axon was traced rostrally toward the internal capsule.

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110.8 PALLIDAL INFLUENCES ON NECK AND SHOULDER MUSCLES IN THE RAT. M.D. Kelland, D. Asdourian, D.J. Zawisa*, and R.Y. Shen*, Dept. of Psychology, Wayne State University, Detroit, MI 48202.

Electrical stimulation of the globus pallidus (GP) in adult male rats (N=25) was used to determine the nature and extent of the control exerted by the GP over activity in the trapezius, biventer cervicis, rectus capitis, and scalenus dorsalis muscles. Muscle activity was recorded electrically.

Experiment 1. Electrical stimulation of the GP resulted in driven contralateral activity in all muscles studied, and weaker ipsilateral activity in the rectus capitis. The parameters of the contralateral activity are as follows: maximal response amplitudes of 0.2-1.7 mV; response latencies of 4-14 msec. (the time between the stimulus artifact and the beginning of the response); durations of 4-13 msec.; and recruitment latencies of 0.3-13.6 sec. (the time it takes for muscle activity to appear following the initiation of stimulation). Spontaneous muscle activity was often inhibited totally by ipsilateral GP stimulation. These results were obtained with optimal stimulation parameters of 15 twin pulses per sec., each pulse having a 1.0 msec. duration with a 1.0 msec. interpulse interval, at 15V (approximately 0.75 ma).

Experiment 2. Animals received a unilateral electrolytic lesion of the substantia nigra pars reticulata (SNr) or the subthalamic nucleus (SubTh) prior to GP stimulation in an attempt to determine the degree to which each of these pathways are involved in the control exerted by the GP over the periphery. Lesions of the SNr had no effect on activity driven by GP stimulation. Lesions of the SubTh blocked activity in all of the muscles studied. Preliminary evidence with ibotenic acid lesions of the SubTh suggests that our results are not due to damaging fibers of the internal capsule. Thus, the pathway through the SubTh seems to be necessary for mediating the control exerted by the GP over the neck and shoulder.

Extensive electrolytic lesions of the motor relay nuclei of the thalamus (VA,VL,VM) were made in order to determine whether these nuclei are part of the pathway mediating the driven activity. These lesions had no effect on activity driven by GP stimulation.

That the muscle activity driven by GP stimulation is primarily contralateral may serve as an explanation for the contraversive motor asymmetry and circling behavior (MAC) seen during GP stimulation. The ipsilateral inhibition of muscle activity during GP stimulation may also contribute to contraversive MAC.

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110.9 NIGRAL INFLUENCES ON NECK AND SHOULDER MUSCLES IN THE RAT. D. Asdourian, C. Geula, M.D. Kelland, R.Y. Shen*, D.J. Zawisa*, W. Lipinski*, Dept. of Psych., Wayne St. Univ., Detroit, MI 48202.

Unilateral electrical stimulation of the substantia nigra pars reticulata (SNr) was used to determine the effects of SNr output on muscles of the neck and shoulder of anesthetised male rats (N=22). Muscle activity was recorded electrically.

Experiment 1. Stimulation of the SNr drove bilateral activity in the trapezius, biventer cervicis, rectus capitis, and scalenus dorsalis. Twin square waves were used with durations of 1.0 msec. and an interpulse interval of 1.0 msec. The voltages needed to drive maximal responses varied from animal to animal and from muscle to muscle and ranged from 6-13V. The ipsilateral responses for a given muscle pair were always greater in amplitude than the contralateral. Ipsilateral responses (over all muscles) ranged from 1.5-10.0 mV while contralateral responses ranged from 0.5-3.0 mV. The most effective stimulation frequencies were between 1-2 twin pulses per sec. and 10-15 per sec. At frequences between 3-9 per sec. responses often became inconsistent and sometimes disappeared altogether. When responses were consistently driven, they appeared with stimulus onset - no recruitment latency delayed the onset of muscle activity. The latency between stimulus onset and driven activity ranged between 2-7 msec.

Experiment 2. Two of the major targets of SNr projection fibers are (1) the middle and deep layers of the superior colliculus (SC) and the reticular formation (RF) immediately ventral to the SC and (2) the thalamic motor nuclei - especially the ventromedial (VM) but also perhaps the ventral anterior (VA) and ventrolateral (VL). Neither unilateral lesions in the SC-RF nor extensive lesions that included VA, VL, and VM had any effect on muscle activity driven by SNr stimulation.

That unilateral SNr output had its greater effect on ipsilateral muscles may serve as an explanation for the finding that SNr stimulation causes ipsiversive rotation in unanesthetised animals. The failure to block SNr output with SC-RF or thalamic lesions leaves unanswered questions concerning the pathways utilized by SNr in gaining access to the motor periphery.

Supported by Neuroscience Small Grants Program (4-48034), WSU, School of Medicine.

110.10 PAIRED-PULSE FACILITATION AND INHIBITION IN RAT STRIATUM IN VITRO. J.L. Stringer*, G.F. Wooten, and E.W. Lothman, Dept. of Neurology, Univ. Virginia, Charlottesville, VA. 22908.

Local electrical stimulation in rat striatal slices evokes two negative field potentials: N1, direct activation of neurons, and N2, synaptic activation. Paired-pulse inhibition has been described for the N2 response when the interstimulus interval (ISI) is less than 25 msec and the $[Ca^{2+}]$ of the medium is at least 2.3 mM (Lighthall et al.,1981). This study examined the responses of N2 field potentials and single units to paired stimuli in media of different $[Ca^{2+}]$. "Low Ca^{2+}" medium contained (in mM): NaCl 127, KCl 2, $MgSO_4$ 1.5, KH_2PO_4 1.1, $NaHCO_3$ 25.7, $CaCl_2$ 1.5, and glucose 10. In this medium, slices (425 μm thick) showed paired-pulse inhibition of N2 responses at 10 msec ISI. At longer ISI, the N2 responses to the second stimulus showed a marked decrease in latency to peak, but no change in amplitude. This observation was confirmed with recordings from N2 neurons; the neuron consistently fired sooner after the second stimulus. Since the latency of N2 neurons depends on stimulus voltage and varies at a given stimulus intensity, it is difficult to quantitate this facilitation using latency measurements. However, we found that paired-pulse facilitation is also characterized by a decrease in threshold for single unit discharges and this is useful analytically. The change in threshold of the neuron exhibits a linear relationship with ISI which is remarkably constant ($r = .99$; $n = 6$). The threshold difference varies from 0.9V at 10 msec ISI to 0.1V at 120 msec. Paired-pulse inhibition of the firing of a single unit was seen in only one neuron at 10 msec ISI. The "high Ca^{2+}" medium was the same as above except the $CaCl_2$ was 3.5 mM. N2 field potentials recorded in this medium showed paired-pulse inhibition at ISI of 20 msec and less. Beyond 20 msec the N2 field showed no significant decrease in latency or change in amplitude. Single unit responses were more complex. Three of 8 cells showed a decrease in latency, but only at 10 msec; only 1 of the 8 showed paired-pulse inhibition, also only at 10 msec ISI. Thresholds for single unit firing were measured in 4 neurons from 10-160 msec ISI. All 4 neurons showed an equal or greater threshold for firing after the second stimulus at 20 msec or greater (ave. increase of 0.4V). One cell demonstrated a decrease in threshold at 10 msec ISI. The data indicate that synaptic behavior in the striatum critically depends on the ambient ionic environment. A change in the calcium concentration from 1.5 to 3.5 mM changes the response to paired-stimuli from facilitation to inhibition. The former value approximates the $[Ca^{2+}]$ in vivo. Therefore, the importance to neuronal function of paired-pulse inhibition found in vitro with high Ca^{2+}, remains to be clarified. Supported, in part, by The United Parkinson Foundation.

110.11 SYNAPTIC ORGANIZATION OF FETAL STRIATAL TRANSPLANTS IN THE NORMAL AND KAINATE LESIONED RAT CAUDATE NUCLEUS. M. DiFiglia, L.Schiff* and W.A. Deckel. Depts. of Neurology, Mass. General Hosp.,Boston, MA 02114 and Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent studies have shown that fetal striatal transplants can reverse locomotor deficits produced by a kainic acid (KA) lesion of the caudate nucleus (Deckel et al, Eur. J. Pharmacol., 93:287, 1983). The anatomical correlates to this functional recovery were examined in fetal striatal transplants in normal and KA lesioned host caudate nucleus. Sprague-Dawley rats (N=8) were anesthetized and given bilateral injections of either KA (0.8 µgs in 0.4 µl PBS) or PBS (Sham lesion), which were placed stereotactically in the anterior region of the neostriatum. Seven days later transplants of 17/18 day fetal striatum (2 mm^3) were introduced into the same injection sites. Following 5-6 wks or 9-10 mos survival, animals were perfused and tissue sections of caudate were processed for anatomical study. In the light microscope transplants in all animals were distinguished from surrounding host caudate by their pale appearance and absence of bundles of myelinated fibers. Cell counts in 1 µm thick sections showed higher proportions of medium-sized neurons with indented nuclei in the 5-6 wk and 10 mos transplants as compared to host caudate. Large neurons were not found in the 5-6 wk tranplants and were present in the 10 mos transplants in higher proportions than in host caudate. At the ultrastructural level, synaptic organization in the 5-6 wk and 10 mos transplants was well developed in KA and sham lesioned animals. Axon terminals contained round or pleomorphic vesicles and made symmetric and asymmetric synapses with cell bodies, dendrites, spines, and axon initial segments. Dendrites ensheathed by synapsing terminals were frequently present in the 10 mos transplants. Compared to host caudate neuropil synaptic density was somewhat higher in the 5-6 wk transplants and lower in the 10 mos transplants and the proportion of axodendritic synapses was higher in both transplant groups. Results suggest that fetal striatal transplants 1) develop a complex synaptic organization in either KA lesioned or normal host caudate; 2) may exhibit neuronal changes over time; and 3) show differences to host neostriatum in their neuronal organization. Supported by grant NS 16367 to M.D.

	transplants				host caudate
	5-6 wk		10 mos		
	KA	Sham	KA	Sham	
medium cells (indented nuclei)	26%	21%	50%	40%	6%
large cells	--		5%	10%	0.8%
density synapses/100μm^2	20		13		17
axodendritic synapses	24%		44%		20%

110.12 MOTOR-LIMBIC INTEGRATION: COLLATERAL NIGRAL PROJECTION TO THE CINGULATE CORTEX AND STRIATUM. M. Takada* and T. Hattori (SPON: R.M. Clavier). Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, Canada M5S 1A8.

A direct projection from the pars compacta of the substantia nigra (SNc) to the cingulate cortex in the rat was examined using several retrograde tracing techniques.

After injecting horseradish peroxidase (HRP) into the cingulate cortex, retrogradely-labeled neurons were found in the SNc as well as the limbic nuclei such as the hypothalamus and the anterior nuclear group, mediodorsal nucleus and laterodorsal nucleus of the thalamus. HRP-positive neurons in the SNc were bilaterally distributed mainly in the rostromedial parts of the nucleus, and to a lesser degree in the caudomedial portions with an ipsilateral predominance. No labeled neurons were detected in the ventral tegmental area of Tsai.

These SNc regions, containing presumed dopaminergic neurons, also project to the medial parts of the caudate-putamen (CPU). When we injected True Blue (TB, 5% suspension) into the cingulate cortex, and Diamidino Yellow (DY, 2% suspension) into the medial CPU, most of TB-positive neurons in the medial SNc were double-labeled with DY. These findings indicate that majority of the SNc neurons sending their axons to the cingulate cortex, also have axon collaterals projecting to the CPU. Moreover, injections of 3H-dopamine (20-40 Ci/mmol) into the cingulate cortex resulted in retrogradely-radiolabeled neurons in the medial SNc.

The present study strongly suggests that the dopaminergic neurons in the medial portions of the SNc have a key role in integrating limbic with motor functions.

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110.13 POTASSIUM EFFECT ON ELECTROTONIC CONSTANTS OF NEOSTRIATAL NEURONS. J. Bargas*, E. Galarraga*, and J. Aceves*. (SPON: J. Alanís). Dept. of Physiology and Neuroscience. Centro de Investigación del IPN. 0700-México, D.F., México.

It has been found (Sugimori et al. J. Neurophysiol. 41: 1662, 1978; Kita et al. Brain Res. 300: 129, 1984) that the membrane time constant of neostriatal neurons is significantly longer when determined in vivo than in vitro. We have explored whether the difference could be mostly explained by differences in extracellular K^+ in one condition as compared to the other. The experiments were done in rat neostriatal slices. Intracellular electrical activity was recorded by conventional microelectrode techniques. The slices were bathed in a medium either with 6.25 or 3 mM K^+. In higher K^+ (n=10), RMP was 67 ± 7 mV; input resistance (Rn): 29 ± 6 MΩ; τ_o : 7 ± 2 msec; τ_1 : 1.3 ± 0.3 msec; electrotonic length (L): 1.2 ± 0.1; ρ : 3.1 ± 1.5. While in lower K^+; RMP was 80 ± 7; Rn : 87 ± 4; τ_o : 16.2 ± 2; τ_1 : 1.7 ± 0.8; L: 0.7 ± 0.1; ρ: 1.4 ± 0.7. As seen, lowering K (in a physiological range) significantly increased input resistance and neuronal time constant. To see whether closing of K channels was involved in the observed changes of electrical constants when was lowered, the effect of TEA was studied at the higher K concentration. The parameters that, as compared to those without TEA, significantly changed were: R_n : 87 ± 9 and τ_o : 16 ± 3. τ_1 tended to be longer, and L and ρ tended to be smaller, as in low K. Other ionic conductances seem not to be as important as K conductance determining the electrotonic parameters of these neurons because TTX, 4-AP (1Mm) and Co apparently did not modify the parameters at 6.25 mM K^+. These results suggest that, at low K concentrations, neuronal electrical "constants" are not constants but depend on extracellular K. They also suggest that the opening of voltage-dependent K channels located mainly on dendrites (see that ρ tended to be smaller) are responsible of the changes in electrical parameters when K is decreased. The differences in time constant in vivo as compared to in vitro might be due to differences in extracellular K in one condition as compared to the other. (Supported by grant PCCBBNA-020884 from the CONACyT of México).

110.14 DOSE-DEPENDENT EXPRESSION OF THE WEAVER GENE ON THE DOPAMINE-CONTAINING INNERVATION OF THE FOREBRAIN AND EFFECTS OUTSIDE OF THE STRIATUM. S. Roffler-Tarlov and A.M. Graybiel, Depts. Neurology and Anat., Tufts Univ. Sch. Med., Boston, MA 02111 & Dept. of Psychology and Whitaker College, M.I.T., Cambridge, MA 02139

Two defects, one obvious and the other quite hidden, are present in the brains of mice carrying the autosomal recessive gene, weaver (wv). In homozygous weavers this mutation results in ataxia and a marked atrophy of the cerebellum consequent to massive loss of granule cells. Several studies point to the granule cell as a direct target of the gene and to dose-dependent effects in cerebellum of heterozygous weavers ($^+$/wv). The second abnormality exists in parts of the forebrain of weaver mice and is concealed from view as it results in no marked atrophy or disruption of cellular architecture. However, the defect in the forebrain can be revealed biochemically as a reduction in dopamine (DA) content in selected regions and anatomically by reduced catecholamine histofluorescence. Our previous report showed that a severe depletion of DA occurs in the dorsal striatum (caudoputamen, CP) of ^{wv}/wv mice relative to $^+$/wv controls, whereas the DA content in the nucleus accumbens (NAc) (part of the ventral striatum) was entirely conserved. A third striatal area, the olfactory tubercle (OT) showed a moderate reduction in DA. (Nature 307, 62-66, 1984).

The present study had two aims. One was to establish whether the affected DA-containing system is likely to be another primary target of the action of the weaver gene or whether the deficits in the DA-containing innervation are secondary, perhaps to cerebellar damage inducing ataxia. We studied striatal DA content in two other cerebellar mutants (Purkinje cell degeneration and staggerer) which are also ataxic and found no changes in the DA content of any striatal region. We compared striatal DA content in adult ^{wv}/wv, $^+$/wv, and $^+$/+ mice. We established that the presence of one weaver gene in the heterozygote results in a reduction of DA in vulnerable striatal areas. Compared to that in $^+$/+ animals, the content of DA in the CP was reduced by 13% in $^+$/wv and by 71% in ^{wv}/wv mice. Values for DA in the NAc were roughly equivalent in the three genetic types. Our second aim was to examine DA-containing regions outside the striatum. We found a 32% reduction of DA in the septum of ^{wv}/wv mice, and a 30% reduction in the hypothalamus. By contrast, the DA content of the frontal cortex, the amygdala, the olfactory bulb and the retina was spared.

The results show that the effects of the weaver gene on the DA-containing innervation of the forebrain are not confined to striatal targets but also extend to the septum and hypothalamus. Lack of DA depletion in other ataxic mutants and the presence of an effect in heterozygote weavers suggests that a subpopulation of DA neurons are likely to be a primary target of the weaver gene. Thus the weaver gene has at least two cellular targets and its effects on these must share some common molecular mechanism. Supported by NIH-NS 20181.

110.15 LATERAL SEPTUM AND CORPUS STRIATUM DIFFER IN THEIR IN VIVO ELECTROCHEMICAL RESPONSES TO CIRCLING BEHAVIOR AND TO AMPHETAMINE ADMINISTRATION. C.R. Freed and P.A. Mason, Depts. of Med. and Pharm., Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

The lateral septum (LS) and anterodorsal striatum (AS) both contain abundant dopamine (DA) nerve terminals but are innervated by different cell groups, lateral septum by A10 and striatum by A9. Previous work in our laboratory has shown an increase in contralateral striatal DA content and apparent release during trained circling for a sucrose water reward (Nature 298:467,1982; Life Sci. 30:2155,1982) but an increase in ipsilateral DA and DOPAC content in lateral septum during circling. (IUPHAR Abstr 1180P, 1984). We have now compared the in vivo electrochemical (EC) responses of the two nuclei to a behavioral stimulus--trained circling--and to amphetamine (AMPH) administration. Male Sprague-Dawley rats 250-350 gm had carbon paste in vivo EC electrodes implanted bilaterally in LS for the circling studies and in both LS and AS for AMPH studies. After 24 hr recovery from surgery, linear sweep voltammetry was performed from -0.2V to +0.5 V at a rate of 10 mV/sec using a DCV-5 cyclic voltammetry device. (Bioanalytical Systems). Electrodes were scanned alternately every five minutes. At both brain sites, a peak was seen at +0.19 V. During 60 min of circling behavior, there was a significant increase ($p<.05$) in the EC peak on the ipsilateral side which was maximally increased by 27±5% over baseline after 35 min and returned to baseline values by the end of circling. In drug experiments, AMPH 5 mg/kg i.p. increased the EC signal in striatum by 88±20% within 40 minutes. By contrast, the peak in lateral septum initially showed a small but not significant reduction and then began to increase at 40 min as the striatal peak reached its asymptote. The septal peak reached a maximum 120 min after AMPH and was 12±2% increased over baseline. The EC peak in LS was characterized by inhibitors of catecholamine synthesis. Animals implanted with in vivo EC electrodes were given pargyline 75 mg/kg, fusaric acid 100 mg/kg, or alpha-methylparatyrosine 300 mg/kg. Pargyline produced an 18±2% decrease in peak height; fusaric acid, a 10±2% reduction; and alpha-methylparatyrosine, a 26±2% decrease. These results indicate that the EC catechol peak in LS may be a composite of norepinephrine, DA and DOPAC. Because of the ambiguity of the EC signal, other animals were implanted with in vivo dialysis catheters masked with epoxy to perfuse either AS or LS and the dialysate was assayed by HPLC with EC detection. AMPH 5 mg/kg caused a large increase in DA release and a reduction in DOPAC release from AS, while in LS no DA release was seen. These data show that motor behavior leads to lateralization of catechol release in LS and AS but on opposite sides of brain. Amphetamine causes an increase in dopamine release from striatum but little or none from lateral septum.

INVERTEBRATE LEARNING AND BEHAVIOR I

111.1 CONTROL OF MOTILITY IN PARAMECIUM BY CYCLIC AMP AND CALCIUM. N.M. Bonini* and D.L. Nelson* (SPON: P. Claude). Neurosciences Training Program and Dept. Biochemistry, Univ. Wisconsin, Madison, WI 53706.

Paramecium, a ciliated protozoan with an excitable membrane, is a model system for studying the molecular mechanisms which regulate motility. The cell has two basic behaviors which reflect the membrane potential: backward and forward swimming. At the resting potential, the cell swims slowly forward; membrane hyperpolarization causes faster forward swimming, while Ca^{++} entry upon membrane depolarization causes backward swimming. We (Bonini, Gustin, and Nelson) have previously shown that increased cAMP correlates with the fast forward swimming of membrane hyperpolarization.

To explore the action of cAMP, and the relationship between Ca^{++} and cAMP in the control of swimming behavior, we are using detergent-permeabilized cells, or "models", to study proteins whose phosphorylation is correlated with specific swimming behaviors. Models are immotile, but the addition of MgATP reactivates them to swim forward. Addition of cAMP causes a two-fold increase in the forward swimming speed, and stimulates protein phosphorylation. The major phosphoproteins are 47, 45, 17, and 15 kD. Some phosphoproteins are associated with the ciliary fraction, including a protein of 55 kD which may be tubulin. These results suggest that at least one of the in vivo effects of elevated cAMP upon membrane hyperpolarization is to increase ciliary beat, possibly through protein phosphorylation.

Ca^{++} addition, which causes backward swimming in models, also stimulates phosphorylation, but of a partially different set of proteins. The major phosphoproteins are 64, 58, 47, 45, 25, and 17 kD. We have also found that, whereas cAMP causes fast forward swimming, and Ca^{++} backward swimming, addition of both cAMP and Ca^{++} results in slow forward swimming. This suggests that cAMP and Ca^{++} may be acting antagonistically to control ciliary beat direction.

In summary, the in vivo fast swimming behavior of the cell in response to membrane hyperpolarization may be mediated by cAMP-stimulated protein phosphorylation. Furthermore, Ca^{++} and cAMP may be acting antagonistically, perhaps both through phosphorylation mechanisms, in the control of swimming direction.

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111.2 PHARMACOLOGICAL AND GENETIC STUDIES OF C.ELEGANS EGG-LAYING. E.J. Wolinsky and H.R.Horvitz. Department of Biology, Massachusetts Institute of Technology, Cambridge MA 02139.

We are using mutations and neuroactive drugs to study the egg-laying behavior of the nematode C.elegans. We believe it is feasible to attempt understanding of the physiology of the egg-laying system because of the small number of neurons involved (only eight innervate the egg-laying muscles), the ease of measuring the behavior (by counting eggs), and the availability of genetic variants and manipulations (e.g., Trent et.al. [1983] Genetics 104:619-47).

Previous experiments suggested that serotonin (5HT) and acetylcholine (ACh) stimulate egg-laying, while octopamine inhibits egg-laying. Additional experiments with the following drugs have strengthened these conclusions: tomoxetine, an inhibitor of 5HT uptake; gramine, a 5HT receptor blocker; synephrine, which interacts with octopamine receptors in other invertebrates. Mecamylamine, a cholinergic blocker, stimulates egg-laying, consistent with earlier results with curare, suggesting that an inhibitory cholinergic input to the egg-laying system exists as well.

Another approach we are taking to studying the regulation of egg-laying is to seek egg-laying constitutive mutants, i.e., mutants that lay more eggs than does the wild-type in the absence of an exogenous stimulus. Unlike the many egg-laying defective mutants previously isolated, many of which are likely to be disrupted in the machinery needed for egg-laying (i.e., the vulva and vulval muscles), egg-laying constitutive mutants seem likely to be disrupted in regulatory functions. In addition, the contrasting phenotypes of the two types of mutants can be used to help define functional pathways based upon whether a particular constitutive mutation can overcome the blockade of egg-laying caused by a particular defective mutation. By screening an existing set of behaviorally "uncoordinated" mutants, we have identified six genes that can mutate to both perturb locomotion and to cause constitutive egg-laying. In addition, we have isolated several new egg-laying constitutive mutants, all of which also have defective locomotion. The mutant che-3(e1124), a chemosensory mutant with normal locomotion, but lacking octopamine, is also constitutive for egg-laying. These egg-laying constitutive mutations are currently being categorized with respect to drug responses and ability to suppress specific classes of egg-laying defective mutations in order to define different functional steps in the egg-laying pathway.

111.3 HABITUATION AND SENSITIZATION OF THE TOUCH-ELICITED SHORTENING REFLEX IN THE LEECH, *HIRUDO MEDICINALIS*. D. S. Stoller* and C. L. Sahley. (SPON: R. J. Contreras) Dept. of Psychology, Yale University, New Haven, CT. 06520.

The two forms of non-associative learning, habituation and sensitization, have been studied extensively in a number of invertebrates. Habituation refers to a decrement in the response elicited by a given stimulus following repeated presentations of that stimulus. Sensitization is defined as the facilitation of a response following the occurrance of a noxious stimulus, such that presentations of a moderate stimulus now elicit a response of increased magnitude. In the leech, habituation of swimming and photo-elicited habituation of shortening have been demonstrated (Debski & Friesen, 1985; Lockery, 1985). We have investigated plasticity of the touch-elicited shortening reflex and of a monosynaptic pathway which may mediate this reflex in *Hirudo medicinalis*.

Thirty leeches were randomly divided into two groups of fifteen. One group received twelve training presentations of a moderate touch stimulus with an interstimulus interval of one minute. A mild shock was presented to the second group four minutes prior to training . A decrease in the magnitude of whole body contraction in response to touchwas observed in the first group. Exposure to the shock stimulus prior to training appeared to retard the response decrement.

To decrease behavioral variability habituation was further studied in leeches with tail sucker denervation. These leeches also demonstrated a decrement in responding to repeated stimulation. Spontaneous recovery of the response was evident 30 min following training.

Using a semi-intact preparation, the activity of both a mechanosensory neuron (P) and a motoneuron (L) in response to skin stimulation delivered according to the temporal parameters used to produce habituation was recorded. The P neuron responds to moderate touch stimulation. The L excites the longitudinal muscles which produce whole-body contractions. The P sensory cell is monosynaptically connected to the L motoneuron via both electrical and chemical synapses (Nicholls and Purves, 1970). No change was seen in the number of action potentials elicited by the P sensory neuron following repeated skin stimulation but a profound decrease was observed in the level of excitation of the L motoneuron. It is suggested that a decrease in the efficacy of the synaptic connections between these particular neurons may be found to account for the habituation of whole body shortening reflex observed in the intact animal.

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111.4 ASSOCIATIVE LEARNING MODIFIES TWO BEHAVIORS IN *HIRUDO MEDICINALIS*. C. L. Sahley and D. F. Ready. (SPON: M. J. Cohen) Dept. of Psychology, Yale University, New Haven, CT. 06520 and Dept. of Biology, Princeton University, Princeton, N. J. 08544.

The simple, stereotyped CNS of the leech makes it a favorable preparation for studying the neuronal basis of behavior (Nicholls, Muller and Stent 1981) including learning (*Macrobdella decora*) (Henderson and Strong, 1972). We report here that the medicinal leech, *Hirudo medicinalis*, can modulate a simple reflex, touch elicited shortening, as well as a complex behavior, stepping, based on a learned association. In the first experiment we asked if leeches could learn to associate a light touch to the head (CS) which normally elicits a shortening response with an electric shock to the tail (US). Forty leeches were randomly assigned to four groups of ten. Leeches in group 1 experienced 20 paired presentations of a light touch to the head (CS) paired with an electric shock to the tail (US) . Leeches in Group 2 experienced 20 explicitly unpaired presentations of the CS and US. Leeches in Groups 3 and 4 experienced 20 presentations of either the CS alone, or the US alone, respectively. Leeches in all groups were tested after every fifth trial. Animals that experienced paired touch and shock showed an increase in shortening to touch. In contrast, leeches in the unpaired, CS alone, or US alone groups all showed decreased responding to the touch over trials. The change in behavior occurs only with an interstimulus interval of 1-2 sec. Thus, associative learning can modify a simple reflex in the leech. Learning is retained for 24 hrs but is not present at 36 hrs.

In a second experiment we asked if associative learning could modulate a more complex behavior such as stepping. A leech in shallow water will reliably step in an inchworm-like fashion away from a shock applied to the tail. Stepping has been characterized by Tomlinson and Kristan (1984). A light touch applied to the mid-body of the leech normally results in only local bending. Following 30 touch-shock pairings, 7 out of 10 leeches stepped to touch alone. To control for nonassociative increases in stepping we compared leeches which had experienced the touch-shock pairings with leeches which had experienced either the CS or US alone, or the CS and US in an explicitly unpaired relationship. Two of ten leeches in the unpaired group stepped to the test touch and none of the leeches which had experienced either the CS or US alone leeches stepped to the touch in the test.

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111.5 ASSOCIATIVE LEARNING IN FLATWORMS. M. Hauser*, B. Kolb* and H. Koopowitz. Developmental and Cell Biology, University of Ca., Irvine, CA 92717.

For the last three decades many researchers have employed a number of classical conditioning paradigms in attempts to demonstrate that planaria can learn associatively. Many conflicting results were reported thus failing to provide conclusive evidence that these primitive metazoans can learn associatively. Since the time that the planarian learning controversy was at its peak, it has been demonstrated that the more advanced marine polyclads can undergo the behavioral modification of habituation. We now report the demonstration of associative learning in the marine polyclad flatworm Notoplana acticola, using an avoidance conditioning paradigm. Notoplana feed regularly on commercially available frozen brine shrimp which was used as the conditioning stimulus (CS). If the supernatant from a shrimp homogenate is placed in a capillary tube and presented to a worm, the normal, unconditioned response (UR) is for the worm to grip the tube. The unconditioned stimulus (US) used was a 0.02 ml squirt of 0.75M KCl delivered to the tail of the animal. In response to this stimulus the animal recoils and rapidly walks or swims away. The paradigm consisted of presenting the shrimp extract (CS) to the animal, and delivering the US when it gripped the tube. Paired trials were delivered at thirty second intervals. The conditioned response (CR) was achieved when the worm no longer reached for the capillary tube or engaged in avoidance behavior upon presentation of the CS. Under these conditions the mean number of trials to response criterion was 4.2 $\pm$ 0.3 (n=33). The mean retention time for this associative change was very short, lasting only 4.1 $\pm$ 0.3 minutes (n=32). Control animals received random, unpaired presentation of the CS and US and did not exhibit CR. There was no evidence of enhancement of learning with testing on sucessive days. Decerebrate animals can be made to habituate to vibration however, they do not demonstrate associative learning in the paradigm studied. This indicates that the presence of the brain is essential if the animal is to learn associatively.

111.6 OCTOPAMINE MAY MEDIATE CENTRAL EXCITATORY STATE RESPONSE IN THE BLOW FLY. Terry R. McGuire* and Lawrence Friedman* (Spon: M.F. MacDonnell). Department of Biological Sciences and Bureau of Biological Research. Rutgers University, Piscataway N.J. 08854

The central excitatory state (CES) is a non-associative component of proboscis extension in the blow fly Phormia regina. Previous work has shown that phenotypic differences in the CES reflect underlying genetic differences (McGuire, Behav. Genet., 11:313, 1981; Tully & Hirsch. Behav. Genet., 12: 395, 1982a). Strains of blow flies have been bidirectionally selected for the expression of high or low CES response. These selected strains differ at a single major genetic locus (Tully & Hirsch, Anim. Behav., 30:1193, 1982b). In addition, the expression of CES is positively correlated with the expression of classical conditioning and CES seems to function as a "primer" or precondition for learning.

We have attempted to determine the role that octopamine plays in the mediation of CES responses through a pharmacological study. Flies from each of the selected strains were injected with 0 (sham), 5, 10, 20 or 100 μg of drugs that specifically affect the octopaminergic system. Two hours later these flies were tested for their CES response. Drugs acting at the level of the beta receptor (propranolol and isoproterenol) had no effect on CES response in either selected strain. Drugs that acted on the alpha receptors (naphazoline, benextramine) showed significant dosage effects in at least one of the selected strains. In the low CES strain, the alpha-agonist, naphazoline, produced a 2 to 4.5 fold increase in mean proboscis extensions over sham-injected flies. The change in mean proboscis extensions in the high-CES strain was in the same direction but was non-significant. The alpha-receptor antagonist, benextramine, produced a significant decrease (0.5 to 4 fold) in CES response in the high-CES strain. Benextramine had no effect on CES response in the low-CES strain.

These results suggest that the octopaminergic system is involved in the mediation of CES at the receptor level. Octopamine may also mediate the expression of classical conditioning.

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111.7 BRAIN BIOGENIC AMINES AND BLOWFLY FEEDING BEHAVIOR. G.L. Brookhart*, R.S. Edgecomb, and L.L. Murdock. Dept. of Entomology, Purdue University, W. Lafayette, IN 47907

The adult blowfly, Phormia regina Meigen, detects food by means of chemosensory hairs on its tarsi. When these hairs contact adequate stimuli, the proboscis is extended. Flies deprived of food require a much weaker stimulus to elicit a proboscis extension than do fed flies. Responsiveness to food stimuli like aqueous sucrose can be expressed quantitatively as the mean acceptance threshold (MAT), the minimum log concentration of sucrose to which the average fly will respond. d-Amphetamine can markedly alter the MAT (Edgecomb, R.S. et al., Soc. Neurosci. Abst. 10, pt 2, p. 1065). When d-amphetamine (12 μg/fly, 0.5 hr.) was injected into three-day-old, unfed flies, the MAT rose from the control value of 3.9 mM sucrose (log value 0.59 ± 0.05 S.E.M.) to about 1600 mM (log value 3.2 ± 0.07). Analysis of brains from the amphetamine-treated flies by HPLC with electrochemical detection revealed reduced levels of octopamine, dopamine, and 5-hydroxytryptamine (5-HT). The time course of d-amphetamine action on the tarsal taste-proboscis extension response and brain amine levels indicated an inverse relationship between MAT and amine levels. By 2 minutes after injection with d-amphetamine (12 μg/fly) the MAT was significantly greater than that of saline-injected flies and brain octopamine was significantly depleted. The highest MAT was observed at 0.5 hr., at which time the octopamine level was reduced from the control value of 2.0 ± 0.29 to 0.30 ± 0.19 picomoles/brain. At 0.5 hr., dopamine had declined from 2.4 ± 0.18 to 0.84 ± 0.16 picomoles/brain and 5-HT had fallen from 1.55 ± 0.12 to 0.44 ± 0.06 picomoles/brain. By 3 hr. the MAT and amine levels of the treated flies had returned to values near those of the controls. dl-Fenfluramine (8 μg/fly, 0.5 hr.) also increased the MAT to 390 mM (log value 2.6 ± 0.39) and depleted octopamine and dopamine, while 5-HT was only weakly affected.

These results suggest that d-amphetamine and dl-fenfluramine, which markedly affect blowfly feeding behavior, act by depleting aromatic biogenic amines in the CNS.

111.8 APPETITIVE LEARNING IN THE TERRESTRIAL MOLLUSC, *LIMAX MAXIMUS*. K. Martin,* A. Diamond*, J. Dubin*, C. Sahley.(SPON: J.A. London. Dept. of Psych, Yale University, New Haven, CT. 06520.

We are interested in the role of learning in the modulation of feeding behavior of the gastropod mollusc *Limax maximus*. Previous work has demonstrated that slugs can learn to associate a food odor and/or taste (CS) with a bitter taste consequence (US), quinidine sulfate (Sahley et al, 1981). This effect has been demonstrated both behaviorally as well as in a semi-intact preparation (Chang & Gelperin, 1981). After an odor-taste pairing slugs no longer show a preference for the odor that had been paired with quinidine. Associative learning in *Limax*, like in vertebrates, is critically dependent upon two inter-event relationships: temporal specificity and predictability (Sahley, et al, 1981). To further study the role of learning in feeding behavior we have been exploring these variables in an appetitive task. In these experiments the CS was a non-food odor and the US was a food. After several CS-US pairings, *Limax* can acquire a preference for the odor paired with food.

Slugs in the appetitive task are trained with a differential training procedure. Each slug is presented with two odors, amyl acetate (AA) and methylcyclohexanol (MCH). For each experimental animal one of these odors was designated as the CS+, during training this odor was consistently paired with food. The other odor, CS−, was never paired with food. The CS-US interval was varied between experimental groups. Slugs in control groups experienced either explicitly unpaired presentations of the CS and US, CS alone or US alone presentations. To assess the effects of this training each slug's odor preference was tested in a two-choice procedure. Each slug was placed in the test chamber in which the two odors, AA and MCH, were localized to opposite sides of the chamber. From a series of 3,60-sec tests the percentage of total time spent over each odor was calculated. We found that only the animals which had experienced an odor-food pairing showed an increase in time over that odor. This result, excitatory conditioning, is only seen when the CS and US are temporally arranged such that the CS precedes by a short time interval, 10-20 sec, the presentation of the US and is terminated shortly after the US begins. If the CS precedes the US by a long period, 30 sec, the animal does not develop a preference for the odor which preceded the food. Also, if the CS both precedes and is presented throughout the US the animal not only does not show a preference for the CS+ but may avoid this odor. This indicates the possibility of inhibitory learning. Further research is exploring the possibility of conditioned inhibition in this paradigm. Support: NIMH 5RO1-MH37902, NIH BRSG 2-507-RR07015, and Sloan BR2486 to C.L.S.

111.9 MAPPING AND PHARMACOLOGY OF NEUROTRANSMITTERS IN THE FEEDING CONTROL SYSTEM OF LIMAX. Ian Cooke* and Alan Gelperin, (SPON: L. Symonds). Dept. Biology, Princeton University, Princeton, N.J. 08544 and Dept. of Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

As part of our study of the associative learning mechanism used to modify information transmission in the taste input-feeding motor program (FMP) output pathway, we are using immunocytochemical and pharmacological techniques to investigate the importance of several neurotransmitters for the operation of this pathway in the cerebral and buccal ganglia of the terrestrial mollusc Limax maximus. Immunohistochemical techniques applied to whole-mount and sectioned tissue revealed a large number of FMRFamide-like immunoreactive (FLI) neurons, ranging in size from 10 to 200 um. FLI axons are present in all the central connectives and commissures and in a large number of peripheral nerves. Varicose FLI fibers ramify extensively throughout the neuropil of central ganglia and in the connective tissue ensheathing the central ganglia.

FMRFamide has a potent inhibitory effect on the expression of FMP by isolated lip-brain preparations. Bath application of $3x10^{-6}$M FMRFamide reduces by 50% the intensity of bouts of FMP elicited by a standard chemostimulus applied to the lips. FMRFamide also has direct inhibitory effects on the autoactive salivary fast burster (FB) neuron. Application of $3x10^{-6}$ M FMRFamide in low calcium, high magnesium saline strongly hyperpolarizes the FB neuron and abolishes the slow ramp depolarization that underlies its spontaneous bursting.

Stimulated by the finding that acetylcholine (ACh) triggers FMP in the marine slug Pleurobranchaea (Davis, personal communication), we applied $3x10^{-6}$M ACh to the cerebral and buccal ganglia of the Limax lip-brain preparation and compared the output elicited by ACh with the output elicited by lip chemostimulation. The buccal outputs elicited by ACh and taste input are very similar. ACh application to the cerebral ganglia alone, but not to the buccal ganglia alone, produced FMP. Dopamine ($5x10^{-5}$M) shows the reverse pattern, producing FMP when applied to the buccal ganglia but not when applied to the cerebral ganglia. Extensions of this work will involve mapping the location of cholinergic neurons in the cerebral ganglia and determination of the transmitter(s) contained in the cerebral-buccal interneurons which can trigger FMP (Delaney & Gelperin, 1985, Soc. Neurosci. Abstr. 11). (Supported by NIMH Grant 39160).

111.10 TRAINING AND TESTING DETERMINANTS OF PHOTOTAXIC BEHAVIOR IN HERMISSENDA. (Spon:M.Lampert) L.Vold*, L.Grover*, and J.Farley. Prog. in Neurosci., Princeton Univ., Princeton, NJ.

Five pairings of light and rotation result in a pairing-specific suppression of Hermissenda's phototaxic behavior which persists for ∿20-25 min. Here, we describe those variables which are important determinants of phototaxic behavior, - and thus the sensitivity of retention tests - and those factors which determine the degree of original learning.

In experiment one, 5,10, or 15 min dark adapted animals received four successive hourly tests of phototaxis in either a horizontal or vertical orientation. Repeated tests increased start & finish latencies in all conditions, more so for animals tested in the horizontal vs. the vertical orientation (6 vs. 2 fold increases). In addition, longer dark-adaptation times resulted in longer phototaxic latencies. Additional experiments indicated that both repeated handling and interruption of the diurnal cycle by the repeated dark-adaptation periods were responsible for the increased latencies. In further experiments, we systematically varied both test orientation (horizontal vs. vertical), and training light intensity (moderate BB:170 μW; 510: 140 μW vs. bright 430 μW; 330 μW), for paired vs. random control animals and obtained measures of both phototaxic as well as negative geotaxic behavior. Animals which received 5 pairings of the moderate intensity training light and rotation, and were tested in the vertical orientation, exhibited significantly greater suppression of phototaxic behavior ($\bar{x}$ ± S.D.; .24 ± .19, n=20) than comparable random controls (.43 ± .24, n=15; $p < .01$). Negative geotaxic behavior was not significantly affected (paired .31 ± .18 random: .39 ± .30; n's=18;). Thus, the changes were both pairing- and stimulus-specific. In contrast, animals trained with the moderate-intensity light but tested in the horizontal orientation (paired: .28 ± .20, n=14; random: .26 ± .21, n=15), or trained with the bright light and tested in either the horizontal (paired: .16 ± .12, n=24; random: .25 ± .20, n=23) or the vertical orientation (paired: .31 ± .21, n=22, random: .27 ± .17, n=18) all showed suppression of phototaxic behavior. It was, however, neither pairing- nor stimulus-specific.

Thus, whether one obtains short-term, pairing- and stimulus-specific reductions in phototaxic behavior resulting from small numbers of training trials depends upon: 1) the use of a moderately intense training light, 2) a sensitive testing orientation. These results may help to explain the failure to obtain pairing-specific suppression of phototaxic behavior (following 5 or 10 pairings) reported by Crow (1983, J.Neurosci.) who tested animals in the horizontal orientation and trained animals with a very bright light (70 times brighter than the one he has previously used and ∿100 times brighter than ours).

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111.11 NEURONAL ANALOGUES OF CONDITIONING PARADIGMS IN APLYSIA . R.E. Clymer* and M.J. McCreery*. (SPON:D.R. Livengood).Dept. of Physio., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

This study examined the conditionability of various abdominal ganglion cells of the Aplysia californica and the role of postsynaptic cell firing in producing any observed conditioning.

Suction electrodes were attached to the left and right connectives of the dissevered ganglion. Intracellular recodings were obtained using potassium acetate filled electrodes and a Dagan Model 8100 single elctrode voltage clamp operating in current clamp mode.

Mild electrical stimulation of one connective was adjusted to produce a postsynaptic potential (PSP) in a cell. The PSP was repeatedly paired with each of 4 forms of stimulation. In the "conditioning" procedure the PSP was followed in 300 msec. by repeated firing of the cell induced by intense stimulation of the other connective . In the "pseudoconditioning" procedure the stimulation induced firing followed the PSP by 10 seconds. In the "clamp" procedure the cell was hyperpolarized to prevent cell firing in response to the intense stimulation of the connective which was presented as in the conditioning procedure. In the "current injection" procedure the PSP was followed in 300 msec. by repeated cell firings induced by injection of depolarizing current into the cell. The order of the 4 procedures was counterbalanced.

Following the conditioning and clamp procedures the PSP was significantly larger. This was not true of the pseudoconditioning procedure. The current injection procedure resulted in a decreased PSP. These results rule out a role for postsynaptic cell firing in producing conditioning.

The pseudoconditioning procedure had an inhibitory effect upon an immediately following conditioning procedure. This appeared to be analogous to the conditioned inhibition observed in behavioral studies. Other demonstrations of a neuronal analogue of conditioned inhbition are unknown to the authors.

Cells were classified according to their response to the conditioning and pseudoconditioning procedures. Cells which did not respond to the conditioning procedure were called unconditionable. If they responded to both the conditioning and the pseudoconditioning procedure they were called pseudoconditionable. If they responded to the conditioning procedure but not the pseudoconditioning procedure, they were truly conditionable. Pseudoconditionable cells were more responsive to the clamp procedure than the other two cell types. This suggests that the mechanism of pseudoconditioning differs from that of true conditioning.

The electrophysiological procedures produced results which were directly analogous to in vivo conditioning experiments. Postsynaptic cell firing does not appear to play a role in producing conditioning. Whatever the mechanism, sophisticated information processing appears to occur in simple neuronal systems.

111.12 DIRECTED DEFENSIVE RESPONSES IN APLYSIA CALIFORNICA. M.T. Erickson* and E.T. Walters (SPON: R. Levine). Dept. of Physiol. & Cell Biol., Univ. of Texas Med. Sch., Houston, TX 77225.

While defensive responses in Aplysia have been used extensively for neurobiological investigations of learning, interesting questions remain about the organization and function of these behaviors. For example, are defensive responses in Aplysia under directional control? We have now found that important features of several defensive responses (inking, siphon withdrawal, and escape locomotion) depend upon the site of noxious stimulation, and that these features are involved in directing behavior either towards or away from an apparent threat.

Animals were placed in the center of a round plastic tub (15" diameter) filled with artificial seawater. After 3 min a noxious tactile stimulus was delivered (40 ma shock or mechanical pinch) to either the left or right side of the body (tentacle, neck, parapodium, or tail) or to the siphon. Videotape analysis showed that the initial jet of ink from the mantle cavity was always directed towards the stimulation site: stimulation of anterior sites elicited anterior ink ejection, tail stimulation elicited posterior ejection, and parapodial or siphon stimulation elicited simultaneous anterior and posterior ejections. Using a 5-point rating scale for direction, we found that anterior vs. midbody, anterior vs. posterior, and midbody vs. posterior stimulation sites produced significantly different directions of initial ink ejection ($p < .001$ in each case). In addition, anterior stimulation caused the siphon to constrict or funnel (presumably to prevent ink ejection through the siphon) and to assume a vertical orientation. By contrast, posterior stimulation caused the siphon to flatten without constricting and to point towards the tail (presumably to direct the ink backwards). Whereas inking is directed towards the threat, escape locomotion is directed away from a source of tactile disturbance: anterior stimulation caused turning and escape locomotion away from the stimulus, while posterior stimulation produced little change in direction (anterior vs. posterior turn angle was significantly different; $p < .001$). Anterior stimulation also produced significantly fewer steps during escape locomotion than did posterior stimulation ($p < .025$).

This directedness of defensive responses in Aplysia adds a new dimension that should be addressed in both neural and functional analyses of these behaviors. It also adds an interesting parameter to explore in studies of learning in this simple preparation. (Supported by grant MH 38726 from the NIMH to E.T.W. and by an NIH summer fellowship to M.T.E).

111.PO *DISRUPTION OF PHEROMONE-GOAL DIRECTED BEHAVIOR WITH AMBIENT CATIONS IN T-CASTANEUM. R.H.Loiselle, A.J. Giannini, W.A.Price, G.F.Ludrik‡ L.Kerr‡ Dept. of Psychiatry, Northeastern Ohio Medical College, P.O. Box 2169 Youngstown, Ohio 44504.

High levels of ambient cations have been shown to produce anxiety and elevated levels of peripheral serotonin in human and other mammalian populations. Some have postulated that this is due to cation-induced inhibition of the enzyme, monoamine oxidase (MAO). To test this hypothesis the authors used the beetle T-castaneum which has repeatedly been shown to possess no MAO activity. One hundred male beetles were placed in a field width measuring 6" x 24". Random movement was observed and nineteen beetles crossed a finish line after twenty minutes. When a species specific pheromone was placed at the finish, seventy-three beetles crossed the line within twenty minutes. When an ion generator was placed behind the finish line and used to raise atmospheric cation levels to 2400 cations/cm^3 (normal: 1050-1150/cm^3), only twenty-eight beetles crossed the line. The avoidance behavior seen indicated that cations also have an adverse affect on beetles. The absence of a MAO system suggest that atmospheric cationic action may be independent of this enzyme.

112.1 INTERACTIONS BETWEEN MUTATIONS AFFECTING CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE IN THE NEMATODE C. ELEGANS. J. B. Rand, C. D. Johnson*, and R. L. Russell*. Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260 and Zoology Dept., Univ. of Wisconsin, Madison, WI 53706.

In C. elegans, the gene cha-1 is the structural gene for choline acetyltransferase (ChAT; Rand and Russell, Genetics 106:227), and the unlinked genes ace-1, ace-2, and ace-3 are the apparent structural genes for for class A, class B, and class C acetylcholinesterase (AChE), respectively (Johnson et al., Genetics 97:261; Culotti et al., Genetics 97:281; and unpublished). Severe cha-1 mutants, which contain less than 2% residual ChAT activity, and are severely uncoordinated and slow-growing, show no apparent alteration in the amount of any of the classes of AChE. Furthermore, the distribution of different molecular weight forms of AChE within each of the classes is unaffected by the presence of a severe cha-1 mutation. We also find that animals homozygous for any one or two of the ace mutations do not show any compensatory changes in the levels of either ChAT or the remaining class(es) of AChE. It therefore seems that the levels of ChAT and AChE activity are not dependent on each other or, presumably, on acetylcholine levels.

Nevertheless, we have observed two cases of phenotypic interaction between ChAT and AChE mutations. The presence of all three ace mutations simultaneously leads to lethality in late embryos or early larvae. However, we have demonstrated that quadruple mutants, containing cha-1 and all three ace mutations, are viable and become fertile adults. We therefore conclude that near-total ChAT deficiency can apparently overcome the lethality caused by near-total AChE deficiency.

We have also found that ace-3 mutations significantly improve the deficient growth rate of severe cha-1 mutations, although such double mutants are clearly not completely wild type. Thus, deficiency of a specific AChE class can partially compensate for secondary effects caused by ChAT deficiency.

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112.2 CLASS D, A FOURTH CLASS OF ACETYLCHOLINESTERASE FROM CAENORHABDITIS ELEGANS. B. D. Stern and R. L. Russell (SPON: G. H. Fromm). Department of Biological Sciences, University of Pittsburgh, Pittsburgh PA 15260.

The free-living soil nematode Caenorhabditis elegans contains three well characterized classes of acetylcholinesterase (AChE) which are designated A, B, and C. AChE forms in each of these classes can be differentiated from those in other classes on the basis of kinetic differences and genetic control, with the genes ace-1, ace-2, and ace-3 the likely structural genes for the three classes. We report here the discovery of a fourth class of AChE, which has been designated class D.

Class D is a relatively minor form representing only a few percent of wild type AChE activity present in extracts. It was first detected in a mutant deficient in class C and was then shown to be present in wild type extracts. All of the biochemical characterizations described below were performed on class D which had been partially purified by an affinity chromatography method from animals deficient in classes B and C.

Only a single class D form has been detected and it has a sedimentation constant of about 3.5s which is distinct from the sedimentation constants of the forms in the other classes (5s, 7s, 11s, and 13s). Class D shows a K_m for acetylcholine of 1 micromolar which is also distinct from the K_m's of the other classes: 15 micromolar, 70 micromolar, and 20 nanomolar for classes A, B, and C respectively. Classes A and B are relatively sensitive to the AChE inhibitor eserine while class C is highly resistant to this inhibitor. Class D also shows a high resistance to eserine, similar to that of class C. However class D is considerably more thermostable than class C. Also, use of a polyclonal antibody to class C indicates that there is no crossreactivity between classes C and D. Double mutant animals deficient in either class A and class B or class B and class C contain class D enzyme, suggesting that the class D is not controlled by the structural genes for any of the other classes.

All of these data taken together suggest strongly that wild type C. elegans contains a class of AChE which has properties which distingish it from the three other known classes. The function of class D is unknown. However it is noteworthy that animals triply deficent in all of the three other classes are inviable while animals deficient in any one or any two of the other three classes are viable. This suggests that class D does not substantially overlap in function with any of the other classes.

112.3 GENETIC MOSAICS OF CAENORHABDITIS ELEGANS WHICH LACK NEURAL EXPRESSION OF THREE GENES FOR ACETYLCHOLINESTERASE ARE VIABLE Carl D. Johnson* and Robert K. Herman*† (SPON: J. Adler) Dept. of Zoology, Univ. of Wisconsin, Madison,WI 53706 and Dept. of Genetics and Cell Biology, Univ. of Minnesota, St. Paul, MN 55108

The acetylcholinesterase (AChE) activity of the nematode Caenorhabditis elegans is heterogeneous. Each of the separable form which has been described, can be assigned to one of three kinetic classes (ACh Kms: 80uM for class A, 15uM for class B and 0.01uM for class C forms). Mutations in the ace-1 gene eliminate the class A forms, ace-2 mutants lack the class B forms. Although ace-1;ace-2 double mutants are uncoordinated and hypercontract abnormally when touched (the Ace-Unc phenotype), strains mutant in either ace-1 or ace-2 alone have no apparent effect on behavior. Recently, mutants which eliminate class C AChE have been isolated; these define the new gene, ace-3. The ace-3 mutants have no visible locomotory defects and display normal responses to touch. Ace-1;ace3 and ace-2;ace-3 double mutants are also apparently normal. In contrast, triple mutant strains are inviable (the Ace-Let phenotype).

Mosaic individuals, in which some cells carry a wild type allele of the ace-1 gene whereas other cells carry only mutant alleles of the ace genes, have been used to investigate the cellular requirements for ace-1 gene expression. Mosaics for the ace-1 gene are generated through somatic loss of a small free chromosomal duplication carrying the wild type allele of ace-1. By using the mutations unc-3, daf-6, osm-1 and sup-10, which are also carried on the duplication, as cell markers (see Herman, Genetics 108, 165) we suggest that the lack of $ace\text{-}1^+$ gene expression in muscle cells is responsible both for the Ace-Unc phenotype (in an ace-1;ace-2 background; Herman and Kari, Cell 40, 509) and for the Ace-Let phenotype (in an ace-1;ace-2;ace-3 background). Animals which lack expression of all three ace genes within all but two of the total 282 somatic neurons, are viable and superficially normal. Although the behavior of such mosaics has yet to be analyzed in detail, this result is consistent with the notion that central cholinergic synapses in this nematode have no requirement for expression of these three ace genes in either pre- or post-synaptic cell.

112.4 IMMUNOAFFINITY PURIFICATION OF CHOLINE ACETYLTRANSFERASE FROM HUMAN BRAIN. D. Casper[a], B. H. Wainer[b], and P. Davies*[a]. [a]Depts. of Neuroscience & Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, and [b]Depts. of Pathology & Pediatrics, University of Chicago, Chicago, IL 60637.

Choline acetyltransferase (ChAT; E.C 2.3.1.6) was purified from human basal ganglia over 35,000-fold in one step using an immunoaffinity column made from F(AB)2 fragments of a monoclonal antibody to ChAT.

Rat monoclonal antibody AB8 reacts with the human form of ChAT by immunoprecipitation assay. This monoclonal was affinity purified on a goat anti-rat Affi-Gel 10 column and the purified antibody was digested with pepsin to yield F(AB)2 fragments, further purified from undigested material on sephadex G-150. F(AB)2 fragments were deemed necessary for the purification because purified AB8 on reducing SDS polyacrylamide gels was shown to have doublet heavy chain bands with apparent molecular weights of 53,000 and 55,000 daltons. Reported molecular weights for ChAT are 50-80 Kd. The use of goat anti-rat serum as a reagent in attempts to develop Western blots of ChAT reveal intense direct staining of the heavy chain doublet transferred to nitrocellulose. In control experiments, both human and rat brain tissues were shown by Western blots to contain appreciable amounts of immunoglobulin with heavy chain bands in the 50-55 Kd region.

In a representative preparation, an acetone powder was made from 319 grams of human basal ganglia, solubilized in PBS with protease inhibitors and spun at 100,000 x g. The supernatant containing ChAT activity was loaded onto a column of anti-Chat F(AB)2 fragments and all of the ChAT activity was retained by the column. After extensive washing, the column was eluted with 3M potassium thiocyanate and the protein-containing fractions were run on a reducing SDS-polyacrylamide gel. The final yield of protein was 0.8mg. The protein profile as seen by silver stain consisted of a doublet at 68,000 and 70,000 daltons, with single bands at 55,000, and 50,000, and lower molecular weight species. Western blotting experiments demonstrated AB8-dependent staining of the 55,000 dalton band, but this is thought to be non-specific binding as this band is also present in the flow through fractions from the affinity column which contain no ChAT activity. Hence an upper limit for the abundance of ChAT is estimated to be 0.003%, assuming the eluted protein to be 100% ChAT. This confirms reports by others that this enzyme is present in extremely low quantities in brain; even those regions where cholinergic neurons are relatively abundant.

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112.5 IMMUNOAFFINITY PURIFICATION OF CHOLINE ACETYLTRANSFERASE FROM CHICK BRAIN AND OPTIC LOBES. M.E. Epstein, D.D. Bloom*†, J.L. Dahl†, and C.D. Johnson*‡ (SPON: C.D. Geisler). Departments of Anatomy, Pharmacology†, and Zoology‡, University of Wisconsin-Madison, Madison, WI 53706.

Choline acetyltransferase (ChAT) has been purified to apparent homogeneity from chick brain and optic lobes. The initial steps involved polyethylene glycol precipitation, followed by chromatography on CM-Sephadex. The final purification step was accomplished by chromatography on Affi-Gel 10 to which had been coupled a monoclonal antibody which binds chick ChAT [Soc. Neurosci. Abstr. 9, 959 (1983)]. More than 90% of the ChAT activity in the CM-Sephadex fraction, applied in the presence of 0.15 M NaCl, was bound to the monoclonal antibody affinity column. After extensive washing of the column with buffer containing 0.5 M NaCl, active enzyme was eluted with 0.1 M citrate, pH 2.2, containing 0.15 M NaCl. Fractions were collected into tubes containing 2.5 M Tris, pH 10.5, so that exposure to acid conditions was minimized. Approximately 25% of the enzyme activity applied to the column was recovered. The specific activity of the purified enzyme was generally in the range of 40 to 60 μmoles/min/mg, although a specific activity of 185 μmoles/min/mg was achieved in one experiment. Affinity-purified enzyme ran as a single band with an apparent molecular weight of 64,000 daltons when subjected to SDS-polyacrylamide gel electrophoresis. The enzyme was stable for months when stored at -50°C in 50% glycerol. The procedure described above enables rapid, large-scale purification of the enzyme; for example, 2000 g of optic lobes can be processed in two weeks to yield 1.2 mg of enzyme. Supported by NIH grant AM32978 to MLE and funds provided by the Research Committee of the University of Wisconsin Graduate School to JLD.

112.6 ACETYLCHOLINESTERASE-CONTAINING NEURONS IN PRIMARY CULTURES OF DISSOCIATED RAT CEREBRAL CORTEX. W.E. Thomas. Dept. of Physiol., Meharry Medical College, Nashville, TN 37208.

The high affinity uptake of choline and subsequent synthesis of acetylcholine (ACh) by primary cultures of cerebral cortical cells has recently been reported (Thomas, W.E., Brain Res., 332:79, 1985). This ACh synthesis was shown to be attributable to the enzyme choline acetyltransferase and supported the existence of cholinergic cells in the cortical cultures. In initial efforts to establish the identity of these cholinergic cells, histochemical staining for cholinesterase enzyme has been employed. Cholinesterase reaction product was observed in the somata and processes of morphologically identified neurons. This neuronal staining was substantially greater than the background staining. While there was some variability in the intensity of neuronal staining, through optimization of the staining reaction time a clearer distinction between stained and unstained neurons was achieved. This allowed the quantitation of stained neurons by direct counting.

To investigate the nature of the activity producing the reaction product, various pharmacological compounds were included in the staining solution. The general cholinesterase inhibitor neostigmine abolished all of the neuronal staining. The pseudocholinesterase inhibitors ethopropazine and tetraisopropylpyrophosphoramide were without effect on the mean number of stained neurons per culture. The acetylcholinesterase (AChE) inhibitor 1,5-bis-[4-allyldimethyl-ammoniumphenyl]pentan-3-One dibromide always reduced the mean number of stained neurons to zero. On the basis of these pharmacological properties, all or most of the neuronal staining was attributable to the enzyme AChE. The mean number of AChE-stained neurons per culture ranged from 113 ± 23 to 555 ± 102 in cultures from twelve different platings. For six of these platings the percentage of AChE-containing neurons was determined after counting the total number of neurons in cresyl violet-stained sister cultures. The mean percentage of AChE-containing neurons in the cortical cultures was 2.17. Finally, the morphology of the stained neurons was evaluated. Entire cultures were scanned and all neurons stained enough to reveal their detailed morphology were classified as pyramidal, stellate, bipolar or bitufted. The accumulative results for the morphological distribution of stained neurons in 34 different cultures (a total of 2,708 cells) was as follows:10.3% pyramidal, 11.1% stellate, 66.9% bipolar and 11.7% bitufted.

These findings provide further support for the presence of cholinergic cells in the cortical cultures and suggest that the upper limit for such neurons is approximately 2.0%. Stained neurons of all morphologies were observed; however, the majority of the AChE-containing neurons exhibited bipolar morphology.

This work was supported by MBRS Grant No. RR-08037 and NSF Grant No. R11-8313599.

112.7 CHOLINE ACETYLTRANSFERASE NEURONS AND THEIR ACETYLCHOLINESTERASE STAINING PATTERNS. E. Gould*, N. J. Woolf, and L. L. Butcher, (SPON: D. W. Zaidel). Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Using a sequential staining protocol (Eckenstein & Sofroniew, J. Neurosci., 3:2286, 1983; Woolf et al., Neurosci. Lett., 40:93, 1983), individual neurons demonstrating choline acetyltransferase (ChAT)-immunofluorescence were further evaluated for acetylcholinesterase (AChE) staining intensity after subsequent counterstaining for the cholinergic degradative enzyme. AChE staining patterns of ChAT-positive neurons were rated as intense, moderate, or light.

Intrinsically organized cholinergic cells found in the caudate-putamen complex, nucleus accumbens, and olfactory tubercle all stained heavily for AChE. 91-96% of cholinergic neurons in these regions stained intensely, and the remainder stained moderately for AChE. No intensely stained AChE cells were found in these regions that were not immunoreactive for ChAT.

In basal forebrain regions slightly lower percentages of ChAT cells stained intensely for AChE. The percentages of ChAT-positive cells that stained intensely for AChE are as follows: 80% in the medial septal nucleus, 80% in the lateral preoptic area, 86% in the diagonal band, 85% in the nucleus basalis, and 93% in the substantia innominata. A few ChAT cells in the basal forebrain were found that stained only lightly for AChE, but these were never more than 2-6% of all ChAT neurons. The remaining ChAT cells of the basal forebrain stained moderately for AChE.

In the various cranial nerve nuclei the majority of the ChAT cells stained intensely for AChE, and the rest stained moderately. 73% of the ChAT cells found in the nucleus of cranial nerve X stained intensely for AChE, which was the lowest percentage found for any cranial nerve nucleus.

The AChE staining patterns of ChAT-containing somata in the pontine tegmentum differed depending on where the cholinergic neurons were found. ChAT cells associated with the pedunculopontine tegmental nucleus were similar to cholinergic basal forebrain cells in that 88% were intensely stained for AChE and the remainder were moderately stained. Unlike cholinergic cells in preceding brain regions however, ChAT cells found in the dorsolateral tegmental nucleus were markedly different in that only 12% stained intensely for AChE, whereas 57% stained moderately, and 30% stained lightly for AChE. This suggests that in certain areas of the brain ChAT cells do not necessarily contain high levels of AChE.

[Support: NS 10928 to L.L.B.]

112.8 ACETYLCHOLINESTERASE DISTRIBUTION AND ANTICHOLINESTERASE-AGENT BINDING IN THE BRAIN OF GUINEA PIG. R.R. Pindzola, Karen R. Olson* and Robert E. Foster. Neurotoxicology Branch, US Army Med Rsch Inst of Cml Def, Aberdeen Proving Ground, MD 21010-5425.

The distribution of acetylcholinesterase (AChE) in the guinea pig brain was assayed biochemically and histochemically. For the biochemical assays, whole brains were dissected (over ice) into ten areas and subsequently kept frozen at -80°C until homogenized. The fresh brain homogenates were then assayed for AChE activity, utilizing the radiometric method of Siakotos et al. (1969). AChE activities (measured at 20°C, pH 7.2) ranged from the high of 159.1±16.1 nmoles ^{14}C-acetylcholine hydrolyzed/min/mg wet weight in tissue from striatum to the low of 2.0±0.2 nmoles ^{14}C-acetylcholine hydrolyzed/min/mg wet weight in cerebral cortex. The ten brain areas listed in descending order of measured AChE activities are striatum, cervical spinal cord, cerebellar neocortex, cerebellar vermis, medulla, midbrain, olfactory bulbs, diencephalon, hippocampus, and cerebral cortex. As expected, a modified Koelle histochemical stain for AChE in brain slices revealed staining density distributions much more complicated than the biochemical assays of acetylcholine hydrolysis could reveal. In a parallel study, the binding of radiolabelled anticholinesterase-agent, ^{3}H-pinacolyl methylfluorophosphonate (^{3}H-soman), to individual 20 micrometer slices of fresh frozen brain was determined via section autoradiography and biochemical assay. The biochemical assay consisted of scintillation counting of radioactivity in tissue sections which were first incubated in ^{3}H-soman alone or soman followed by ^{3}H-soman, dehydrated, defatted, dryed and then scraped into vials. Tritiated-soman binding to parasagittal 20 micrometer slices from fresh frozen brains revealed an average of 45 femtomoles of covalently bound soman per tissue slice (or, 1 DPM/ug tissue using 1.25Ci/mmole ^{3}H-soman; ave. weight/slice = 130 ug). This amount of bound soman is approximately 2 times greater than the amount of binding sites attributable to AChE. Thus, approximately 50% of covalently bound soman in brain tissue slices is distributed to "non-specific" sites. The calculated 1:1 ratio of soman binding to AChE sites versus non-specific binding sites in brain slices (in vitro) is supported by the results of experiments where, prior to ^{3}H-soman incubation, the non-specific binding sites in the tissue sections were blocked by pretreatment with CBDP (cresylbenzodioxaphosphorin oxide). Finally, from the autoradiographic analysis of ^{3}H-soman binding, an areal distribution of bound ^{3}H-soman was revealed that corresponded, in part, to the density distribution of AChE (determined by the Koelle stain).

112.9 VARIABILITY OF CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN NEOCORTEX OF SQUIRREL MONKEYS. L. C. Walker, K. R. Brizzeet, M. B. Kaack*† and D. L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; †Delta Regional Primate Research Center of the Tulane University Medical Center, Covington, LA 70433.

In primate neocortex, the majority of the activity of choline acetyltransferase (ChAT), the enzyme which synthesizes acetylcholine, resides in axons and terminals emanating from neurons in the basal forebrain cholinergic system. On the other hand, the hydrolytic enzyme acetylcholinesterase (AChE) is associated with both cholinergic and noncholinergic elements. It is important to specify the levels of these enzymes in various neocortical regions of primates for the following reasons: to estimate the degree of innervation of heterogeneous cortical areas by neurons of the basal forebrain cholinergic system and to relate ChAT levels to AChE content in those areas; to determine the normal variability of the two enzymes among cortical regions; and to establish the interindividual variation in overall levels of ChAT and AChE in neocortex of a New World primate. To determine interindividual and interareal patterns of variation, we analyzed the activities of ChAT and AChE in five neocortical regions of eight 30-day old squirrel monkeys (Saimiri sciureus). Fresh tissue blocks were removed from right motor, somatosensory, auditory, visual, and prefrontal association cortices, and ChAT and AChE were measured in homogenized samples (Kaack, B., Walker, L., Brizzee, K. R. and Walker, M., J. Neurochem., 34:1772-1775, 1980). Results show that ChAT levels vary significantly across cortical areas ($p < 0.01$, ANOVA), the activity being highest in motor and auditory cortices, lowest in visual cortex, and intermediate in somatosensory and frontal cortices. This pattern closely parallels that in rhesus monkeys (Lehmann J., Struble, R. G., Antuono, P. G., Coyle, J. T., Cork, L. C. and Price, D. L., Brain Res., 322:361-364, 1984). Whereas AChE levels also differ significantly across the five cortical regions and correlate significantly with ChAT levels, the ratio of AChE to ChAT was generally lowest in motor cortex and highest in frontal association cortex. Differences among individuals in overall ChAT levels and AChE levels were not significant, although some variation in individual levels and patterns of enzyme activity was apparent. Thus, cholinergic innervation of the neocortex of Saimiri is not uniform, and AChE activity is a significant, but somewhat variable, correlate of cortical cholinergic innervation.

112.10 BRAIN ACETYLCHOLINE - A VIEW FROM THE CEREBROSPINAL FLUID. R. Becker, E. Giacobini, R. Elble, T. Mattio*, M. McIlhany* and G. Scarsella* (SPON: J. Couch). Depts. Pharmacology, Psychiatry, Medicine and Surgery, Southern Illinois University School of Medicine, Springfield, IL 62708 USA and Dept. Cell Biology, University of Rome, Rome, ITALY

Our understanding of the origin of the four major cholinergic components in human CSF has become increasingly more important in connection with early diagnosis and treatment of Alzheimer's disease (AD). Our studies of the molecular forms of acetylcholinesterase (AChE) in CSF, plasma and brain of the dog, as well as in CSF of normal controls and AD patients have shown that 1) most of the cholinesterase (ChE) activity present in CSF is AChE (more than 85%); 2) AChE in CSF originates primarily from brain tissue as a result of secretory process; and 3) the major form found in human CSF is the 10S molecular form corresponding to the globular tetramer G_4. The origin of choline acetyltransferase (ChAc) activity in CSF is probably neuronal while acetylcholine (ACh) might be, in part, the product of local (CSF) synthesis. The origin of choline (Ch) is multiple and only a small percent might reflect neuronal ACh synthesis. In order to correlate variations in levels and activity of the four main cholinergic components in CSF, brain and plasma, we have compared the effect of two routes (intravenous and intracerebral ventricular) of administration of physostigmine (Phy) in beagle dogs. The dosage has varied between 20 and 1,000 ug. Implanted reservoirs communicating with the respective lateral ventricle through a silastic catheter were used. Significant (50-90%) and persisting (3-9 hrs) inhibition in CSF and brain AChE activity as well as an increase in ACh levels were seen only with i.c.v. administration. At comparable dosages, intravenous administration produced only small and short-lasting effects. Therefore, intracerebral administration of Phy appears to be the route of choice if maximal central AChE inhibition and ACh levels increases are the goal of the treatment. Three of the cholinergic CSF components showed a decrease in our group of AD patients. In addition, changes seen in AChE, and ChAc activities and in Ch levels at 3-6 month intervals in the same patient seem to be indicative of the progression of the disease. In conclusion, our studies, both in dogs and humans, support the concept that ACh metabolism in brain is reflected by variations of cholinergic parameters in CSF. The diagnostic value of such variations in pathological conditions such as AD is under scrutiny. (Supported in part by funds for a pilot project from S.I.U. School of Medicine; E.F. Pearson Foundation and S.I.U. Alzheimer Research Fund.)

112.11 KITTY-ChAT: IMMUNOHISTOCHEMISTRY OF CHOLINE ACETYLTRANSFERASE IN THE FELINE BRAIN. S.R. Vincent and P.B. Reiner. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

The distribution of choline acetyltransferase (ChAT) in the feline brain was examined using monoclonal antibodies (Eckenstein, F. & Thoenen, H., EMBO J. 1:363, 1982). The well characterized cholinergic systems previously described in various species were readily detected. These include the ventral horn motoneurons and the intermediolateral preganglionic sympathetic neurons of the spinal cord, the motor nuclei of cranial nerves III, IV, V, VI, VII, XI and XII, and the preganglionic parasympathetic nuclei of nerves III, VII, IX and X.

The distributions of the large striatal neurons, the magnocellular cells of the rostral column in the basal forebrain, and the caudal column in the central tegmental field and the laterodorsal tegmental nucleus were examined in detail. Furthermore, the topographic relationship of the caudal cholinergic column to the catecholamine cells in the midbrain and pons was determined by comparing the localization of ChAT-positive neurons with that of tyrosine hydroxylase and dopamine-β-hydroxylase immunoreactive cells.

More controversial cholinergic cell groups were also detected. In the basal forebrain, smaller cells were intermingled with the magnocellular neurons, often capping the islands of Calleja. Positive neurons were also found in the central nucleus of the amygdala and intercalated within adjacent fiber tracts. The ventral medial habenula contained a dense cluster of ChAT-positive neurons, and a few cells were present in the lateral habenula. Many weakly stained cells were scattered throughout the lateral hypothalamus, some extending medially to the paraventricular and arcuate nuclei. Small cells were found in the superficial layers of the superior colliculus which also contained a well organized terminal field. Virtually all neurons in the parabigeminal nucleus showed moderate ChAT immunoreactivity, and cells showing similar staining were seen in the parabrachial nuclei, which were clearly separate from the more intensely staining cells of the caudal column. In the medulla a cluster of cells was noted in the prepositus hypoglossal nucleus, and small intensely stained cells were found just ventro-lateral to the abducens nucleus along the 7th nerve and in the periolivary portion of the superior olivary complex, while scattered larger cells were present in the gigantocellular tegmental field. Neurons were also observed in laminae III-V of the dorsal horn of the spinal cord and around the central canal. These results suggest a more widespread central cholinergic system than has been previously indicated.

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112.12 IS THE VERATRIDINE INDUCED HYDROLYSIS OF CYTOPLASMIC ACETYLCHOLINE IN CENTRAL CHOLINERGIC NERVE ENDINGS MEDIATED BY THE BACKWARD REACTION OF CHOLINE-O-ACETYLTRANSFERASE EC 2.3.1.6? P.T. Carroll, M. Badamchian and P.J. Craig Dept. Pharmacol. Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

The depolarizing agent veratridine not only stimulates the release of acetylcholine (ACh) from a vesicular fraction (P_3) by a Ca^{2+} dependent process but also causes the breakdown of cytoplasmic (S_3) ACh (Carroll & Benishin, 1984; Carroll, 1984). The purpose of the present study was to trace the fate of the choline derived from hydrolyzed S_3 ACh; also to determine whether this hydrolysis might be caused by the soluble, cytoplasmic fraction of ChAT running backwards. Rat hippocampal minces were loaded with (N methyl [^{3}H]ACh), post-incubated with veratridine in the absence or presence of Ca^{2+}, and the effect of veratridine on the subcellular levels and release of both [^{3}H]ACh and [^{3}H]choline determined. In some experiments the poorly penetrating acetylcholinesterase (AChE) inhibitor echothiophate was used to prevent the extraterminal breakdown of [^{3}H]ACh. The results indicated that veratridine stimulated the release of [^{3}H]ACh from the P_3 fraction of the minces by a Ca^{2+} dependent process and simultaneously caused the breakdown of the [^{3}H]ACh in the S_3 fraction. Some of the [^{3}H]choline derived from this hydrolyzed [^{3}H]ACh was transferred to the P_3 fraction where it was acetylated and released as [^{3}H]ACh by a Ca^{2+} dependent process. Some of the [^{3}H] choline was released from the S_3 fraction independently of Ca^{2+}. When the "penetrating" AChE inhibitor paraoxon was substituted for echothiophate during the loading and release phases, veratridine no longer stimulated the Ca^{2+} dependent release of [^{3}H]ACh or the Ca^{2+} independent release of [^{3}H]choline. Rather, it then stimulated only the Ca^{2+} independent release of [^{3}H]ACh and this occurred from the S_3 fraction. Paraoxon was also found to inhibit the backward reaction of soluble ChAT in a dose dependent fashion. These results indicate that veratridine causes the breakdown of S_3 ACh and that at least a portion of the choline derived from this hydrolyzed ACh is used at vesicular site of synthesis to form releasable ACh. They also suggest that the veratridine induced hydrolysis of S_3 ACh may be mediated by either a soluble fraction of S_3 ChAT running backwards or by an intraterminal form of AChE. (Supported in part by NINCDS grant NS 2121289-01 to P.T.C. and a Tarbox Postdoctoral Fellowship to M.B.)

112.13 CHOLINERGIC STIMULATION RELEASES STRIATAL ASCORBATE AND DOPAMINE IN THE CONSCIOUS RAT. James A. Clemens and Lee A. Phebus*, The Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

Several studies have demonstrated that cholinergic agents can stimulate the release of dopamine from neostriatal tissue in vitro. Cholinergic neuronal dendrites in the neostriatum form synaptic connections with dopaminergic axons and are believed to be involved in the modulation of dopamine release. The effects of the cholinergic agonist, pilocarpine, and the acetylcholine esterase inhibitor, physostigmine, on dopamine and ascorbate release in conscious animals were investigated using in vivo electrochemistry.

Experiments were conducted in awake, freely-moving rats that were previously implanted unilaterally in the neostriatum and nucleus accumbens with graphite paste-tipped, electrochemical working electrodes. Reference (Ag/AgCl) and auxiliary electrodes were implanted in the contralateral neocortex. On the day of an experiment, semi-differentiated linear sweep voltammograms were recorded at half-hour intervals until a stable baseline was reached. A scan rate of 5 mV/sec was used. Drugs or saline were then injected i.p. and changes from this baseline observed. When this electrochemical measurement system is utilized, the resulting voltammogram consists of three distinct peaks. The first peak is mainly ascorbic acid (AA), the second contains uric acid and 5-hydroxyindoles, and the third peak is homovanillic acid (HVA).

Administration of 10.0 mg/kg of pilocarpine nitrate produced a significant elevation in both ascorbate and HVA voltammetric peaks in the striatum that lasted several hours. A significant elevation in HVA but little effect on AA was observed in the nucleus accumbens. Similar changes in AA and HVA were observed after physostigmine (0.5 and 1.0 mg/kg) treatment. Although dopamine was not directly measured, the rise of its principle metabolite, HVA, indicates that dopamine release must have occurred. The rise in AA in the neostriatum may have been related to dopamine release because dopamine agonists release AA in the neostriatum. The results of this study are consistent with the hypothesis that acetylcholine can induce the release of dopamine in the neostriatum and also provide evidence that cholinergic stimulation releases dopamine in the nucleus accumbens.

112.14 DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE (ChAT) IN THE INTERPEDUNCULAR NUCLEUS (IPN). T.C. Eckenrode*, W. Battisti, and M. Murray (SPON: L. Greenberg). Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The IPN is a complex nucleus comprised of several cyto-architectonically distinct subnuclei. Substance P (SP), norepinephrine (NE), and serotonin (5-HT) have different patterns of localization to specific subnuclei. The origins of these neurotransmitters have been identified, and the effects of lesions on their distribution among the subnuclei have been determined. Acetylcholine (ACh) and ChAT levels in the IPN are among the highest in the brain. Most of the ACh is thought to be conveyed to the IPN via the fasciculi retroflexi (FR). We have used an antibody to ChAT, kindly obtained from Bruce Wainer, to identify its subnuclear distribution and to examine the effects of FR lesions on its localization.

We examined the localization of ChAT in adult rats using the unlabelled antibody (PAP) method of Sternberger. In intact animals, staining is present in the FR. Within the IPN, moderately dense ChAT staining is seen in the rostral and central subnuclei. The staining is distributed in mediolaterally oriented parallel bands that occupy most of these two subnuclei. This pattern resembles the pattern of axons revealed with a Bodian stain. The intermediate subnuclei, which demonstrate no SP staining, contain dense, coarse ChAT staining. The dorsal subnucleus contains sparse ChAT immunoreactivity. The lateral subnuclei, which contain exceptionally dense SP staining, are virtually devoid of ChAT staining.

Ten days after bilateral FR lesions the FR still show ChAT staining, however it has lost its fascicular appearance. Within the IPN, the ChAT in the central, rostral, and intermediate subnuclei decreases markedly, indicating that this staining is associated with FR axons. Staining in the dorsal subnucleus is less depleted, indicating that there may be an additional source of ACh to this subnucleus. Unilateral lesions produce a symmetrical pattern of staining which suggests that the ACh projection to the IPN, from the FR is bilateral and overlapping.

The pattern of staining thus differs from the pattern of SP staining and 5-HT staining but is similar to the localization of NE. The axons in the FR which contain SP and those which contain ACh therefore diverge in the IPN. The pattern of overlapping projections suggests an interaction between the ACh and NE systems in the IPN.

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112.15 MODULATION OF HIPPOCAMPAL CHOLINE ACETYLTRANSFERASE BY PEPTIDES: POSSIBLE INVOLVEMENT OF ADENOSINE-3',5'-MONOPHOSPHATE. P.A. Lapchak* and B. Collier. Dept. of Pharmacol. & Ther., McGill University, McIntyre Medical Bldg., Montreal, Quebec, Canada, H3G 1Y6.

Choline acetyltransferase (ChAT) and numerous peptides have been shown to coexist in various regions of the rat CNS, including the cerebral cortex and hippocampus. Vasoactive intestinal polypeptide (VIP) has been reported to activate ChAT; in addition, VIP has also been shown to enhance adenylate cyclase activity in hippocampal homogenates. The present work was undertaken to study the effects of various peptides on ChAT activity, and to test whether these effects may be mediated by adenylate cyclase.

First, we studied the possible modulation of ChAT in hippocampal homogenates by the following related peptides: peptide histidine isoleucine (PHI), VIP, secretin and glucagon. Both PHI and VIP rapidly activated ChAT, with a maximal effect occurring at 10 minutes of preincubation. This effect was dose-dependent, with a maximum activation of 18.0 ± 2% (n = 4) and 14.0 ± 1.5% (n = 6) for PHI (10^{-6}M) and VIP (10^{-7}M), respectively. Secretin and glucagon were ineffective over the concentration range tested (10^{-8} to 10^{-5}M).

Second, adenosine-3',5'-monophosphate (cAMP) was shown to activate ChAT over a similar time course as did PHI and VIP. At 10^{-8}M, cAMP enhanced ChAT activity by 25-38% (n = 3). In addition, a less polar derivative of cAMP, dibutyryl cAMP, also enhanced ChAT activity at a concentration of 10^{-8}M (12-17%, n = 6) and 10^{-6}M (32-44%, n = 4). The increase in ChAT activity corresponded to a decrease in the K_M for choline (50-63%), with no change in the apparent V_{max}.

In conclusion, the results suggest the possibility that peptides like VIP or PHI might have a modulatory role in cholinergic transmission as the result of activating ChAT and that this activation might occur by an adenylate cyclase/cAMP dependent mechanism. (Supported by MRC, Canada and FCAC, Quebec.)

112.16 ^{3}H-ACETYLCHOLINE SECRETION IN GUINEA-PIG ILEUM MYENTERIC PLEXUS IS EQUALLY ENHANCED BY FORSKOLIN, 8-BROMO ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE OR 3-ISOBUTYL-1-METHYLXANTHINE. P. Alberts and V. Ögren*. Division of Experimental Medicine, National Defense Research Institute, S-901 82 Umeå, Sweden.

The possible involvement of cyclic nucleotides in receptor-mediated control of ^{3}H-acetylcholine (^{3}H-ACh) secretion was studied. The guinea-pig ileum longitudinal muscle preparation was preincubated with ^{3}H-choline and subsequently mounted in an organ bath and superfused with Tyrode's solution containing eserine and hemicholinium-3. The preparation was stimulated with trains of 150 monophasic shocks (25 V/cm) at 0.5 Hz (0.5 ms). Two control stimulations preceeded two stimulations made in the presence of drug. The effect of drugs on the secretion is expressed as a quotient of the secretory response obtained in the presence and absence of drug. After incubation with choline kinase the ^{3}H-ACh collected in the superfusate was separated from ^{3}H-choline in tetraphenylboron-containing 3-heptanone.

The potent adenylate cyclase activator forskolin enhanced the ^{3}H-ACh secretion in a concentration-dependent manner. From the linear regression analysis of the double-reciprocal plot of the data (10^{-6} - 10^{-4} M, n=8) the 'maximal enhancement' at 'infinitely' high concentration of forskolin was calculated to be 205 ± 11% of control. The concentration yielding half-maximal enhancement (EC_{50}) was (2.3 ± 0.4) x 10^{-6} M. The secretion of ^{3}H-ACh was enhanced by 8-bromo adenosine 3',5'-cyclic monophosphate (8-Br cyclic AMP), also in a concentration-dependent manner. The effects of 8-Br cyclic AMP in the range 0.25 - 2 mM (n=9) yielded a 'maximal enhancement' of 270 ± 45% of control and an EC_{50} value of 1.3 ± 0.43 mM. Addition of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (1 - 5 mM, n=9) enhanced the secretion of ^{3}H-ACh yielding a 'maximal enhancement' of 239 ± 27% of control. The EC_{50} value was 2.6 ± 0.7 mM. Simultaneous addition of forskolin (2.5×10^{-5} M) and IBMX lowered the EC_{50} value for IBMX to 0.45 ± 0.24 mM (n=5) without changing the 'maximal enhancement' for IBMX (248 ± 30% of control).

Since the 'maximal enhancement' values for forskolin and IBMX, and for IBMX in the presence of forskolin, are similar to that for the 'exogenous' cyclic AMP compound used, the results suggest that forskolin and IBMX accomplish their effects on ^{3}H-ACh secretion by elevating 'endogenous' neuronal cyclic AMP levels.

The results suggest that regulation of intraneuronal cyclic AMP levels is a possible mechanism for receptor-mediated control of ^{3}H-ACh secretion in the cholinergic terminals of guinea-pig ileum myenteric plexus.

112.17 ENZYMATIC SYNTHESIS OF A FALSE CHOLINERGIC NEUROTRANSMITTER: DIETHYLAMINO ETHYLACETATE AS A SPECIFIC MUSCARINIC AGONIST ANALOG OF ACETYLCHOLINE. Peter K. Chiang, George A. Miura, Richard K. Gordon, Michelle M. Richard, Felipe N. Padilla, and B. P. Doctor. Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Upon incubation with bovine brain choline acetyltransferase (acetyl-CoA: choline O-acetyltransferase; EC 2.3.1.6), diethylamino ethanol and acetyl-CoA, an acetylcholine analog, diethylamino ethylacetate was formed. The new product co-chromatographed with authentic diethylamino ethylacetate on silica thin layer plates, and its formation was proportional to the duration of incubation and protein concentrations. When tested on guinea pig ileum, diethylamino ethylacetate was found to be an agonist, albeit weak, with an ED_{50} of 1.3×10^{-4} M, compared to an ED_{50} of about 2.0×10^{-7} M for acetylcholine. The contraction of guinea pig ileum induced by diethylamino ethylacetate could be blocked by atropine. Moreover, diethylamino ethylacetate induced a secretion of α-amylase from isolated pancreatic acini cells; this effect was blocked by atropine. Diethylamino ethylacetate could also block the binding of radioactive quinuclidinyl benzilate to the muscarinic receptors of N4TG1 neuroblastoma cells, with an I_{50} of 0.5 mM. It was very interesting that diethylamino ethylacetate has neither agonist nor antagonist activity on the isolated frog rectus abdominis muscle, and is hence not a nicotinic cholinergic neurotransmitter. Judging from our data, diethylamino ethylacetate can be a false cholinergic neurotransmitter generated in vivo when drugs such as aprophen or procainamide are administered to animals or patients, since either of these drugs can undergo enzymatic hydrolysis to generate diethylamino ethanol as one of the products.

BRAIN METABOLISM I

113.1 POSSIBLE MECHANISMS OF POST-HYPOXIC HYPEREXCITABILITY IN HIPPOCAMPAL SLICES. S.J. Schiff and M. Balestrino*. Division of Neurosurgery and Department of Physiology, Duke University Medical Center, Durham, NC 27710.

In rat hippocampal slices exposed to 30 min moderate hypoxia at 29°C, irreversible increases in CA1 population spike amplitude are observed during reoxygenation, often accompanied by the appearance of a second population spike (Schiff, S.J. and Somjen, G.G., Brain Res., in press). This study examined the changes in neuronal excitability and recurrent inhibition that accompany these phenomena.

In response to orthodromic Schaffer collateral stimulation, presynaptic volley, focal-EPSP (fEPSP), and population spike were recorded in stratum radiatum and stratum pyramidale of CA1. In response to alveus stimulation, antidromic population spikes were recorded in CA1. Recurrent inhibition was evaluated using paired antidromic and orthodromic pulses. Only those slices demonstrating intact recurrent inhibition were studied. In hypoxia experiments, data were collected prior to the induction of 30 min hypoxia and after 45 min reoxygenation; in control experiments, data were collected over the same time span without hypoxia.

Recurrent inhibition was lost in most slices following hypoxia. This loss correlated well with the appearance of a second population spike, but correlated poorly with the increase in first population spike amplitude; indeed, in some slices an increase in first population spike amplitude was observed despite the preservation of recurrent inhibition. The population spike evoked by a given amplitude of presynaptic volley was increased after hypoxia. The fEPSP evoked by a given presynaptic volley was not changed by hypoxia, while the population spike evoked by a given fEPSP was increased. The amplitudes of antidromic population spikes were not changed after hypoxia.

These data suggest that changes in the excitability of postsynaptic neurons, rather than changes in presynaptic fibers or transmitter release, are responsible for the increased first population spike amplitude. Although the loss of recurrent inhibition may be the cause of the appearance of a second spike, recurrent inhibition loss appears to occur independently of the increase in first population spike. A decrease in action potential threshold, perhaps due to slight pyramidal cell depolarization, may be important in increasing the first population spike; in addition, enhanced conduction in the postsynaptic dendritic tree, or reduced feed-forward inhibition, might also conceivably play a role in this phenomenon.

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113.2 CHLORPROMAZINE IMPROVES RESISTANCE OF HIPPOCAMPAL SLICES TO HYPOXIC DAMAGE. G.G. Somjen and M. Balestrino*. Department of Physiology, Duke University Medical Center, Durham, NC 27710.

Chlorpromazine (CPZ) has been shown to have a protective effect against hypoxic damage in the liver and in the heart (Am. J. Pathol.,88:539-553,1977; J. Mol. Cell. Cardiol.,15:603-620, 1983; J. Mol. Cell. Cardiol.,15:621-628, 1983). We have investigated its possible usefulness in brain tissue hypoxia. Rat hippocampal slices 400μ thick were incubated in an interface chamber at 35°C. The perfusion fluid was bubbled with 95% O_2, 5% CO_2 and the same mixture was used for the gas phase above the slices. CPZ was administered i.p. (30 mg/Kg) to rats 30 minutes prior to sacrifice and added (7 μM/l or 70 μM/l) to the perfusion bath. Control slices, obtained from age-matched rats having received a comparable amount of i.p. saline solution 30 minutes prior to sacrifice, were incubated in CPZ-free medium. Extracellular potentials were evoked in stratum radiatum and stratum pyramidale of the CA1 region by stimulation of the Schaffer collaterals. DC sustained potentials in stratum pyramidale were also recorded. Two hours were allowed for recovery after dissection, following which hypoxia was induced by switching the gas phase to 95% N_2, 5% CO_2. After 9 minutes of hypoxia, reoxygenation was started. In both treated and untreated slices, evoked responses failed 1-3 minutes after induction of hypoxia. Shortly thereafter, a slow, slight negative shift in sustained potential was evident, and eventually an abrupt, spreading depression-like fall in this potential occurred. In CPZ-treated slices, the latter tended to occur later than in control ones (controls: 237 ± 51 seconds after induction of hypoxia; CPZ 7 μM/l: 311 ± 75 seconds; CPZ 70 μM/l: 442 ± 138 seconds; 1-way ANOVA: $p<.02$). Upon reoxygenation, no postsynaptic potential recovery was seen in control slices (two hours follow-up). In contrast, 50% of slices treated with 7 μM/l CPZ and 100% of slices treated with 70 μM/l CPZ showed full recovery of postsynaptic responses ($\chi^2 = 7.47$, df = 2, $p<.03$). Therefore, CPZ seems to counteract the hypoxia-induced SD-like response and to enhance the chances for functional recovery in this preparation.

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113.3 SELECTIVE NEURONAL VULNERABILITY TO HYPOXIA IN VITRO. P.G. Aitken and S.J. Schiff, Dept. of Physiology (PGA) and Division of Neurosurgery (SJS), Duke Univ. Med. Ctr., Durham, NC 27710.

Different regions of the brain are selectively vulnerable to ischemia. In the hippocampus, the CA1 region is particularly vulnerable while the fascia dentata (FD) is relatively resistant. It has not been possible to determine which aspect(s) of in situ ischemia (e.g., hypoglycemia, tissue acidosis, or hypoxia) is responsible for the differential vulnerability. Using the in vitro hippocampal slice, we have demonstrated that the CA1 region is more vulnerable to damage from a purely hypoxic insult than is FD.

Hippocampal slices 400μM thick were prepared with standard techniques and maintained at 35.5°C in an interface chamber, superfused at 2cc/min with oxygenated artificial cerebrospinal fluid. Except during hypoxic periods, the gas phase above the slices consisted of warmed, humidified 95% O_2 - 5% CO_2 flowing at 225cc/min. Hypoxia consisted of replacing the gas phase with 95% N_2 - 5% CO_2 flowing at the same rate. Extracellular field potentials were recorded in stratum pyramidale of CA1 (in response to Schaffer collateral stimulation) and in st. granulosum of FD (in response to perforant path stimulation). Ninety minutes after dissection, baseline recordings were taken and then the slices were exposed to a sequence of hypoxic periods of increasing duration: 2, then 3, then 4 minutes. Each hypoxic period was followed by a 15-20 minute recovery period of full oxygenation. Starting 10 minutes into each recovery period, recordings were taken. A region was considered responsive if any postsynaptic population spike or excitatory postsynaptic potential could be evoked, and unresponsive if neither could be evoked. If a region was unresponsive, stimulation intensity was increased and electrode positions changed to verify the lack of postsynaptic response. Following the last hypoxic period slices were oxygenated for at least 1 hour to verify the permanence of any loss of postsynaptic response.

In experiments on 14 slices, postsynaptic activity disappeared during all hypoxic periods in CA1 and FD. This loss was always temporary in FD and sometimes temporary in CA1; the loss was permanent in CA1 (within the time periods studied) after 2 minutes of hypoxia (1 slice), after 3 minutes (7 slices), or after 4 minutes (6 slices). CA1 presynaptic fiber volleys were always preserved.

These data demonstrate that the relative vulnerabilities of CA1 and FD to ischemia in situ can be replicated by a purely hypoxic insult in vitro. Since this preparation lacks a vascular system, the differential vulnerability must derive from factors intrinsic to the neural tissue. Other components of ischemia may, of course, play a role in situ. (Supported by the Research Foundation of the Amer. Ass'n. of Neurological Surgery, and by NIH grants NS 18670 and 17771.)

113.4 ELECTROPHYSIOLOGICAL CORRELATES OF ANOXIC DAMAGE TO HIPPOCAMPAL CA1 PYRAMIDAL CELLS: ROLE OF OXYGEN FREE RADICALS. J.J. Miller and P. Leung*, Department of Physiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Alterations in the electrophysiological properties of hippocampal CA1 pyramidal cells during and following anoxia were investigated using the in vitro slice preparation. During the initial phase of anoxia (95% N_2, 5% CO_2) the stratum radiatum evoked field potentials recorded in dendritic and somatic regions exhibited a slight increase in amplitude followed by multiple spike discharges and then complete blockade of synaptic transmission. Upon reperfusion with normal oxygen concentration (95% O_2, 5% CO_2) the extracellular EPSP response exhibited a relatively early recovery beginning 4-5 min post-anoxia and attained control levels usually by 7-8 min. Reappearance of the population spike was considerably delayed (8-9 min) and the magnitude of recovery was variable. In some cases only partial recovery (<50%) in the amplitude was observed by 45 min following the anoxic insult. In others there was a brief period of potentiation 8-18 min post-anoxia following which the response rapidly deteriorated such that the population spike could not be elicited using pre-anoxia stimulation intensities.

Intracellular records show a parallel sequence of events. The resting membrane potential remained relatively stable during the initial phase of anoxia followed by a rapid depolarization of approximately 40-60 mV. Full recovery was usually observed by 20 min post-anoxia. The synaptically evoked IPSP reappeared first following anoxia (9-10 min) while the late hyperpolarizing potential was slightly delayed. There was a striking increase in the threshold for both current and synaptically evoked spike generation. No long-term alterations were observed in either extra- or intra-cellular paired pulse inhibition.

In an attempt to reverse the anoxic damage, as reflected by the delayed onset and deterioration of somatic spike generation, slices were pre-treated with the free radical scavenger allopurinol (10 uM) for 10 min prior to the onset of anoxia. In almost all cases, both dendritic and somatic responses exhibited a rapid recovery to control levels and no evidence of prolonged anoxic damage was observed. On the basis of these data, it may be suggested that the long term decrease in somatic excitability induced by anoxia results from the production of oxygen free radicals and thus may ultimately be involved in the degeneration of neuronal elements.

113.5 MEDIATION OF HYPOXIC DAMAGE BY FREQUENCY OF SYNAPTIC TRANSMISSION IN MOUSE HIPPOCAMPAL SLICES. H.Itagaki and J.Parmentier. Anesthesia Res. Lab., Univ. of South Alabama, Mobile, AL 36688.

Recent studies using dispersed cultures of rat hippocampal neurons have shown that prevention of depolarization by exogenous glutamate, or the blockade of synaptic transmission by Mg++ can reduce the neuronal death caused by anoxic exposure (Rothman, J. Neuroscience 4(7):1884-1891). In order to study the degree to which synaptic activity in mature central neurons may protect or contribute to hypoxic damage, we have monitored the loss and recovery of synaptic transmission in slices of mouse hippocampus exposed to conditions of hypoxia and reflow. 400u thick slices of mouse hippocampus were allowed to stabilize for 60 minutes in an interface chamber under conditions of 95% oxygen, 5% CO_2. Extracellular recordings of transmission from stimulated Schaffer collaterals to CA1 pyramidal dendrites were used to monitor synaptic activity. EPSP amplitudes were monitored and periodic input-output curves were determined. Following 40 minutes of baseline recording the chamber gas supply was changed to a mixture of 95% N_2, 5% CO_2 for seven minutes and then returned to the original 95% O_2/5% CO_2 mixture for a recovery period of 40 minutes. During the course of these experiments, intervals of synaptic stimulation of 200 msec, 500 msec, 1 sec, 5 sec, 10 sec and 30 sec were examined. Frequency facilitation was noted in almost all cases over the 40 minutes of baseline recording, although slices stimulated at 200 and 500 msec intervals showed an initial decrease in their EPSP amplitudes prior to facilitation. While a high degree of variability was noted in EPSP amplitudes for both the hypoxic and recovery periods, our results show that most slices stimulated at 1 sec or longer intervals recovered from hypoxic exposure to their facilitated pre-hypoxic amplitude levels. However, the mean amplitudes of EPSPs in slices stimulated at 200 and 500 msec intervals did not recover to their initial, unfacilitated levels within the 40 minute observation period. A stimulus interval of 1 sec appeared to produce the most efficient recovery. These observations agree with the suggestion that some component of synaptic activity mediates the degree of hypoxic damage to central neurons, and that increased levels of this component or its characteristics result in enhancement of hypoxia damage. These results also provide support for using anticonvulsant or other drugs which depress neuronal firing frequency in the treatment of stroke or hypoxic brain injury. We are presently investigating the protective effects against hypoxia of various synaptic blockers in this system.

113.6 EFFECTS OF BLOCKING CALCIUM ENTRY WITH NIMODIPINE AND MAGNESIUM IN ANOXIC HIPPOCAMPAL BRAIN SLICES. I.S. Kass and J.E. Cottrell*. Department of Anesthesiology, SUNY/Downstate Medical Center, Brooklyn, New York 11203.

Increased cell calcium concentration has been implicated in producing irreversible neuronal damage. We have utilized the in vitro hippocampal slice model of anoxia (Kass + Lipton 1982, J. Physiol 332:459) to examine agents that block calcium entry for their ability to improve recovery from anoxic damage in vitro.

High Mg physiological buffer (10mM Mg) protected against anoxic damage; the tissue recovered to 76±5% of its preanoxic amplitude. This protected better than normal physiological buffer (0±0) but not as well as either Zero Ca - High Mg buffer or Cobalt buffer with which we have previously showed complete recovery.

Nimodipine is an organic Ca entry blocker that has been shown to improve neurological outcome following ischemia in vivo (Steen et al 1983 J. Cereb Blood Flow and Metab. 3:38). We tested its direct effect on neuronal recovery. Nimodipine (10-5M), added to the perfusate one hour before and continued until 10 minutes after the anoxic epoch did not improve recovery post anoxic (12±12). Thus Nimopidine does not protect neurons directly against anoxic damage.

Data from the studies of others indicate that nimodipine may not block Ca entry into neurons. Nimodipines inability to block Ca entry into neurons is supported by our studies. The size of the population spike is only slightly effected by Nimodipine before anoxia (90±6). If Ca channels in neurons, were blocked a greater reduction or a complete loss of the population spike after Nimodipines addition would occur. Such a loss was seen in Cobalt buffer, Zero Ca - High Mg buffer and High Mg buffer.

In order to determine whether blocking Ca entry protects by preventing the fall in ATP during anoxia we measured ATP levels in the dentate region. A 10% increase in ATP levels during anoxia was found in studies with 0 Ca - 10 mM Mg buffer when compared to studies with normal buffer. Other experiments indicate that this small increase in ATP can account for only a small part of the increased protection seen with 0 Ca-10mM Mg buffer. Studies with nimodipine indicate it also causes a 10% increase in ATP during anoxia, however additional studies are needed to account for shifts in control values with nimodipine.

Blocking Ca entry with High Mg, during anoxia protects against the irreversible anoxia damage. Nimodipine does not protect in vitro and probably does not block calcium entry into neurons.

113.7 GM_1 TREATMENT IN VIVO PROTECTS MEMBRANE ATPase ACTIVITIES AND PREVENTS MITOCHONDRIAL DAMAGE IN HIPPOCAMPAL SLICES. R. Bianchi*+, F. Milan*°, D. Janigro*°, D. Di Gregorio*° and A. Gorio*°. (SPON: L. Valzelli).
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Ganglioside incubation of hippocampal slices in vitro was recently reported to double the latency between the onset of hypoxia and dramatic cell depolarization simultaneously involving all the neurons (spreading depression). Similar protection against Na^+,K^+-ATPase inhibition was obtained in muscles incubated in the presence of ganglioside mixture ($5x10^{-8}$M) or dissected from mice injected with 10 mg/kg ganglioside mixture daily for three days) in K^+-free physiological solution for several hours or in hypoxic medium. These observations suggest a protective effects of gangliosides on membrane structures under stress. To check this hypothesis, we measured Na^+,K^+-ATPase specific activity, which can be considered a marker of membrane integrity, in hippocampal slices frozen immediatly after a preparation or after 35 minutes incubation. The loss of enzymatic activity after incubation was over 30% in control hippocampi, but lower than 10% in hippocampal slices from GM_1 pretreated rats (10 mg/kg i.p. daily, for three days). These biochemical data correlated with the ultrastructural evidence. The most dramatic difference between the two groups at 35 minutes was in mitochondrial structure. Untreated animals showed a high percentage of swollen mitochondrial profiles almost completely lacking cristae, while the remaining mitochondria (18%) had a normal configuration, were minute and had a dense matrix. In GM_1 treated animals the vast majority of mitochondria (85%) showed normal ultrastructure though minor modifications such as swelling were detectable in a small percentage (6%). These data suggest that maintenance of healthy hippocampal slices in vitro might be improved by pretreating the animals with GM_1.

113.8 SLICE CHAMBER FOR BIOCHEMISTRY AND MORPHOLOGY. F. Hospod*, P. Wu* and G. Newman (SPON: A. Sterman), Department of Neurology, SUNY, Stony Brook, New York 11794.

The in vitro slice chamber used for physiology is not well-suited to biochemistry or morphology because of its small volume, open atmosphere and difficulty with rapid, atraumatic slice removal. A slice chamber that overcomes these problems has been designed and tested. Up to 5 slices rest between 2 Nylon mesh screens stretched on nested glass rings separated by 500 μ Teflon spacers. The slices are immersed in 6 ml of buffer flowing at 2.2 ml/min and covered with 24 ml of 95% 02-5% C02 flowing at 50 ml/min. A drain permits rapid buffer changes for drug or substrate exposure and rinsing. The ring system is easily removed without disturbing the slices allowing rapid fixation or freezing followed by cryostat-sectioning or punch (modified Palkovitz technique).

Using this chamber we have established that rat suprachiasmatic nucleus in hypothalamic slices continues to take-up 14C-2-deoxyglucose consistent with the diurnal phase of the sacrificed animal even after 8 hours of incubation. (See Abstract by Newman et al.). Slices incubated with 35S-methionine (9.8μCi/ml) incorporate radioactivity linearly. After 4 hours, over 200,000 cpm are incorporated into proteins of an SCN punch sample, allowing resolution of over 80 proteins by SDS-PAGE.

Examination of incubated slices by light and electron microscopy, after immersion in Bouin's fixative or 4% paraformaldehyde-2.5% glutaraldehyde, reveal moderate disruption of neuronal perikarya at the slice surface but little disruption of the slice interior after 1 hour. After 8 hours, these changes increase considerably at the slice surface but much less so in the interior. In contrast to the perikaryal changes, SCN axon terminals of all types appear intact from surface to surface after either 1 hour or 8 hours of incubation. Similarly, the 14C-2-deoxyglucose experiments reveal no differences in uptake between the slice surface and interior. Thus, at least for adult rats, these in vitro studies suggest, but do not prove, that uptake of 2-deoxyglucose correlates with synapse metabolism rather than events in the perikarya.

In summary, an inexpensive and versatile chamber has been designed to optimize homogeneity of incubation conditions for biochemical and morphologic studies.

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113.9 COMPARISON OF IODOANTIPYRINE AND NEUTRAL RED AS INDICATORS OF RELATIVE PERFUSION IN BRAIN FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION. T.S. Whittingham, W.D. Lust, J.C. LaManna(#), G. Rodziewicz*, R.A. Ratcheson and C.A. VanDerVeer*. Div. of Neurosurgery and (#) Dept. of Neurology, Case Western Reserve Medical School, Cleveland, OH 44106.

The study of metabolic events during focal ischemia in the rat brain is being facilitated by the use of neutral red, a diffusible dye. The staining pattern following occlusion of the left middle cerebral artery generally shows three distinct types of neutral red labelling: 1) uniform red, 2) white (unstained) and 3) mottled or lightly stained tissue. ATP, PCr, glucose and lactate analyses from different portions of the neutral red staining pattern indicate that dye intensity can be used as a qualitative indicator of tissue perfusion. The possibility of using neutral red as a quantitative marker of cerebral perfusion is currently being investigated. Labelling patterns identical to that of neutral red are obtained in autoradiographs when [14-C]-iodoantipyrine is infused just prior to fixation by funnel freezing. The present technique involves injection of neutral red following MCA occlusion in rats and 30 min prior to in situ brain fixation for metabolite analysis. In other animals, the neutral red procedure is followed by infusion of radiolabelled iodoantipyrine for the 30 sec prior to decapitation and serial blood samples are obtained. The frozen brain is then sectioned in a cryostat and the sections are either mounted on glass slides for autoradiography or freeze dried for microdissection and metabolite analyses. The tissue block face is photographed and the following section is used for autoradiography. The photographs provide a record of neutral red staining, and the autoradiographs a record of iodoantipyrine labelling. Comparison of neutral red photographs and corresponding iodoantipyrine autoradiographs suggests that increased resolution can be obtained from neutral red dye intensity. A monochromatic densitometer can be used to quantitate both neutral red staining intensity throughout a given tissue photograph and iodoantipyrine distribution in the corresponding autoradiograph. The two results can then be compared to determine the validity of using neutral red as a quantitative marker of cerebral perfusion.

113.10 ROLE OF POLYPHOSPHOINOSITIDE IN FREE FATTY ACID PRODUCTION DURING BRAIN ISCHEMIA. M. Ikeda*, S. Yoshida*, R. Busto*, M. Santiso* and M.D. Ginsberg (SPON: P. Scheinberg). Cerebral Vascular Disease Research Center, Univ. Miami School of Medicine, Miami, FL 33101.

The possible contribution of polyphosphoinositide degradation to the release of free fatty acids (FFAs) in the brain during ischemia was evaluated in the rat. Ischemia was induced by decapitation. Major portions of triphosphoinositide (TPI) and diphosphoinositides (DPI) were extracted with chloroform/methanol (2:1) containing 0.25% HCl after bulk lipids including monophosphoinositide (MPI), phosphatidic acid (PA), diacylglycerol (DG) and a portion of TPI and DPI had been extracted with chloroform/methanol (1:1). The lipids were separated by one-dimensional TLC. The fatty acid composition of the isolated lipids was quantified by GLC following transmethylation with anhydrous HCl-methanol. FFAs were methylated by diazomethane.

The fatty acid composition and the contents of isolated lipid in control animals are shown in the Table.

	Fatty acid composition (mol%)					Content (μmol/g)
	C16:0	C18:0	C18:1	C20:4	C22:6	(mean ± SD, n=6)
TPI	10	47	2	41	0	0.41 ± 0.09
DPI	10	49	2	39	0	0.29 ± 0.01
MPI	10	50	2	37	0	1.40 ± 0.10
PA	22	28	41	7	1	0.86 ± 0.07
DG	37	20	27	13	1	0.035 ± 0.002
FFA	36	26	21	12	5	0.146 ± 0.024

Ischemia caused a rapid and time-dependent decrease of TPI and DPI and a concurrent increase of DG and FFAs. At 1 and 3 min of ischemia, the decrease of TPI and DPI corresponded in amount to the increase of DG and FFAs. TPI, DPI and DG reached their respective plateau levels at 10 min of ischemia: (% of control) TPI, 50%; DPI, 40%; DG, 500%. Thus there appeared to be two pools of polyphosphoinositide which have different metabolic rates, as suggested by several authors. However, FFAs continued to increase up to 30 min of ischemia. There was no change in MPI and PA. Among individual fatty acids in the FFA pool, increases of C18:0 and C20:4 were prominent at 1 and 3 min. Free C16:0, C18:1 and C22:6 increased slowly but linearly up to 30 min. DG containing C18:0 and C20:4 increased markedly during the first 3 min. These results indicate that at the onset of ischemia, hydrolysis of TPI and DPI by phosphodiesterase (phospholipase C) and subsequent deacylation of DG by DG lipase and/or monoacylglycerol lipase are primarily responsible for the production of FFAs. At later times during ischemia, deacylation of other lipids likely contributes to the continuous increase of FFAs as well. Supported by NIH grant NS 05820.

113.11 METABOLISM OF ARACHIDONOYL-DIGLYCERIDE IN RAT BRAIN. J.A. Kelleher* and G.Y. Sun. (SPON: W. G. Wood) Biochem. Dept. and Sinclair Comparative Med. Res. Farm, Univ. of Missouri, Columbia, MO 65203.

The receptor-mediated phosphodiesteric hydrolysis of poly-phosphoinositides (PI) has recently been recognized to be the key step for transduction of extracellular signals to intracellular events mediated through protein kinase C. According to this scheme, diacylglycerols (DG) generated from poly-PI breakdown would serve as a second messenger for modulating the protein kinase C activity. The DG of neuronal membranes is enriched in 18:0 and 20:4, reflecting a metabolic relationship with the poly-PI. The functional role of DG may be regulated by enzymes metabolizing the DG, namely, DG-kinase and DG-lipase. We have generated membrane-bound DG labeled at the arachidonoyl-group by two methods: (1) Activation of endogenous phospholipase C acting on [^{14}C]arachidonoyl-PI by incubation of brain membranes with deoxycholate and Ca^{2+}. The DG-enriched membranes were then washed and heat-treated before use. (2) Use of phospholipase C from B. cereus. This enzyme effectively converts the arachidonoyl-labeled PI and PC to DG, thus allowing one to examine endogenous DG-lipase and DG-kinase activity in the same membrane preparation. Using the heat-treated DG-enriched membrane as substrate, DG-lipase and DG-kinase activities were found in brain cytosol as well as in the membranes. DG-kinase activity was observed when the fresh DG-labeled microsomes or the heat-treated membranes with brain cytosol were incubated in the presence of Mg^{2+}, ATP and NaF. In general, DG-kinase more actively metabolized the DG than did the DG lipase. DG-lipase, which is more active in the cytosol, may be enhanced slightly by Ca^{2+}. Using the DG-labeled membranes generated from B. cereus, we clearly demonstrated that DG-kinase is activated by deoxycholate. This latter assay system is suitable for elucidation of membrane active agents which may be affecting the kinase and lipase activities in brain.

113.12 DETERMINATION OF INTRACELLULAR pH FROM FREEZE TRAPPED RAT BRAIN. J.C. LaManna, T.S. Whittingham, K.A. McCracken, and W.D. Lust. Dept. Neurology and Div. Neurosurgery, Case Western Reserve University Medical School, Cleveland, Ohio 44106.

Intracellular pH (pHi) is a critical factor in brain function in normal and pathological states. There is a need for reliable methods for measuring pHi that are quantitative and regional, do not require equilibrium conditions, and that also allow determination of other metabolic and functional variables such as high energy phosphates, substrate levels, and extracellular ion concentrations. We have shown previously that the pH indicator dye, neutral red, can be administered intravenously or intraperitoneally to rats (Anal Biochem, 142:117, 1984). This dye is taken up and concentrated in the brain parenchyma, acting as an in vivo brain pH indicator that can be quantitated by scanning reflectance spectrophotometry of the cortical surface. In order to study regional variations in pHi, we have developed a method whereby this information can be obtained from frozen in situ rat brains. Neutral red was administered intravenously to anesthetized rats 30 minutes prior to funnel freezing with liquid nitrogen. Frozen brains were removed and mounted in a microtome at -30°C. Color photographic slide transparencies were made of each block face at different levels through the brain as it was being coronally sectioned. The sections were later processed for microregional histochemistry of glucose, glycogen, lactate, ATP, PCr, ADP, and total adenylates. The color slides were analyzed for neutral red wavelength specific absorption by projection onto the entrance slit of a rapid-scanning spectrophotometer. The ratio of the absorption by the acid form of neutral red at 560nm to that of the base form at 430nm was determined. Intracellular pH was then calculated from the equation relating this ratio to pH generated from a standard curve prepared from color slides of sections of frozen rat brain homogenates. Using the ratio of these two absorption peaks makes the method independent of dye concentration. Constructing a standard curve by this method was necessary to control for interference in pH measurements caused by temperature and dye binding influences. Current pH resolution using this method is better than 0.1 unit and spatial resolution is limited by the projector magnification and the size of the entrance slit of the monochromator. For these studies, we adjusted the size of the region from which pH was determined to match that of the samples taken for histochemistry, about 0.5mm x 2mm. This method allows regional comparisons of tissue metabolic state and corresponding acid-base status.

113.13 ACTIVITY-DEPENDENT ACID TRANSIENTS IN THE RAT OPTIC NERVE: A DEVELOPMENTAL STUDY. W.G. Carlini and B.R. Ransom. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Extracellular pH, pH_o, was measured in the isolated rat optic nerve (RON; Connors et al., Science 216:1341, 1982), a pure white matter tract of the CNS, using double-barreled H^+-sensitive microelectrodes with tip diameters of approximately 1-4 μm. Trains of supramaximal stimuli were delivered via suction electrodes to RONs of different postnatal ages (1 to 30 days). In RONs older than 14 days, stimulus trains produced an acid shift in pH_o which could be as large as 0.3 pH unit. The time course and magnitude of the pH_o decrease were positively related to stimulus frequency, duration and intensity. The time course of the neural activity-dependent acid transient was slower in its development and recovery than simultaneously measured increases in $[K^+]_o$; the $t_{1/2}$ recovery of pH_o was 3 to 4 times greater than the $t_{1/2}$ of $[K^+]_o$ recovery. No initial alkaline shift was seen, as was reported in the cerebellum (Kraig et al., J. Neurophys. 49:831, 1983) and this may relate to the absence of synaptic interactions in the RON. After very prolonged periods of stimulation (> 30 sec) an alkaline undershoot sometimes developed following the acid shift. Changes in pH_o (± 0.5 pH) produced by altering the CO_2 environment had little effect on the compound action potential, although brief exposure to an alkaline environment often produced a transient enhancement of the field potential.

Carbonic anhydrase, which may play an important role in pH_o buffering, is localized mainly in glia and, like gliogenesis, this enzyme's expression develops postnatally. Hence, it was anticipated that activity in RONs prior to gliogenesis would produce an acid shift larger than that produced in older nerves which contained mature glia. Contrary to expectation, acid transients were smaller in neonatal RONs, with nerves less than 4 days of age showing no acid shift whatsoever. Thereafter, the magnitude of the maximum activity-evoked acid shift increased progressively with age, reaching stable adult levels at about two weeks of age. In contrast, evoked increases in $[K^+]_o$ were much larger in neonatal, as opposed to adult, RONs. Exogenously applied K^+ (10 to 30 mM) produced rapid acid shifts by as much as 0.4 pH_o in adult RONs while similar applications in RONs less than five days of age produced much smaller acid shifts. The reduced acid shifts seen in neonatal nerves, relatively devoid of glia, could indicate a role for glia in producing these shifts. Alternatively, a metabolic system in optic nerve axons may be exclusively responsible for acidification of the extracellular space, and this system may be absent in neonatal nerves. Studies are in progress to answer these questions. The isolated RON preparation appears to be a useful model system in which to analyze activity-dependent changes in pH_o.

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113.14 REGIONAL DISTRIBUTION OF CALCIUM IN THE RAT BRAIN. H.W. Sampson and M.E. Trulson. Department of Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843.

The importance of calcium in physiological functions such as muscle contraction, gland secretion and nerve impulse conduction is becoming increasingly evident. Although large numbers of investigators have examined the activities of calcium at the cell level in nerve tissue, very little work has been directed toward the similarities and differences in calcium content of brain regions in toto. In this study, 11 male Sprague-Dawley and 3 Fisher rats were sacrificed by decapitation. Three of the Sprague-Dawley rats were six months old and averaged 174 g in weight. The remaining Sprague-Dawley rats were 1 year in age and averaged 450 g. The 3 Fisher rats were 2 years of age and averaged 410 g. The brain was rapidly removed and dissected over ice into 10 regions. The regions were cerebellum, hippocampus, caudate, frontal cortex, remaining cortex, thalamus, hypothalamus, tectum, tegmentum, and medulla. Total calcium was determined by atomic absorbtion spectrophotometry using a nitrous oxide-acetylene fuel mixture. One-way analysis of variance indicated that there is a significant difference in the experimental groups ($P<0.005$). Tukey's HSD test indicated a significant difference between cerebellum and the 5 other brain regions. In order to discern these variations more fully, the brain regions were grouped into forebrain, hindbrain, and cerebellum plus spinal cord. A two-tailed student's t-test indicated that there is a significant difference ($P<0.001$) between these regions when grouped in this manner. It is not clear as to why this variation exists since there is no major differences in cell population density. If the brain regions are ranked according to embryological derivation there is a Spearman rank order correlation coefficient of 1 ($P<0.01$). If the prosencephalon is further divided into diencephalon and telencephalon, this highly significant correlation coefficienct is retained. One surprising finding was that there was no significant difference in brain regions relative to advanced age of the animal. There was a large variation in calcium levels in the cerebellum among animals. It is not known if this variation is present in the "normal" state or a function of the behavior of the animal immediatley prior to death. Equally interesting is the extremly small variation between hippocampal regions, indicating possibly the need to keep the calcium level tightly controlled in this region of the brain. This is supported to some extent by reports that low and high calcium levels cause epileptiform activity in hippocampal neurons. Also, it has been suggested that calcium has a special role in long-term potentiation and cognitive memory which is located in certain areas of the hippocampus.

113.15 COPPER DISTRIBUTION IN THE NORMAL HUMAN BRAIN. E. Bonilla, E. Salazar*, J.J. Villasmil*, R. Villalobos*, M. Gonzalez*, and J.O. Davila*. FUNDACITE-ZULIA, Inst. Invest. Clinicas. LUZ. Apartado 376 Maracaibo, Venezuela.

Copper deficiency affects the central nervous system mainly during infancy. Menke's kinky hair syndrome is due to a genetically determined defect in copper absorption and is characterized, among other symptoms, by progressive mental deterioration. On the other hand, Wilson's disease is an inborn error of metabolism that affects copper homeostasis. The genetic abnormality leads to excessive copper accumulation in the liver, brain, kidney, and cornea. In order to get a better insight about the pathophysiology of the nervous system alterations produced by copper deficiency in humans, it is necessary to know the regional distribution of this metal in the brain. In the present work we include results from 38 areas of 7 normal human brains.

Samples weighing between 5 and 55 mg were taken from unfixed brain in which macroscopic examination showed no signs of disease. The autopsies took place from 2 to 4 hours after death. The copper content was determined by flameless atomic absorption spectrophotometry. Brain regions of seven males ranging in age from 11 to 75 years were analyzed. Two of them died from multiple traumata without brain lesions. Three died as a consequence of myocardial infarctions. Among the other two, one died by haemorrhage after a gun shot and the other by drowning. None of the persons had a previous disorder of the nervous system. The general linear model from Statistical Analysis System was used for the analysis of variance.

Copper was found to be unevenly distributed in the human brain. The gray matter yielded higher concentrations of copper than the white matter (16.73 $\pm$ 0.89 ug/g dry weight for gray matter; 10.74 $\pm$ 2.07 ug/g for white matter). The difference was statistically significant. The copper concentration ratio of cerebral gray matter to white matter was 1.56. Identical areas of the gray and white matter of the cerebral cortex showed significant difference between individuals. In the caudate nucleus, the highest concentration was found in the tail, followed by the body and the head, respectively of the same region. No difference was observed for the concentration of copper in the gray matter of the frontal lobe (18.80 $\pm$ 1.15 ug/g) when compared with the occipital (22.34 $\pm$ 1.61 ug/g), parietal (17.84 $\pm$ 1.59 ug/g) and temporal (16.30 $\pm$ 2.32) lobes, but when comparing the content of the occipital lobe with that of the parietal and temporal lobes the difference was significant at the 5% level. It was found a negative lineal regression between age and brain copper concentrations. With increasing age a decrease in brain copper content was observed.

113.16 PALMITATE UPTAKE INTO THE HYPOTHALAMIC-NEUROHYPOPHYSIAL REGIONS OF OSMOTICALLY STRESSED BRATTLEBORO (DIABETES INSIPIDUS) RATS. J.M. Gnaedinger* and S.I. Rapoport, Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20205.

Previous reports indicate increased glucose metabolism in certain hypothalamic-neurohypophysial regions of osmotically stressed and Brattleboro rats (Schwartz et al., Science 205:723, 1979; Kadekaro et al., Brain Res. 275:189, 1983; Sutherland et al., Brain Res. 271:101, 1983). This increased uptake of 2-deoxyglucose is presumably due to increased energy demands on this neural pathway due to stress or due to a genetically related abnormality (absence of vasopressin in Brattleboro animals). However, vasopressin treatment does not reverse this increased energy use in the pituitary of Brattleboro rats, suggesting that there may be structural changes associated with the pituitary. We therefore examined the turnover of stable membrane compartments in this neural pathway by assessing the incorporation of 14C labelled palmitate as described by the protocol of Kimes et al., Brain Res. 274:291, 1983.

Six groups of animals were examined which included Long-Evans controls, heterozygous Brattleboro, homozygous Brattleboro and their corresponding water-deprived groups. 14C-palmitate was injected IV, as a bolus, and animals were killed 4 hours later. Brains were frozen at -50°C, cut into 20 micron sections and autoradiographed. Films were densitometrically scanned and a ratio was calculated for each region which consisted of the optical density for the region/optical density for white matter. Results were analyzed using Bonferroni statistics for multiple groups.

Normally hydrated and water-deprived homozygous animals demonstrated significantly decreased ($p<.05$) palmitate uptake ratios, as compared to hydrated Long-Evans controls, into the following regions which are outside the blood-brain barrier: anterior and posterior pituitaries, subfornical organ, median eminence, and pineal gland. As in the 2-DG studies, there were few statistically significant differences in palmitate uptake in barrier-protected hypothalamic regions.

These decreases in palmitate uptake contrast with the increases seen in the 2-DG results, and may be due to several factors. The most likely explanation is that these non-barrier regions utilize palmitate for energy (Vanucci and Hawkins, J. Neurochem. 41:1718, 1983). Thus, an elevation in the quantity of palmitate used for energy purposes would result in smaller amounts available for uptake into stable membrane compartments. This competitive effect might mask any changes in palmitate uptake due to membrane alterations.

113.17 REGIONAL PALMITATE INCORPORATION INTO RAT BRAIN FOLLOWING UNILATERAL VISUAL DEPRIVATION, J.C. Miller*, O. Tone*, J.M. Bell*, and S.I. Rapoport, Laboratory of Neurosciences, National Institute on Aging, Bethesda, MD 20205, (spon: S.B. Waller).

Regional palmitate flux as determined by the quantitative autoradiographic technique of Kimes, et al.(Brain Res. 274,(1983) 291-301) has been positively correlated with the regional cerebral metabolic rate for glucose (rCMRglc) and is not limited by regional cerebral blood flow. Palmitate incorporation is increased from 15-25 days of age coincident with rapid brain growth and peaks at 20 days, when myelination is maximal, such that flux into white matter exceeds that into gray matter during this period (H. Tabata, et al., in preparation). In adult rats, incorporation into gray matter exceeds that into white matter and does not change with age from 3-34 months of age.

Because the visual system is 80-90% crossed at the optic chiasm, monoocular deprivation in the rat removes most of the visual sensory input to the contralateral cerebral hemisphere. Previous studies with 14C-2-deoxyglucose in rats and monkeys demonstrated marked decrements in rCMRglc in the contralateral superior colliculus, lateral geniculate, and visual cortex after enucleation (L. Sokoloff, J. Cereb. Blood Flow Metabol. 1,(1981) 7-36).

Regional incorporation of labelled palmitate was measured up to 12 weeks after unilateral deprivation of 11 day and 3 month old Fischer-344 rats. Enucleation and catheterization were done under halothane anesthesia followed by a 4 hour recovery period. 14C-palmitate was then injected into the femoral vein, timed arterial plasma samples collected to determine the integrated specific activity of plasma palmitate, and the animal killed after 4 hours by pentobarbitol overdose. The brain was then removed, frozen, sectioned, and exposed to x-ray film for autoradiography.

Preliminary results indicate that compared with the ipsilateral side small decrements in palmitate incorporation occur in the contralateral superior colliculus (3%, $p<0.02$) and the lateral geniculate (2%, $p<0.05$) 4-12 weeks after enucleation of 11 day old rats. No significant change in palmitate incorporation was observed in the visual cortex.

113.18 REGIONAL PALMITATE INCORPORATION INTO THE BRAIN FOLLOWING UNILATERAL AUDITORY DEPRIVATION IN RATS. O.Tone*, J.C.Miller*, J.M.Bell* and S.I.Rapoport. Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20205. (spon: N. Azzam)

The effects of acute and chronic unilateral auditory deprivation in immature and adult rats on the regional incorporation into the brain of plasma palmitate were studied, using the quantitative autoradiographic method of Kimes et al.(Brain Res. 274;291-301, 1983)

Two age groups were selected for this study to measure palmitate incorporation after cochlear destruction in immature (11 day old) and mature (3 month old) awake Fischer-344 rats. Palmitate incorporation into the brain is closely related to increased lipid synthesis during brain development (Tabata et al., in preparation). Cochlear function in rat increases quickly from 11-16 days after birth (Bosher,J.Physiol.212:739-761,1971). Incorporation of 14C-palmitate was measured 1 day, 1 week, and 6 weeks to 3 months after cochlear destruction in both age groups. The destruction of the left cochlea by the trans-meatal approach was done under halothane anesthesia. Catheters were inserted into the femoral artery and vein under halothane anesthesia, then allowed to recover for 4 hr. 14C-palmitate (450 uCi/kg) in 5 mM HEPES buffer, pH 7.4, containing 5 mg/ml BSA was injected into the femoral vein. Plasma samples were collected from the femoral artery for 240 min following injection to measure plasma palmitate radioactivity. The brains were removed 4 hr after isotope injection, following pentobarbital overdose and decapitation. The brains subsequently were frozen, sectioned, and exposed to film for quantitative autoradiography.

Palmitate incorporation into the left cochlear nucleus of the immature group, 6 weeks to 3 months after deprivation, was lower than that of right side by 7% ($p<0.01$). Right inferior colliculus and medial geniculate of individual animals had less incorporation than did the corresponding regions on the left side by 6% ($p<0.02$) and 5% ($p<0.02$) respectively. A similar pattern was observed in the adult group, but to lesser degree.

The present study indicates that chronic auditory deprivation reduces the palmitate incorporation into brain structures along the auditory pathway.

113.19 CHOLINERGIC INNERVATION OF THE CEREBRAL CIRCULATION:CHARACTERIZATION OF SPECIFIC UPTAKE AND RELEASE PROCESSES IN ISOLATED PIAL VESSELS.

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Major cerebral arteries appear to be endowed with high affinity choline uptake and acetylcholine release mechanisms(1);however,very little is known about these specifically neuronal characteristics in small pial (arteriolar) vessels believed to be the most important vascular segment in the regulation of regional blood flow. In the present study, we have assessed the capacity of perfused, isolated rabbit pial vessels to accumulate ^{3}H-choline;furthermore,we examined the effects of potassium-induced depolarization on the spontaneous efflux of vessels pre-loaded with ^{3}H-choline. For comparison, these parameters were measured in the cerebral arteries of the circle of Willis and in the choroid plexus.

All preparations exhibited a high capacity to accumulate ^{3}H-choline at a low concentration ($5x10^{-8}$M). In pial vessels, the high affinity choline uptake was saturable, time- and temperature-dependent with the accumulation of choline at 4°C being less than 10% of that measured at 37°C (0.76 ± 0.14 vs 12.73 ± 1.4 pmol/mg protein/hr). Hemicholinium-3, a selective inhibitor of the high affinity choline uptake, induced a dose-dependent inhibition of ^{3}H-choline accumulation in pial vessels with maximal inhibition (> 90%) at 10^{-3}M.

Pial vessels and large arteries pre-incubated with ^{3}H-choline reached a stable spontaneous efflux of tritium after continuous superfusion of the tissues for 20 to 30 min. Addition of potassium to the medium induced a concentration-dependent increase in tritium outflow which, at 50 mM, was approximately double that of the basal release. The efflux returned to basal levels following 20 min superfusion with a normal potassium medium; after this period, the vessels were still responsive to stimulation of 50 mM potassium,though with a slight decrease in tritium outflow. After removal of calcium from the superfusion solution, addition of 50 mM potassium failed to evoke an increase in tritium efflux. The choroid plexus, preloaded with ^{3}H-choline, also exhibited a continuous efflux of tritium; however, this release was not stimulated by potassium, even in the presence of calcium.

These findings suggest that part of the choline accumulated in pial vessels is taken up by perivascular nerve terminals, transformed into acetylcholine which can be released upon depolarization. Also, the results confirm previous observations on the presence of functional mechanisms for the uptake and release of acetylcholine in large cerebral arteries but would argue against such an innervation in the choroid plexus.

(1) Duckles, S.P. (1981), J. Pharmacol. Exp. Ther. 217(3): 544-548.

113.20 HISTOCHEMISTRY AND EFFECT OF CHRONIC METHAMPHETAMINE ON MICROVESSELS OF RAT CAUDATE NUCLEUS, SUBSTANTIA NIGRA AND VENTRAL TEGMENTAL AREA. M.S. Cannon,* E.D. Kapes* and M.E. Trulson (SPON: J.B. Gelderd). Dept. of Anatomy, College of Medicine, Texas A&M Univ., College Station, TX 77843.

Young adult male Sprague-Dawley rats served as either saline-injected controls or as test animals receiving methampethamine HCl (20 mg/kg, i.p.) twice daily for ten consecutive days. All rats were then decapitated, brains removed and appropriate areas of forebrain and midbrain selected for histochemical examination of microvasculature. The histochemical reactions utilized represented a number of metabolic pathways, including; glycolysis, the hexose-monophosphate-shunt, Kreb's tricarboxcylic cycle, beta-oxidation of fats, and the respiratory chain. Enzymes were evaluated from fast, frozen material, while substrates such as glycogen, neutral fat and free fatty acids were examined from fixed and/or frozen tissue.

No differences in metabolic profiles of arterioles from rat caudate nucleus, ventral tegmental area and the zona compacta and zona reticulata of the substantia nigra were observed between control and amphetamine-treated animals. Arterioles from all three regions are metabolically active vessels with the capacity for aerobic and anaerobic metabolism. These arterioles, and in particular the larger microvessels of the caudate nucleus and ventral tegmental area, appear to possess a significant potential for nucleic acid and protein synthesis, while arterioles from these two regions and the substantia nigra demonstrate little intramural lipid storage; any fatty acids utilized by the arteriolar wall are provided by the blood supply. Furthermore, although arterioles of the rat ventral tegmental area and from both zones of the substantia nigra show similar metabolic profiles, when comparing the latter with the ventral tegmental area, a lower metabolic capacity is seen in the substantia nigra. In the ventral tegmental area and substantia nigra, it is not known if these metabolic differences are related to possible differential roles of dopamine neurons and the regulation of vascular tone. Also, this study indicates that metabolic differences do occur between microvessels of different calibers within the same vascular bed.

LEARNING AND MEMORY: PHARMACOLOGY I

114.1 A SPECIFIC DEFICIT IN SPATIAL LEARNING IS CAUSED BY REPEATED BUT NOT ACUTE TREATMENT WITH A CHOLINERGIC AGONIST. S. E. Wade* and S. F. Maier (SPON: M. L. Laudenslager). Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

The watermaze task (Morris, R. G. M., Learn. Motiv., 12:239, 1981) is used to assess spatial learning in rats: normal rats quickly learn to escape from a cool swimming pool onto a platform hidden at a fixed location just under the surface of the water, by reference to distal spatial cues. Hippocampal lesions severely impair performance on this task, without affecting performance on a task identical except in use of a visible rather than hidden escape platform (nonspatial task) (Morris, R. G. M. et al., Nature, 297:681, 1982). This disassociation is taken as evidence of a specific effect of hippocampal lesions on spatial learning, as opposed to motivational, reinforcement, or motor-performance factors. Cholinergic blockade given just before acquisition trials has also been shown to impair spatial watermaze performance (Sutherland, R. J. et al., J. Comp. Phys., Psych., 96:563, 1982). We demonstrate that while a single acute treatment with the cholinergic agonist, oxotremorine (OXO), has no effect upon acquisition of spatial watermaze performance, 2 treatments with OXO produce a profound and specific impairment of spatial learning that is observable 48-72 h after the last drug injection.

Male albino rats were removed from individual home cages, injected i.p. with OXO (2.5 µmole/kg), and returned to home cages. When given 9 acquisition trials in the spatial watermaze task 60-100 min later (the time of maximum drug effect upon body temperature), they showed normal performance, with mean escape latency on trials 7-9 (MEL) of 10-15 sec. Acute oxotremorine was similarly without effect on the nonspatial task, with normal MEL in the same range.

When identical OXO treatment was given on 2 successive days, and watermaze acquisition was tested 48 h after the last drug injection, performance on the spatial task was profoundly impaired (MEL 37 ± 5 sec) while latencies on the nonspatial task were normal. (MEL 14 ± 2.8 sec). Similar results were seen when OXO injections were alternated with saline injections over 4 days and testing was 72 h after the last injection. Saline injections alone were without effect.

These results suggest that a specific spatial learning deficit can be produced in the rat in response to repeated treatment with OXO, a cholinergic agonist. Both the absence of an acute drug effect and the observed time course of the impairment make a residual drug effect unlikely, and the impairment is therefore tentatively attributed to a regulatory change induced by repeated cholinergic insult. This change is similar in its effects on spatial learning to hippocampal lesions and cholinergic blockade.

114.2 EFFECTS OF SCOPOLAMINE ON PERFORMANCE OF RATS IN A 14-UNIT T-MAZE. E. Spangler*, P. Rigby*, and D. Ingram* (SPON: E. Bresnahan) Gerontology Research Center, NIA, NIH, Francis Scott Key Medical Center, Baltimore, MD 21224.

Relative to the performance of young adult rats, aged rats are impaired in many learning and memory tasks. Evidence has been presented that these age-related deficits are associated with declines in central cholinergic neurotransmitter systems. Previously, we had demonstrated age differences in both rats and mice in learning an automated, 14-unit T-maze task (Soc. Neurosci. Abstr., 10:452). By administering scopolamine, a muscarinic antagonist, to young animals, we sought to determine whether the cholinergic system was involved in learning this task. Male F-344 rats were given preliminary training in one-way active avoidance (US=1.0 mA) in a straight runway (1 m long). The criterion was 8/10 successful avoidances on the last of three consecutive 10-trial daily sessions. Testing in the automated maze task began 24 hr later. Animals were randomly assigned to one of six groups in which they received 30 min prior to testing an IP injection of either saline, scopolamine (0.1, 0.3, 1.0, or 3.0 mg/kg) or methylscopolamine (1.0 mg/kg). Each animal was provided with two 10-trial daily sessions in the maze task separated by 24 hr. The rat was required to traverse each of five maze segments within 10 sec to avoid footshock. All groups demonstrated learning in the maze task; however, the 1.0 and 3 mg/kg scopolamine groups learned at a slower rate and their asymptotic level of performance was higher. Based on a regression analysis, the variance accounted for by scopolamine dose was 62% - error scores; 42% - run time; and 38% - for the number of shocks. Scopolamine dose did not account for a significant amount of the variance for amount of shock. For the error score measure, one-way ANOVAs for each of four blocks of five trials revealed significant impairment for the 1.0 and 3.0 mg/kg scopolamine groups relative to the saline group (Dunnett's test; $p < .01$). For the run time measure, significance appeared in the final three blocks but not in the first block (p's < .01). Analyses of total shock time and number of shocks indicated no differences in block one for either measure. Differences were noted for the 1.0 mg/kg group only in block two of the number of shocks measure, while the 3.0 mg/kg group was impaired in blocks 2, 3, and 4 of this measure (ps < .01) and in blocks 2 and 4 for the amount of shock measure (ps < .01). These findings suggest that run time, amount of shock and number of shocks were influenced by error performance in this task. Thus, scopolamine had a deleterious effect on performance, particularly on error scores. These findings indicate that cholinergic neurotransmitter systems may influence learning of this complex maze task, but at doses higher than those previously observed to affect performance in a radial arm maze (Physiol. Behav., 26:845).

114.3 SCOPOLAMINE-DISRUPTION OF RADIAL ARM MAZE PERFORMANCE: MODIFICATION BY NORADRENERGIC DEPLETION. M. W. Decker and M. Gallagher. Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27514.

Cholinergic deficits characteristic of Alzheimer's disease (AD) are correlated with cognitive deficits, and experimental disruption of this system interferes with memory in both humans and laboratory animals. Cholinergic deficits in AD, however, typically occur in the context of depletions of other neurotransmitters such as norepinephrine (NE), so it is important to consider the possibility of interactions between the cholinergic system and other affected neurotransmitter systems.

We investigated the interaction of the ascending noradrenergic system and the cholinergic system in learning and memory by comparing the effects of the muscarinic antagonist, scopolamine, on radial arm maze performance of rats with 6-hydroxydopamine lesions of the dorsal noradrenergic bundle (n=7) with scopolamine's effects on the performance of operated control animals (n=6). Following training on both a continuous performance version of the 8-arm maze and on a version in which a 30 sec. delay was imposed between the 4th and 5th arm selections, surgery was performed. Two weeks post-operatively, rats were retrained on the no delay version of the task prior to beginning drug studies.

A pretraining injection of scopolamine (0.25 or 0.5 mg/kg) did not differentially disrupt performance in the continuous version of the task, but did impair the performance of lesioned animals more than control animals when a 45 min. delay between arms 4 and 5 was imposed. In both of these procedures, no differences were noted between the saline control performances of the two groups. In a post-training injection procedure, scopolamine was injected immediately after the 4th arm selection and a 5 hr. delay was imposed before the animal was allowed to complete the task. Here, NE-depleted animals performed significantly more poorly than control animals when 0.5 mg/kg scopolamine was administered, whereas no group differences were noted when saline or lower doses of scopolamine (0.25 or 0.125 mg/kg) were administered.

This study shows that NE depletion intereacts with blockade of the cholinergic system in a learning and memory task, and suggests that further consideration of the role of this interaction in the etiology of the cognitive deficits associated with Alzheimer's Disease may be worthwhile.

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114.4 THE EFFECT OF PHYSOSTIGMINE AND/OR SCOPOLAMINE ON RADIAL ARM MAZE PERFORMANCE IN RATS. G. Vincent, R. Harney*, and E. Gamzu, Dept. of Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ 07110

The effects of acute and/or repeated administration of cholinergic agonists and antagonists were evaluated on working memory of Wistar rats (4-8 months of age) in an eight-arm radial maze task. All drugs were administered intraperitoneally. Scopolamine (1 mg/kg), administered 30 minutes before testing, significantly decreased accuracy (i.e. more errors), but not rate (i.e. time to complete eight new choices) as compared to performance on non-drug control days. Methylscopolamine (1 mg/kg) had no effect on either accuracy or rate, suggesting that the effect produced by scopolamine is central rather than peripheral. The next study was undertaken to determine whether repeated exposure to scopolamine would produce a reliable deficit in working memory. Daily administration of scopolamine (1 mg/kg) for up to six days disrupted accuracy, but not rate, of performance compared to that observed on non-drug control days. However, the level of disruption decreased as a function of the number of days the drug was administered, suggesting an up-regulation of cholinergic receptors and/or an increased receptor sensitivity (Loullis, C.C. et al. Soc. for Neurosci. Abstr. 7:925, 1981).

Few studies have attempted to pharmacologically reverse drug-induced deficits in spatial memory performance. We administered physostigmine in an attempt to reverse the deficits in accuracy produced by scopolamine. In the first studies, a dose response curve for physostigmine was established by administering a constant dose of scopolamine (1 mg/kg) versus varying doses of physostigmine (0.03, 0.1, 0.3, and 1 mg/kg). Physostigmine, when administered 25 minutes before testing at doses of 0.3 and 1 mg/kg, reversed the scopolamine-induced deficit in accuracy of performance but had no effect on rate. The number of errors was not significantly different from control performance values. Doses of 0.03 and 0.1 mg/kg physostigmine did not reverse the scopolamine-induced deficit in accuracy of performance. When a constant dose of physostigmine was given (1 mg/kg) and the dose of scopolamine was varied (0.1 or 0.3 mg/kg), no effect on accuracy was observed at 0.1 mg/kg, but some disruption in accuracy was seen after the higher dose of scopolamine. These results support previous suggestions that the central cholinergic system is, directly or indirectly, involved in spatial memory, and that cholinergically induced deficits of this system are pharmacologically reversible.

114.5 EFFECTS OF SCOPOLAMINE AND KCL INJECTIONS INTO THE CAUDATE NUCLEUS ON THE PERFORMANCE OF CONTINUOUS AND FIXED RATIO REINFORCED BEHAVIORS. R.A. Prado-Alcalá and M. E. Archundia*. Dept. of Physiology, Faculty of Medicine, Natl. Univ. of México, P.O. Box 70250, México, D.F., México 04510.

Male Wistar rats were trained for 15 sessions to press a bar in order to obtain water. One group of animals was submitted to a continuous reinforcement schedule (CRF) and another to a fixed ratio-6 reinforcement schedule (FR-6). The sessions ended when the animals had pressed the bar 120 times or when 30 minutes had elapsed. Six minutes before sessions 10, 12 and 14 bilateral injections into the antero-dorsal aspect of the caudate-putamen were made (scopolamine (30 ug/3 ul), isotonic saline solution (3 ul) and 3M KCl (1 ul), respectively) to half the groups under each reinforcement condition. A significant impairment in performance was found only in the CRF animals injected with KCL.

The lack of effect of KCl injections to the FR-6 group and of scopolamine injections to the CRF anf FR-6 groups on learned performance is interpreted as being due to overtraining.

These results further support the hypotheses that: a) cholinergic activity of the caudate nucleus is not critically involved in the maintenance of overtrained tasks, and b) after overtraining the control of instrumentally conditioned tasks is transfered to a non-cholinergic system, within or outside the caudate nucleus.

114.6 EFFECTS OF CHOLINESTERASE INHIBITORS ON ONE-TRIAL PASSIVE AVOIDANCE RETENTION DEFICITS INDUCED BY IBOTENIC-ACID LESIONS OF THE NUCLEUS BASALIS OF MEYNERT. L.J. Thal[*1], C.P.J. Dokla[*1], E.L. Gardner[1], and M. Weinstock[2] (SPON: W.R. Salafia). [1]Departments of Neurology and Psychiatry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461 and [2]Department of Pharmacology, The Hebrew University-Hodassah Medical School, Jerusalem. Ibotenic acid (IBO) lesions of the nucleus basalis of Meynert (NBM) produce extensive loss of choline aceyltransferase (CAT) in cortex and currently serve as a primary animal model for Alzheimer's disease. NBM lesions impair retention on a number of behavioral tasks, particularly one-trial passive avoidance (PA) (e.g., Flicker et al., Pharm. Biochem. Behav. 18: 973-981, 1983). The present investigation examined the effects of IBO lesions on retention of PA, a reference-memory task, and sought to determine if the memory deficits could be eliminated or attenuated using two cholinesterase inhibitors: physostigmine and RA-6. RA-6 is a unique, potent, specific, and reversible cholinesterase inhibitor (Weinstock et al. 30th Oholo Biological Conference, Eilat, Israel, 1985) that may be useful in the treatment of Alzheimer's disease. Fischer 344 rats underwent bilateral stereotaxic NBM injections (two-stages) using 18 ug of IBO in 1 ul of buffered saline. Rats were trained on PA using a single 0.5 mA (0.5 sec.) footshock following two pretraining days. Retention of PA was assessed on the first two postshock days (600 sec. cutoff criterion), and dose-finding drug trials commenced on the next day and continued for 11 consecutive days. Independent IBO lesioned groups (n=9/group) were given physostigmine (in 0.04 mg/kg increments starting at 0.04 mg/kg); RA-6 (in 0.5 mg/kg increments starting with 0.5 mg/kg); or vehicle (distilled water). An additional group served as sham-operated controls (n=9). The IBO groups displayed the expected robust PA retention deficit on the first two postshock (no drug) days ($p<.001$) and the retention deficit persisted strongly for the first 8 days of dose-finding (p's <.01 to .05). While physostigmine failed to significantly attentuate the retention deficit (best dose 0.32 mg/kg), RA-6 produced significant recovery of PA at the 2.0 mg/kg dose ($p<.05$). The present results confirmed that IBO lesions of the NBM produce robust PA deficits and further suggest that the novel cholinesterase inhibitor, RA-6, may have potential value as a memory-enhancing agent following damage to the NBM (supported by the Westchester ADRDA).

114.7 ORAL TACRINE ADMINISTRATION IN MIDDLE AGED MONKEYS: EFFECT ON DISCRIMINATION LEARNING. A. Kling, L.J. Fitten*, K. Perryman* and K. Tachiki*. Departments of Psychiatry and Medicine, Sepulveda VA Medical Center, Sepulveda, CA 91343, and UCLA School of Medicine, Los Angeles, CA 90024

Animal studies testing the cholinergic hypothesis of age-related cognitive decline have usually employed acute, short-term parenteral administration of positive memory enhances. Our lab is developing a non-human primate model (macaca radiata) for assessing the effects of chronic, oral administration for tetrahydroaminoacridine (THA, Tacrine), a cholinomimetic with properties similar to physostigmine but with substantially more clinically desirable pharmacokinetic charasteristics.

All subjects (13-19) (N=4) were adapted for two weeks to the Wisconsin General Training Apparatus (WGTA). Each monkey then learned a series of five or seven different color pair discriminations. Learning criteria was reached when the subject scored 90% correct responses on three successive trials. Each trial consisted of 25 consecutive presentations of the discriminanda. The number of trials to criteria (TTC) were recorded. Two different dosing paradigms were used. In the first, a subject served as his own control (N=1). He received four successive THA dose regimens (2.5, 5.0, 7.5 and 10.0 mg/day). Each was preceded by placebo testing and followed by a five day drug-free interval before the next pair. A different color pair corresponded to each dose regimen or placebo trial. THA Serum levels 1 hour after ingestion were obtained for each dose and 24 to 48 hours after the beginning of the drug-free interval. In the second paradigm (n=3), subjects learned five discriminations on one dose and on placebo. Monkeys were either tested on 5.0 mg/day THA and then retested on placebo following a drug-free interval; or they were first tested on placebo and then on 5.0 mg/day THA. THA serum levels were monitored during the active (1 hour post injestion) and placebo periods.

Results in the first case indicate that 5.0 mg/day and 7.5 mg/day were particularly effective in reducing TTC (TTC=25, for THA vs 250, 200 for placebo) while 10.0 mg/day proved behaviorally toxic (TCC=175). In the second model, all subjects showed improvements on THA vs placebo but only one was statistically significant (p=.01). serum THA levels showed substantial variability throughout the dose range and from subject to subject. No signs of peripheral toxicity were noted.

We conclude that THA is well tolerated and possibly beneficial in enhancing discrimination learning. However, serum THA variability under our testing conditions may have significantly impacted on performance.

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114.8 EFFECTS OF SCOPOLAMINE ON RECOGNITION MEMORY IN MONKEYS. D. Walker*, T. Aigner*, P. Cantor*, & M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205.

Previous work from our laboratory showed that the anticholinergic agent scopolamine (SCOP) impairs visual recognition in monkeys as measured by delayed nonmatching-to-sample (DNMS) with trial-unique objects. To test whether the drug has a retrograde effect equivalent to its anterograde effect, we administered SCOP to three rhesus monkeys either before (task 1) or after (task 2) the acquisition phase of DNMS. In the acquisition phase of both tasks, the animal was shown a series of 40 baited sample objects at the rate of 1 every 30 sec. In the test phase, the animal was given 40 choice trials in which each sample object, now unbaited, was paired with a novel baited object. During the test phase, the order of presentation of the sample objects was reversed (i.e. the last sample object in acquisition was the first to be paired with a novel object and vice versa). In task 1, the retention interval ranged from 1 to 40 min. In task 2, in order to allow for testing retrograde drug effects, a 20-min interval was imposed between acquisition and test, and, consequently, the retention interval ranged from 20 to 60 min. Drug testing on each task was preceded by 15 practice sessions on each. SCOP was injected 20 min before the start of acquisition in task 1 and immediately after acquisition in task 2. SCOP doses of 10 and 17.8 ug/kg were tested on task 1 and doses of 10, 17.8, and 32 ug/kg were tested on task 2. Each dose was tested 8 times and at least 1 nondrug session preceded each drug session. Equivalent doses of neostigmine were given just prior to SCOP to block adverse peripheral effects.

On both tasks, linear forgetting curves were obtained. In task 1, the average percent correct ranged from 98% for the shortest retention interval (1 min) to 70% for the longest (40 min), yielding a mean score of 80%. In task 2, the values ranged from 83% (20 min) to 65% (60 min), for a mean of 73%. In task 1, 10 ug/kg of SCOP produced a marginal impairment and 17.8 ug/kg produced a significant impairment. In task 2, in which SCOP was administered after acquisition, neither 10 nor 17.8 ug/kg produced an effect, impairment being observed only with the highest dose, 32 ug/kg. The results suggest that SCOP has mainly an anterograde effect on recognition and hence probably affects storage more than retrieval. Furthermore, in each task, the effective dose of SCOP produced a forgetting curve shifted downward but parallel to the control curve. The latter result suggests that the anterograde effect of SCOP is equivalent to reducing the strength of the memory trace by increasing the passage of time following acquisition.

114.9 EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL (THC) ON RECOGNITION MEMORY AND HABIT FORMATION IN MONKEYS. T. Aigner*, R. Brown*, D. Walker*, & M. Mishkin (SPON: S. Mitchell). Laboratory of Neuropsychology, NIMH, Bethesda, MD 20205 and Preclinical Research Branch, NIDA, Rockville, MD 20852.

Although the cognitive effects of marijuana, or its active metabolite THC, are still poorly understood, evidence from studies in animals suggests that THC may be exerting its effects through actions on the limbic system. To explore this possibility, we investigated the effects of orally administered THC in monkeys on two learning abilities, one known to be dependent on the limbic system, and the other known to be independent of it.

Six rhesus monkeys were divided into two groups of three each. Group I was trained in delayed nonmatching-to-sample (DNMS) with trial-unique objects, the limbic-dependent memory task. After receiving pretraining on easy versions of DNMS, the animals were tested for their ability to remember a series of 40 (baited) sample objects, presented at the rate of one every 15 sec. In the choice test, each sample object (now unbaited) was paired with a (baited) novel object, again once every 15 sec. Doses of THC (1, 2, & 4 mg/kg) were administered either 1 or 2 hrs prior to the session. Each dose was tested twice at each time point in a nonsystematic order. At least one nondrug control session preceded each session in which THC was given. Group II was trained in 24-hr concurrent discrimination learning (CDL), a task that monkeys with limbic lesions can perform normally. A set of 20 object pairs was presented once every 24 hrs, with the positive and negative objects in the pairs and the order of pair presentations remaining constant across sessions. Criterion was set at 90 correct responses in 100 trials. Three doses of THC (4, 8, & 16 mg/kg) and two nondrug control treatments were tested, each with a different set of 20 object pairs. THC was given 2 hrs prior to each daily session. Tests were conducted 7 days per week, with at least 1 week intervening between sets.

THC had a significant effect on the DNMS performance of Group I. The largest effect was seen 2 hrs after a 4 mg/kg dose, the highest dose tested on DNMS, with performance decreasing to 80% of control levels under this condition. No significant impairment was found on the CDL performance of Group II at any dose, though one of the three monkeys appeared to be slightly impaired at the dose of 16 mg/kg. The results suggest that THC interferes with memory more than with habit formation, and this in turn may reflect a selective action of THC on limbic structures.

114.10 A STUDY OF NICOTINE (NIC), DESGLYCINAMIDE ARGININE VASOPRESSIN (DGAVP), AND SCOPOLAMINE (SCOP) IN AN AUTOSHAPED LEVER-TOUCH MODEL OF "LEARNING" IN RATS. Edgar T. Iwamoto and William Mundy* (SPON:W.R.Martin) Dept. of Pharmacology and the Tobacco and Health Research Instit. Univ. of Kentucky, Lexington, KY 40536.

Nicotine reportedly increases or decreases "learning" and "memory" in animals and humans. Since nicotine is known to alter vasopressin plasma levels, and vasopressin purportedly modifies "learning" and/or "memory" as estimated by acquisition and resistance to extinction of conditioned avoidance behavior, the ruling hypothesis has evolved that "nicotine improves cognitive function because it releases vasopressin from the posterior pituitary". Messing and Sparber (EJP 89:43, 1983) reported that DGAVP improved acquisition and slowed extinction of a food-reinforced autoshaped lever touch response. Since most of the previous work with nicotine used avoidance paradigms, we decided to test the hypothesis that nicotine improves "learning" and "memory" using this positively reinforced task.

Methods: Adult male Sprague-Dawley rats, and heterozygous and homozygous male Brattleboro rats were food-deprived to 80% of original b.wt., 306±41g SD, N=150. Modular test cages held a retractable lever that was presented on a random interval 48s schedule. The lever retracted after 15s or until it was touched at which time one 45mg food pellet was delivered ("discrete trial"). In paradigm I, magazine trained rats were injected SC with saline, 5 or 10 µg/kg of DGAVP prior to each of two daily acquisition sessions. Three days after acquisition training, rats received saline or DGAVP prior to two daily extinction (feeder disabled) sessions. In paradigm II, the acquisition sessions consisted of 10 discrete trials/da for 5 or 10 da. Non-magazine trained rats were autoshaped to a criterion of 10 correct lever touches. The number of correct lever touches (lever extended), "nose-pokes" (touching retracted lever), and the time spent near the lever were recorded.

Results. Paradigm I: Five µg/kg (one experiment) and 10 µg/kg (2 experiments) of DGAVP did not alter the acquisition or the extinction of the lever touch response, the incidence of nose-pokes, nor the time spent near the lever. Paradigm II: Saline-trained rats acquired the 10 correct/10 trial criterion in 4 to 6 sessions. Ten µg/kg of DGAVP did not alter acquisition. NIC and SCOP, (0.1-0.8 mg/kg), given 15 min prior to each session caused significant dose-related depressions of acquisition compared to saline controls. Acquisition by homozygous Brattleboro rats was not different from heterozygous littermates (2 experiments). These data suggest that pharmacologic and physiologic manipulations of vasopressin do not affect, while both SCOP and NIC impair, the acquisition of a positively-reinforced, autoshaped lever touch response in rats. (Supported by the Kentucky Tobacco Research Board and BRSG).

114.11 ARGININE VASOPRESSIN (AVP) RETARDS THE ACQUISITION OF BEHAVIORAL TOLERANCE TO TWO EFFECTS OF ETHANOL IN RATS. D.L. Hjeresen, D. Brief, D.L. Amend, and S.C. Woods. (SPON: R.H. LOVELY.) Department of Psychology, University of Washington, Seattle, WA. 98195.

There is extensive evidence that AVP and related peptide fragments affect learning processes and increasing evidence that learning mechanisms contribute to the development of drug tolerance. The present study was conducted to determine the influence of AVP on the acquisition and extinction of tolerance to the ataxic and hypothermic effects of ethanol (EtOH).

Twenty-eight male Long-Evans rats were tested in 4 groups: 1) EtOH/NaCl, 2) EtOH/AVP, 3) NaCl/NaCl 4) NaCl/AVP. EtOH (2.0 g/kg, 15% v/v, i.p.) and/or AVP (6 ug/kg, s.c.) was administered after an initial rectal temperature was taken and 10 m prior to testing on a moving belt (10 cm/s) test for ataxia. Testing consisted of 3 1-m trials (30-s ITI) and a second rectal temperature. These procedures were repeated for 12 consecutive days (ACQUISITION). On day 13 all rats were injected with EtOH and NaCl to test for possible differences in tolerance development. On Days 14 to 20 (EXTINCTION) rats received the following treatments: Group 1) NaCl/NaCl, 2) NaCl/AVP, 3) NaCl/NaCl, 4) NaCl/AVP, and on Day 21 the procedures of Day 13 were repeated.

Results confirm that AVP is a hypothermic agent and that its effect is additive to the hypothermic effect of EtOH. AVP significantly retarded the acquisition of tolerance to both the ataxic (Grp 1 vs. Grp 2, $F=5.6$, $p<.01$) and hypothermic ($F=15.4$, $p<.01$) effects of EtOH, although the level of tolerance attained on Day 13 was not significantly different between Groups 1 and 2 (i.e., both groups were significantly more tolerant than rats in Groups 3 and 4 which did not differ from each other). During extinction Groups 2 and 4 were rendered significantly more hypothermic than Groups 1 and 3 while no significant differences were noted in treadmill performance. On Day 21 rats in Group 2 were significantly more tolerant to the hypothermic effect of EtOH than rats in any other group. Differences between groups on the treadmill task were not significant.

That AVP interferes with the acquisition of two different measures of EtOH tolerance suggests that this effect is due to a generalized interference with learning. However, results of the extinction phase indicate that AVP delays the extinction of tolerance once it has been acquired. Both results suggest a role for learning mechanisms in the development of ethanol tolerance.

114.12 EPINEPHRINE EFFECTS ON MEMORY STORAGE: INTERACTION WITH β-ENDORPHIN AND NALOXONE. I. Introini* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

There is extensive evidence that epinephrine modulates memory. Low doses facilitate memory and high doses induce amnesia in a variety of tasks. Opioid peptidergic systems also modulate memory: Retention is enhanced by naloxone and impaired by β-endorphin. The present study examines the interaction between peripheral epinephrine and opioid peptidergic systems on retention. Male CFW mice (60 days old) were trained on an inhibitory avoidance response (IAR) or a visual discrimination response (VDR), injected (IP) immediately posttraining and tested 24 hours later. No significant effects on retention were observed when a facilitatory dose of epinephrine (IAR: 10 µg/kg; VDR: 300 µg/kg) and an impairing dose of β-endorphin (IAR: 1.0 or 3.0 µg/kg; VDR: 1.0 µg/kg) were given together. Further, a subeffective dose of epinephrine (IAR: 3.0 µg/kg; VDR: 30 µg/kg) did not modify the dose-response curve of naloxone (0.3-3.0 mg/kg).

In contrast, a facilitatory dose of naloxone (IAR: 1.0 mg/kg; VDR: 3.0 mg/kg) completely reversed the impairing effect of epinephrine (IAR: 0.3 mg/kg; VDR: 1.0 mg/kg) on memory, suggesting that the amnestic action of epinephrine may be due to an increase release of an opioid peptide, possibly β-endorphin. In view of pharmacological evidence that a novel experience depletes brain β-endorphin (Izquierdo and McGaugh, unpublished), we also examined the effects of posttraining epinephrine (IAR: 0.3 mg/kg; VDR: 1.0 mg/kg) on mice given a novel experience one hour prior to training of both tasks. With these procedures, otherwise amnestic doses of epinephrine, significantly enhanced retention. These findings suggest that different mechanisms of action mediate the facilitatory and impairing effects of epinephrine on memory modulation.

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114.13 ADRENAL DENERVATION ALTERS THE EFFECT OF MET-ENKEPHALIN AND NALOXONE ON MEMORY CONSOLIDATION. S. Zhang*, I. Introini*, R. Juler* and J.L. McGaugh (SPON: G. Novack). Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717 U.S.A.

Previous evidence indicated that endogenous opioid peptides are involved in modulation of memory. The site of action, however, is not yet clear. It is well known that enkephalins are released from adrenal medulla after stress, but the findings regarding enkephalins and memory are conflicting. The present study examined the effect of naloxone and Met-enkephalin (ME) on memory consolidation and assessed the involvement of adrenal medulla in such effect.

Two experiments were performed in male CFW mice (50-60 days old). In Experiment I, intact mice were trained in a one-trial inhibitory avoidance task (IA, 0.6 mA, 2sec footshock) and 10-14 days later in a Y-maze discrimination (YMD, 0.4 mA footshock). Immediately after training, animals received i.p. saline or one of the following drugs: naloxone, morphine, ME, naloxone + morphine or naloxone + enkephalin. Retention was tested 24 hr later. In both tasks, naloxone (0.3 mg/kg) enhanced retention while morphine (3 mg/kg) or ME (2 µg/kg in IA, 0.5 µg/kg in YMD) produced a retention deficit. For IA, morphine (3 mg/kg) or ME (2 µg/kg) attenuated the enhancement of naloxone on retention. For YMD, naloxone (0.3 mg/kg) antagonized the memory impairment caused by ME.

In Experiment II, mice were bilaterally adrenal denervated (ADXN) or sham-operated (SHAM). Two weeks after surgery the mice trained in IA and two weeks later in YMD. Approximately half the mice in each group immediately received posttraining injections of saline and the remainder were injected with naloxone (in IA) or ME (in YMD). Retention was tested 24 hr later. For IA, naloxone enhanced memory in SHAM, but did not enhance retention in ADXN. For YMD, ME significantly impaired retention in SHAM but did not significantly affect retention in ADXN.

The present data suggest that the effect of naloxone and ME on memory consolidation may be mediated in part by the release of ME from the adrenal medulla.

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114.14 EFFECTS OF OPIATE ADMINISTRATION INTO THE MEDIAL SEPTAL AREA ON LATENT INHIBITION. M. Gallagher, E. Bostock, and M.W. Meagher.* Psychology Department and Neurobiology Program, University of North Carolina, Chapel Hill, NC 27514.

Studies using classical conditioning procedures have reported that opiates and some opioid peptides impair the performance of learned responses. In addition, opiates and opioid peptides administered after training disrupt retention of recent learning. The present investigation examined the effects of intracranial administration of an opiate agonist, levorphanol, on latent inhibition of classically conditioned heart rate responses in rabbits.

New Zealand albino rabbits in the latent inhibition (LI) groups received 15 tone (1KHz, 82dB, 5 sec) presentations per day for 2 consecutive days. On Day 3 all rabbits received a standard Pavlovian conditioning session in which 15 tone alone presentations were followed by 30 trials in which the tone was paired with eyelid shock (1.2 mA, 500 msec). Tone pre-exposure produces latent inhibition reflected in a decrement of subsequent conditioned responses. Compared to a normal conditioning group (Day 3 session only), both an unoperated LI group and a LI group receiving vehicle injections into the medial septal area (MSA) exhibited significant latent inhibition of classically conditioned heart rate responses ($ps<.002$). These LI control groups, however, did not differ from one another. Opiate administration into the MSA significantly reduced retention for tone pre-exposure. Two LI groups which received levorphanol (3 ug) administered into the MSA either prior to or immediately following each tone pre-exposure session on Days 1 and 2 differed significantly from the LI control groups ($ps<.01$).

The effect of post-training levorphanol injection into the MSA exhibited both pharmacological and neuroanatomical specificity. Administration of dextrorphan (3 ug), the inactive enantiomer of levorphanol into the MSA immediately following each tone pre-exposure session or administration of levorphanol (3 ug) 1.5 mm dorsal to the original injection site did not significantly alter subsequent latent inhibition of conditioned heart rate responses.

These results complement previous findings that systemic administration of opiate agonists and antagonists alter retention for exposure to non-reinforced presentation of stimuli. Morphine was found to impair long-term retention of habituation and post-training naloxone has been reported to enhance retention of habituation in rats and mice (Izquierdo, Psychopharmac., 66, 199, 1979; Rodgers et al., Psychopharmac., 82, 322, 1984) as well as latent inhibition of conditioned heart rate in rabbits (Bostock and Gallagher Soc. Neurosci. Abstr. 8, 148, 1982). The MSA appears to provide a brain site sensitive to the effect of opiates on retention of non-reinforced presentations of a tone as reflected in latent inhibition.

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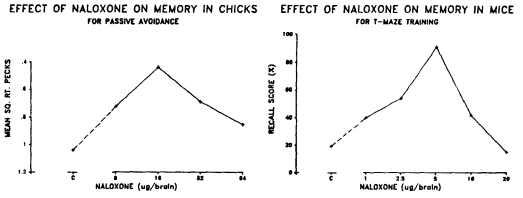
114.15 **INTRACEREBRAL NALOXONE ENHANCES MEMORY RETENTION IN CHICKS AND MICE.** **J.F. Flood, A. Cherkin and J.E. Morley. Psychobiology Res. Lab. and GRECC, VA Med. Center, Sepulveda, CA 91343.**

Naloxone, an opioid peptide antagonist, enhances memory retention in rodents after peripheral administration. Intravenous naloxone in human subjects produces conflicting effects on memory. One interpretation is the dose-dependence of memory-enhancing drugs, complicated by dose-dependent bidirectional effects, i.e., enhancement by moderate and impairment by high doses. The studies reported here examined the effect upon memory of central administration of naloxone over a wide dose range in two classes, Aves and Mammalia. All experiments were blind. Chicks (N = 30-38/gp) were trained in one 10-sec trial to suppress their spontaneous pecking at a normally attractive target (3-mm steel bead) by coating it with an aversive liquid, methyl anthranilate, in a passive avoidance paradigm (Physiol. Behav. 14:151, 1975). Naloxone hydrochloride (NAL) in 2 ul of saline was injected into each forebrain hemisphere 2 min after training, with the chick restrained in a head-holder. Moderate doses (16 or 32 ug per brain) enhanced 24-hr memory retention ($p < 0.05$, compared to saline, C in figure), as indexed by the reduced peck score (mean sq. root of pecks) during the 10-sec test presentation of the dry target. A lower dose (8 ug) or higher dose (64 ug) was ineffective (see figure; note inverted scale of ordinate and log scale of doses). In mice, a moderate dose (5 ug per brain) of NAL enhanced 168-hr memory retention ($p < 0.01$) when injected intracerebroventricularly 2 min after training in a T-maze active avoidance paradigm (Neurobiol. Aging 4:37, 1983). Lower and higher doses (1.0, 2.5, 10, 20 ug per brain) were not effective (see figure). We conclude that post-training central administration of NAL enhances memory retention in chicks and mice. The dose-response curve in each case is an inverted-U, i.e., low and high doses are ineffective. The "therapeutic window" is narrow from a pre-clinical point of view, suggesting that individual dose titration be considered in evaluating NAL for human memory enhancement.



114.16 OPIOID ANTAGONISTS ENHANCE AN ACQUIRED IMMOBILITY RESPONSE IN MICE. Gaila S. Opotowsky-Gulack*, Joe L. Martinez, Jr., and Marianne Leslie*. Psychology Department, University of California, Berkeley CA 94720

Naloxone (Nx) is known to enhance memory in several species. In contrast, Nx reportedly impairs learning of an acquired immobility task (the behavioral despair test) in rats and mice (Eur J Pharm 1984, [103] 205-210; Physio & Beh 1982, [28] 249-251). In the present study, mice were placed in a 1000 ml beaker containing 650 ml of 22 C water for 10 m. Nx or sal was administered either 5 m before training or immediately following training on Day 1. Retention was tested 24 h later (Day 2). Immobility was defined as floating, either upright or sideways, with no activity beyond that required to maintain balance. The number of s that a mouse remained immobile in each m was recorded. The number of s immobile in m 2-7 (posttrial administration) or m 1-10 (pretrial administration) was considered to be a measure of acquisition (Day 1) or retention (Day 2).

In the first experiment, Nx (1mg/kg, 10mg/kg) or sal was administered immediately posttrial on Day 1. Immobility in the Nx group (10mg/kg) was greater than that of the saline group [$t(76)=2.47, p<.01$]. The lower Nx dose was without effect. Nx (10mg/kg) or saline administered 5 m prior to training (Day 1) also enhanced Day 1 performance [$t(38)=2.88, p<.005$]. Further, Nx-treated animals showed an even greater immobility response than did animals in the sal group on Day 2 [$t(38)=6.88$, $p<.0005$].

Methylnaloxonium (MeNx) was administered to assess the contribution of peripheral opioid receptors. MeNx was given in doses equimolar to Nx (1.04mg/kg, 10.4mg/kg) immediately posttraining (Day 1). Neither MeNx dose produced a significant effect. Therefore, MeNx was administered at a dose three times the effective dose (30.12mg/kg) to compensate for the lower affinity of MeNx for opioid receptors. MeNx then produced a significantly greater immobility than sal [$t(36)=2.14, p<.05$].

These data demonstrate that Nx facilitates the retention of the immobility response, in general agreement with known actions of Nx in other tasks. Facilitation on Day 2 occurs whether Nx is administered before or after training on Day 1. Since MeNx also facilitates this response, it may be that Nx and MeNx affect learning by acting at the same peripheral receptor site. (Supported by ONR N00014-83-K-0385).

114.17 LEU-ENKEPHALIN: EFFECTS ON PLACE PREFERENCE CONDITIONING AND LACK OF EFFECT OF DES-TYR-LEU-ENKEPHALIN IN ACTIVE AVOIDANCE CONDITIONING. (SPON: M.R. Rosenzweig) Stephen Heinrichs,*Sandra Brown,*and Joe L. Martinez, Jr., Psychology Department, University of California, Berkeley, Ca 94720.

For place preference (PP) conditioning mice were injected (i.p.) with either Leu-enkephalin (LE) or sal and placed in a distinctive environment for 30 m. Drug and sal administration were alternated on consecutive days for 6 days. Before training the mice were allowed to freely explore the 2 environments for 10 m each day to determine preference scores. Following training the mice were given a 10 m testing session where they were allowed to choose between the 2 compartments. Mice were trained against and in the same direction as their initial preference. A dose of 300 ug/kg LE produced a positive PP [$t(56) = 2.1$, $p = .040$], if the mice were trained against their initial preference. Conversely, the opposite effect was observed with the 300 ug/kg dose, if the mice were trained towards their initial preference [$t(66) = 2.78$, $p = .007$]. The next experiment replicated the positive PP conditioning effect produced by 300 ug/kg LE [$t(21) = 1.91$, $p = .03$, one-tail]. This positive effect was partially attenuated by methylnaloxonium. Mice that received 10 mg/kg along with LE (300 ug/kg) were not different from the control group. These data extend the generality of enkephalin actions on conditioning to another non-shock motivated task. The partial attenuation by methylnaloxonium suggests that some aspect of the enkephalin effect is mediated by peripheral opioid receptors.

In the one-way active avoidance task mice were placed in the shock compartment of of a two-compartment alley and given 10 s to shuttle into the safe compartment. If no avoidance occurred in 10 s, a shock came on (800 uA), which was terminated when the mouse escaped. Four training trials were given on Day 1 and 14 trials were given on Day 2. The mice were injected with LE (saline 50 or 100 ug/kg, i.p.) or des-tyr-LE (saline 35.4 or 75.7 ug/kg) 5 min before training on Day 2. The number of avoidances the animal made on Day 2 was the measure of performance. LE at a dose of 100 ug/kg impaired acquisition of the avoidance response on Day 2 [$t(54) = 2.64$, $p = .019$]. Des-tyr-LE did not produce any significant effect [$ts < .98$]. This finding suggests that LE produces its effects through an action at opioid receptors, since des-tyr-LE is a non-opioid peptide. (Supported by ONR N00014-83-K-0408).

114.18 INTRASEPTAL β-ENDORPHIN IMPAIRS NOVEL SPATIAL MEMORY. E. Bostock and M. Gallagher, Neurobiology Program and Psychology Department, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514

Recent work from this lab has demonstrated that opiate antagonists facilitate acquisition of novel spatial information. The involvement of the hippocampal formation and it's connections in spatial behavior suggests that opiates may influence novel spatial memory by acting at sites within this neural system. These studies examine the effect of intraseptal β-endorphin (β-end) administration on performance of a previously acquired spatial task and on a task requiring the acquisition of novel spatial information.

In Experiment I male Long Evans rats were trained to visit each arm of a radial 8-arm maze once during a session to obtain food reward. Rats received 1 session/day of training until they achieved criterion performance of no more than 2 errors/session for 3 consecutive sessions. An error was a return visit to an arm that had been previously visited within a session. A within-subject design was used to test the effect of intraseptal injections of 1 μg β-end or vehicle solution on performance. Treatments were administered 10 min. prior to a maze session for 3 consecutive days. Following a 5-7 day interval the alternate treatment was administered. Treatment order was counterbalanced among animals. The total number of errors for each treatment condition was recorded for each rat. Intraseptal β-end injections had no effect on the number of errors made suggesting that intraseptal β-end does not influence performance of a previously acquired spatial task.

In Experiment II rats were trained on the maze as above. During further training a 5 hr. delay was inserted between the 4th and 5th arm choices within a session and training continued until rats achieved criterion performance. Experiments consisted of testing rats on the maze in novel spatial environments. Treatments were administered immediately following the 4th arm choice for the 1st 4 sessions in a new room. Using a within-subject design rats received 500 ng β-end in one room and vehicle treatment in another. Treatment order was counterbalanced among animals. The number of errors and sessions to acheive criterion performance was recorded for each animal in each new room. The results of this experiment reveal that animals commit more errors and require more sessions to acheive criterion performance when they receive β-end treatment. A further experiment revealed that intraventricular injections of β-end do not alter the aquisition of novel spatial information. These findings suggest that intraseptal β-end injections impair the acquisition of novel spatial information in rats and that the septal region may be a site sensitive to the memory altering properties of opiate substances.

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114.19 PASSIVE AVOIDANCE CONDITIONING INHIBITED BY PRETRAINING INJECTION OF LEUPEPTIN. J.L. Davis and R.M. Pico. Aging and Behavioral Biology Res. Lab., VA Med. Ctr., Sepulveda, CA 91343; Dept. of Anatomy, College of Medicine, Univ. of California, Irvine, Irvine, CA 92717.

A one-trial passive avoidance paradigm using young cockerels has been employed in our laboratory to investigate the effects of certain amino acids and peptides (e.g., L-proline and vasopressin) on the avoidance response (Cherkin, A. & Van Harreveld, A., Br. Res., 156, 265-273, 1978; Pico, R.M. & Davis, J.L., IRCS Med. Sci., in press, 1985). Our work with neuroactive peptides has involved the assessment of L-leucyl-L-leucyl-L-arginyl (leupeptin); an inhibitor of proteases, including those known as calpain. Initial results indicated that it inhibited the avoidance response. A recent report described the performance of rats on several memory tasks after receiving leupeptin (Staubli, U., et.al., Behav. Neural Biol., 40, 58-69, 1984), and concluded that memory for delayed spatial alternation and not conditioned avoidance learning is disrupted by this peptide. We have therefore extended our investigation to determine the specificity of leupeptin in avoidance learning by testing it along with two analogues.

Male chicks received intraventricular injections of isotonic saline (N=29), leupeptin (N=30), L-leucyl-L-leucyl-L-leucine (LLL) (N=30) or L-leucyl-L-arginyl (LA) (N=28) 15 min prior to training (200 ug/chick). Each chick was presented with the stimulus probe (a 3-mm steel bead attached to a wire rod) which had been coated with an aversive liquid. After 1-4 pecks at the coated bead, chicks retreat and avoid the probe. Retention of this training is tested 24 hr later by presenting an uncoated test probe for a 10-sec period. The mean peck rate/10-sec (MPR) serves as an index of avoidance conditioning: Saline group MPR= 0.93, 72% pecked at the probe only 1 time or not at all (avoidance ratio: AR): LLL group MPR= 1.36, AR= 70%: LA group MPR= 2.21, AR= 46%: Leupeptin group MPR= 4.10, AR= 10%. These results indicate that leupeptin is the most effective inhibitor of the avoidance conditioning, although LA appears to have 50% of its potency. In light of these findings, we are currently examining the effects of leupeptin and analogues on avoidance conditioning in the rodent.

114.20 PHARMACOLOGICAL INVESTIGATIONS OF THE RELATIONSHIP BETWEEN LUMBAR SPINAL CORD CYCLIC AMP AND THE ACOUSTIC STARTLE REFLEX IN RATS. J.H. Kehne, D.I. Astrachan, J.F. Tallman, and M. Davis, Dept. Psychiat., Yale Univ. Sch. Med., 34 Park St. New Haven, CT 06508

Cyclic AMP has been widely implicated as an intracellular second messenger, though few experiments have shown a direct linkage between cAMP and behavior. In the present study, drugs which increase cAMP levels were infused intrathecally into the subarachnoid space of the lumbar spinal cord in behaving rats and the effects on the acoustic startle reflex were measured.

Dibutyryl cAMP (12.5 - 100 µg), a membrane-permeable and phosphodiesterase-resistant cAMP analog, markedly and dose-dependently increased startle following intrathecal infusion, with peak activation occurring at approximately one hour after infusion. Local infusions of dibutyryl cAMP at more rostral levels of the neuraxis failed to mimic the excitatory effect of lumbar infusion, indicating that the primary site of action was in the lumbar spinal cord. Furthermore, no activation was seen following intrathecal administration of dibutyryl cGMP (50-100 µg). Intrathecal infusions of the precursor ATP, the metabolite 5'-AMP, or cAMP itself, did not substantially alter startle, indicating that extracellular actions or non-specific changes in energy metabolism probably do not account for the observed activation by dibutyryl cAMP.

Forskolin has been shown to be a selective and reversible activator of adenylate cyclase. However, when infused intrathecally, forskolin (5.0 - 100 µg, dissolved in DMSO), had no consistent effect on startle amplitude. In contrast, a water-soluble forskolin derivative, 7-deacetyl-7-O-hemisuccinic acid (forskolin-DHA; 12.5-100 µg, dissolved in artificial CSF) increased startle following intrathecal infusion.

In summary, the excitatory effects of lumbar intrathecal infusions of dibutyryl cAMP and forskolin-DHA indicate a link between spinal cord cAMP and the acoustic startle response. Furthermore, the lack of effect of forskolin relative to forskolin-DHA indicates that the water-soluble derivative is a better pharmacological tool for investigating behavioral correlates of cAMP activation, at least in studies which involve drug administration directly into cerebrospinal fluid. Current studies are investigating the spinal cord receptor system(s) which are linked by cAMP to activation of the startle reflex.

BIOLOGICAL RHYTHMS I

115.1 CORTICAL GRAFTS IN III VENTRICLE OF SUPRACHIASMATIC LESIONED RATS INDUCES RECOVERY OF THE PHOTO PERIOD OF DRINKING RHYTHM+. R. Aguilar-Roblero*, F. García-Hernández*, R. Aguilar*, and R. Drucker-Colín. Departamento de Fisiología, Centro de Investigaciones en Fisiología Celular, UNAM, México 04510, D.F.

We have previously shown that fetal Suprachiasmatic Grafts can restore Diurnal Rhythms in Drinking behaviour in Suprachiasmatic Nucleus (SCN) lesioned adult rats. The present study attempts to establish whether the Drinking Rhythm of grafted animals is an endogenous Circadian Rhythm and also whether the recovery can only be induced by fetal SCN or whether grafts from other brain regions have similar effects.

Once complete abolishment of Drinking Rhythm was achieved by bilateral electrolitic lesions of the SCN of rats, the animals were divided in three groups. Group A, receiving fetal SCN grafts placed on the floor of the III ventricle, Group B, receiving fetal Occipital Cortex grafts placed on the floor of the III ventricle and, Group C receiving fetal Suprachiasmatic Nucleus in a cavity prepared on the Occipital Cortex. All the animals were placed in a room with a 12:12 L-D schedule. Six weeks later continuous recording of Drinking behaviour was carried out during 4 weeks. Illumination schedule was then changed to constant light (L-L), and the recording continued through the next 4 weeks. At the end of the experiment the animals were anestethized and the Brain removed and processed for Histological verification of lesion site and Graft characteristics.

With the L-D schedule both Group A and Group B showed restoration of the Drinking Rhythm, in phase with L-D cycle. Group C, however, did not show any diurnal periodicity in Drinking behaviour. During L-L schedule there was total desorganization of the Rhythm in both Group A and Group B animals, as if animals were SCN lesioned without grafts.

The results confirm previous observation that SCN fetal grafts can restore diurnal Rhythm of Drinking Behaviour in SCN lesioned animals and indicate that the rhythm is passively driven by the L-D schedule. Since Occipital Cortex grafts have similar effects as SCN grafts, the phenomenon seems to be unspecifically induced. This observation can be explained either by proposing that the graft functions merely as a bridge between the retina and other hypothalamic areas, or by proposing that the retinal fibers impinging upon grafter tissue, drives the rhythm either through direct or indirect outputs. The fact that SCN grafts to the Occipital Cortex cavity cannot restore the Diurnal Rhythm in Drinking behaviour suggests that direct Retinal inputs are needed to induce the photoperiod.

+ This work was partially supported by Grant No. PCSABNA-022539 from the Consejo Nacional de Ciencia y Tecnología (CONACyT).

115.2 PERTURBATIONS OF CIRCADIAN RHYTHMICITY INDUCED BY MICROINJECTION OF αBUNGAROTOXIN INTO THE AREA OF THE SCN. J.R. Pauly* and N.D. Horseman* (SPON: R. Quock). Dept. of Biology, Marquette University, Milwaukee, WI 53233.

For skeletal muscle and fish electric organ acetylcholine (ACh) receptors, the binding of αbungarotoxin (αBT) is highly specific to nicotinic sites and is essentially irreversible. This ligand has also been used extensively to identify putative nicotinic cholinergic sites in the central nervous system and those sites have been proposed to be critical to the circadian timekeeping system of mammals. The highest concentrations of hypothalamic nicotinic receptors identified by αBT binding are in the suprachiasmatic nuclei (SCN), putative circadian pacemakers. However, the pharmacology of ACh transmission in the brain is proving to be quite different from more classically defined peripheral ACh systems. The identification of neuronal αBT binding sites and nicotinic receptors is still controversial because in most vertebrate preparations, αBT fails to inhibit neuronal nicotinic transmission. In the SCN, although there are a large number of αBT binding sites, the concentrations of choline acetyltransferase is very low. Therefore it is plausible that these αBT binding sites are important to the circadian system in ways not directly explained by ACH transmission. We have examined the effects of microinjection of αBT into the area of the SCN on circadian locomotor activity rhythms in the rat.

Animals were anesthetized with Chloropent and stainless steel guide cannula assemblies were implanted into the area just dorsal to the SCN. After surgeries, animals were entrained for a period of 2 weeks to LD12:12 and then blinded by bilateral orbital enucleation. Total locomotor activity rhythms were monitored through the use of plexiglass activity monitors. Infusions of αBT (10 µg in a volume of 1 µl) were given at either of two time points. Twelve animals were treated 2 hr after the onset, of the daily active interval. Phase delays were induced in 6 of these animals with a maximum phase shift of about 3 hr. Seven of 11 animals injected with αBT 2 hr prior to the offset of their daily active interval showed phase advances in their locomotor activity rhythms. Maximal response was again about 3 hr. In other cases the injections caused dramatic alterations of free-running period or caused dyschronism (loss of rhythmicity). These results indicate that αBT produces perturbations in circadian rhythmicity that in some cases are similar to those reported for light pulses. Recent studies in our lab have demonstrated that depletion of brain ACh stores does not alter the phase-shifting effects of light. This suggests that the previously hypothesized roles of ACh may not be correct. In light of the results presented here, it seems plausible that the effects of carbachol injections could be mediated through activation of αBT binding sites. These discrepancies may be resolved by comparing the effects of cholinergic agents of varying specificities.

115.3 THE DEVELOPMENT OF THE SUPRACHIASMATIC NUCLEUS IN AN EMBRYONIC EXPLANT SYSTEM. M. H. Roberts, M. F. Bernstein and R. Y. Moore, Depts. of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794

The suprachiasmatic nucleus (SCN) of the hypothalamus has been implicated as a controlling pacemaker in the rodent circadian system. Anatomically, the nucleus can be divided into ventrolateral and dorsomedial portions based upon the area of termination of the direct retinohypothalamic tract (RHT), a secondary visual projection from the intergeniculate leaflet, the geniculohypothalamic tract (GHT), and upon the distribution of distinct, peptide containing neurons. Vasoactive intestinal polypeptide (VIP) immunoreactive cell bodies and fibers are found in the ventrolateral subdivision. Vasopressin (VP) immunoreactive cell bodies and fibers are found in the dorsomedial subdivision. The VP positive fibers also extend ventrally to form a capsule around the region of VIP immunoreactivity and the zone of RHT and CHT termination. The current study addressed the question of whether the SCN will develop when isolated from its normal environment.

Embryonic hypothalamic explants were placed in the anterior chamber of the eye of young adult rats. Embryonic day 13 explants allowed to develop for 14 or 30 days did not contain structures identifiable as SCN, but after 45 days, explants contained SCN-like cell groups, which appeared as dense, roughly elipsoid masses of tightly packed, small cells (10-15um) in Nissl stains. Immunohistochemistry showed a group of VIP positive cells and fibers that were in close apposition to a group of VP positive cells and fibers. The VP fibers extended in a cap around the region of VIP immunoreactivity in a pattern quite similar to that seen in the normal rat SCN. The SCN-like structure was closely apposed to a bud of cartilage. After 60 days of development, one explant also contained an SCN-like structure. In this case the VP staining was lighter and only partly surrounded the region of VIP immunoreactivity. In addition, there was no adjacent piece of cartilage.

Explants taken from E14 embryos also showed the development of an SCN-like structure. This was not evident at 45 days, but at 60 days, all explants contained SCN-like neuronal groups which contained dense VIP immunoreactivity adjacent to a region of VP immunoreactivity. Explants taken from older embryos (E15-16) demonstrated distinct fields of differentiated neurons which did not exhibit either VP, VIP or LHRH immunoreactivity and did not contain discrete identifiable nuclei.

These data can be summarized as follows: 1) the SCN will develop in an in vivo explant system; 2) the nuclei differentiate normally as expressed by the organization of VP and VIP containing neurons; 3) the cells of the germinal epithelium that give rise to the SCN are committed to form the nuclei as early as day 13 of embryonic development. Supported by USPHS Grant NS17600.

115.4 IN VITRO SUPRACHIASMATIC NUCLEUS RHYTHM FOR UPTAKE OF 14C-2-DEOXYGLUCOSE. G. Newman, P. Wu* and F. Hospod*. Department of Neurology, SUNY, Stony Brook, New York 11794.

The suprachiasmatic nucleus (SCN) appears to contain the endogenous circadian rhythm generator in mammals. In vivo and in vitro studies reveal high SCN neuronal firing rates during (subjective) day and low rates at night. 14C-2-deoxyglucose (2DG) uptake into the SCN in vivo also is high in the day and low at night. However, further insight into the biochemical mechanism of circadian rhythm generation is inhibited by the inaccessibility of the in vivo SCN and lack of an in vitro biochemical model.

Rats were maintained in a 12:12 light-dark cycle for 3 weeks. After decapitation, 400μ slices were prepared from hypothalamus and incubated in a chamber designed for biochemical and morphological studies of slice preparations (see Abstract by Hospod et al). After incubation the slices were exposed to 14C-2DG (0.5μCi/ml), rinsed, cryostat-sectioned to 20μ and developed 1 week on x-ray film. Sections with SCN were identified by Nissl staining. Densitometry measurements were made in a "blind" manner for 3 SCN regions and adjacent hypothalamus, 1mm from the SCN, (AHT) throughout the length of the SCN.

In the first experiment, slices were incubated for 1 hour and than exposed to 14C-2DG for 6 minutes. All middle-SCN sections from 3 rats were used for each time point. Slices exposed to 14C-2DG at CT0300 and CT0900 (lights on at CT0000) showed SCN radioactivity 75% greater than AHT. The SCN of slices exposed at CT1600 and CT2000 were less than 30% and those at CT2300 were 37% above AHT.

In the second experiment, 3 rats were sacrificed at CT1900 with exposure to 14C-2DG for 6 minutes at CT0300 and 3 were sacrificed at CT0800 with 14C-2DG exposure at CT1600. Thus radioactive exposure occurred in the phase opposite sacrifice. Slices exposed at CT0300 showed SCN radioactivity 75% above AHT and SCN of slices exposed at CT1600 were 18% of AHT. Microscopic results are presented in our companion abstract.

These results demonstrate that the SCN, in an in vitro hypothalamus slice preparation, continues to take up 2-deoxyglucose consistent with the circadian phase of the sacrificed animal even when incubation times cross phase transitions. Data is being analyzed to identify regional differences. Biochemical studies of protein synthesis and phosphorylation, ion concentrations and cyclic nucleotides are in progress.

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115.5 CIRCADIAN PATTERNS OF VASOPRESSIN SECRETION AND SCN NEURONAL ACTIVITY IN VITRO. Steven M. Reppert and Martha U. Gillette Children's Service, Massachusetts General Hospital Boston, MA 02114 and Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61810.

The nonapeptide arginine vasopressin exhibits a prominent circadian rhythm in the cerebrospinal fluid (CSF) of numerous mammals. Brain lesion studies in rats show that the hypothalamic suprachiasmatic nuclei (SCN), the site of a circadian pacemaker, are necessary for the generation of the CSF rhythm. Since the SCN are capable of synthesizing vasopressin, the nuclei may be the actual source of the CSF vasopressin rhythm. We investigated this by examining the pattern of vasopressin release in vitro from perifused hypothalamic slices in which the SCN show a rhythm in single unit firing rate.

Adult male Long-Evans rats housed in diurnal lighting (12 hr light per day) were sacrificed, their brains were removed, and the hypothalami were blocked and cut into 400 um sections. Slices (n=3) containing only SCN (and not supraoptic and paraventricular nuclei) were put into a chamber that was perifused with Earl's balanced salts medium supplimented with Na_2CO_3 and glucose and bubbled with 95% O_2 and 5% CO_2. Single unit firing rates were recorded and analyzed over a 30-hr period (Gillette, this volume). The perifusate was collected as 4-hr fractions, and each fraction was acidified and passed through a C 18 Sep-Pak column. Vasopressin was eluted off the column, the effluent was dried, and the residue was reconstituted in assay buffer. Vasopression concentrations were determined by RIA.

Both vasopressin release and firing rates manifested clear daily rhythms. The rhythms expressed proper circadian timing based on the circadian time of the donor animal; that is the peak amplitude of both rhythms occurred during what would have been the daytime (subjective day) of the donor animal. The phase of the vasopressin rhythm was close to that expected in CSF with levels rising before lights on and peaking 4 to 8 hr into the subjective day. Interestingly, the peak in vasopressin secretion preceded the peak in single unit firing rate.

The results suggest that the SCN provide both the timekeeping information and secretory stores of vasopressin that appear in the CSF. The demonstration of two phase distinct oscillatory patterns raises interesting questions about possible interrelationships between neuronal electrical activity and vasopressin secretion.

115.6 CIRCADIAN RHYTHMS OF VASOPRESSIN RELEASE FROM PERIFUSED RAT SUPRACHIASMATIC EXPLANTS IN VITRO. D.J. Earnest and C.D. Sladek. Depts. of Anatomy and Neurology, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Numerous studies have established that the suprachiasmatic nucleus (SCN) of the anterior hypothalamus is an important component of the circadian system in mammals. Consistent with its role in the generation of a variety of physiological and behavioral rhythms, recent work suggests that intrinsic properties of the SCN oscillate on a circadian basis. In this regard, we have demonstrated that vasopressin (VP), which constitutes a major peptidergic component of the rat SCN, is released in a circadian fashion for at least four cycles from SCN explants in static organ culture. Using a perifusion culture technique, the present study was designed to extend these findings and to develop a system capable of providing better resolution of the waveform of the VP rhythm.

Suprachiasmatic explants were excised from the brains of decapitated male Sprague-Dawley rats (125-150g) that had been maintained on LD 12:12. The explants consisted of tissue blocks approximately 1mm on a side and included only the paired suprachiasmatic nuclei, their rostral projections to the organum vasculosum of the lamina terminalis and the underlying optic chiasm. Each of these preparations was cultured in a polysulfone chamber in constant darkness and perfused with medium at a constant flow rate (i.e., 0.25 or 0.4ml/hr). Following a period of equilibration, serial samples of the perfusate were collected at 2hr intervals for four days. VP concentration in the medium was determined by radioimmunoassay.

The total daily VP output from the SCN explants remained constant throughout the four-day sampling period; individual explants released an average of 120pg of VP per 24hr. However, VP concentration in the perfusate fluctuated rhythmically during the course of a day; the VP rhythm, which persisted for 2-3 cycles, was characterized by peak levels of release during the subjective day and minimal levels during the subjective night (3- to 5-fold difference). Importantly, the rhythmic pattern of vasopressin release was quite uniform among individual SCN explants; the daily increase in vasopressin release began late in the subjective night while the decrease occurred near the middle of the subjective day. These results indicate that the rhythm of vasopressin release from individual perifused rat SCN explants is similar to that observed with static organ cultures and thus provide a basis for utilizing the perifusion culture technique to examine the biochemistry and physiology of circadian oscillators in mammals.

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115.7 OSCILLATION OF CYCLIC NUCLEOTIDE AND MELATONIN RELEASE IN THE DYNAMIC CULTURE OF AVIAN PINEAL. S.S. Nikaido* and J. S. Takahashi (SPON: D. Ferster). Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.

The avian pineal gland expresses a circadian oscillation of serotonin N-acetyltransferase activity and of melatonin release in vitro. In addition, light-dark cycles can entrain the rhythm of melatonin release in culture. Previous studies, in which drugs were applied and cyclic nucleotide and enzyme levels were measured in vitro, have suggested that cyclic nucleotides may be involved in the regulation of the enzyme, serotonin N-acetyltransferase. Measurement of cyclic AMP and cyclic GMP levels in cultured chick pineal glands has shown a reduction in the levels of both nucleotides with light exposure of the glands. The reduction of cyclic AMP and cyclic GMP levels by light preceded the reduction of serotonin N-acetyltransferase activity by light. Pharmacological experiments have strongly suggested that regulation of this enzyme occurs with changes in cyclic AMP rather than cyclic GMP.

To determine whether or not cyclic AMP levels oscillate in a light-dark cycle, chick pineal glands were cultured in a flow-through superfusion system. Both melatonin and cyclic AMP were measured by radioimmunoassay from samples collected every two hours over a 86-hour period. In this dynamic culture system, chick pineal glands expressed an oscillation of cyclic AMP release in a 12:12 LD cycle. The cyclic AMP oscillation is in phase with the entrained rhythm of melatonin release also seen in the organ culture. Levels of cyclic AMP release vary from 500 fmoles of cyclic AMP per hour during light to 2000 fmoles of cyclic AMP per hour during darkness. Addition of isobutylmethyl xanthine (IBMX) to the superfusion medium resulted in a loss of the rhythm of melatonin release. However, a rhythm of cyclic AMP release was sustained at levels which were approximately ten times the levels seen without IBMX. Preliminary experiments show that the oscillation of cyclic AMP release persists for up to three cycles in constant darkness.

Although a clear relationship between cyclic nucleotide levels and serotonin N-acetyltransferase activity has not been established, the concurrent oscillations of cyclic AMP and melatonin release suggests that cyclic AMP has a role in driving the circadian rhythm of melatonin release. The mechanisms governing the oscillation of cyclic AMP are not known, but they should be tractable to experimental analysis with currently available methods.

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115.8 THE SUPRACHIASMATIC NUCLEI (SCN) OF THE CAT AND GUINEA PIG. J. Speh*, V.M. Cassone and R.Y. Moore (Spon. A. Rosen). Departments of Neurology and Neurobiology and Behavior, SUNY @ Stony Brook, Stony Brook, New York, 11794.

The SCN is a circadian pacemaker in the mammalian brain (Moore, '83). In the rat (Moore and Card, '85), hamster (Card and Moore, '84) and monkey (Moore and Card, '82), the SCN has a distinctive organization. Visual afferents from the retina, the retinohypothalamic tract (RHT), and from the intergeniculate leaflet of the thalamus (GHT), terminate in the ventrolateral subdivision of the SCN, an area that also contains a large population of vasoactive intestinal polypeptide (VIP)-immunoreactive neurons. In contrast, the dorsomedial division of the SCN receives no direct visual input and contains a large population of vasopressin (VP) immunoreactive neurons. Glutamic acid decarboxylase (GAD)-immunoreactive neurons are present throughout the SCN.

The present study was carried out to determine whether the organization of the SCN in two crepuscular mammals, the guinea pig and cat, is similar to that in the species noted above. The distribution of the RHT was shown by the autoradiographic tracing method following injection of tritiated proline into the eye. The distribution of the GHT, a neuropeptide Y (NPY)-containing pathway, was analyzed by the immunohistochemical demonstration of NPY. The distribution of VIP, VP and GAD-containing neurons was also shown by immunohistochemistry.

As in other mammals, the RHT and GHT in the guinea pig and cat terminate selectively within a subdivision of the SCN which is distinguished by a dense packing of neurons and the presence of a large population of VIP-immunoreactive cells. The VIP neurons are restricted to the portion of the nucleus receiving visual input and give rise to an axonal plexus which extends throughout the SCN and dorsally through the anterior hypothalamic nucleus to terminate at the ventral border of the paraventricular nucleus. Although there appear to be fewer VP-immunoreactive neurons in the guinea pig and cat SCN than are apparent in the rat or monkey, these cells are confined to the dorsomedial aspect of the SCN. Axons exhibiting VP-like immunoreactivity are similar to the VIP immunoreactive plexus in that fibers not only arborize throughout the SCN, but also project dorsally to terminate along the ventral border of the paraventricular nucleus. Neurons exhibiting GAD-like immunoreactivity occur in both SCN subdivisions and give rise to an extremely dense axonal plexus which is continuous into the immediately adjacent periventricular nucleus.

These data reiterate the consistency in organization of the SCN in divergent mammalian species and also provide evidence for two functionally distinct subdivisions of this nucleus. Supported by NIH grant NS-16304.

115.9 MAGNOCELLULAR NEURONS ADJACENT TO THE SCN ARE LABELLED BY INTRAVENOUS INJECTION OF HRP. T.G. Youngstrom and A.A. Nunez. Dept. of Psychology and Neuroscience Program, Michigan State University, E. Lansing, MI. 48824.

Studies of the retino-hypothalamic tract (RHT) in mammalian species utilizing horseradish peroxidase (HRP) have reported reaction product (RXP) in regions dorsal and lateral to the boundary of the suprachiasmatic nuclei (SCN). The SCN have, to date, been the only hypothalamic nuclei reliably reported as receiving RHT fibers. Intravenous (I.V.) application of HRP was used to determine the likelihood of HRP so transported, contributing RXP to that observed within or just outside the SCN after intraocular injections of the enzyme. Adult males of three species (house mouse-Mus musculus; deer mouse-Peromyscus maniculatus bairdi; white-footed-Peromyscus leucopus) were injected with 9 µl of 50% (w/v) HRP (Sigma Type VI) in physiological saline. Survival periods of 1 to 24 hr were used. Brains were prepared for histological examination using the chromogen 3,3',5,5'-tetramethylbenzidine. In all cases of 4 hours or greater survival, a large number of magnocellular neurons within the supraoptic and paraventricular nuclei were labelled with RXP, and RXP was also seen in a few magnocellular neurons immediately adjacent to the third ventricle. Compared to those of house mice, brains of deer and white-footed mice contained more labelled magnocellular units immediately dorsal-lateral to the SCN , and in lateral hypothalamus. These anatomical differences may be related to species differences in response to changes in photoperiod. The present results indicate that following intraocular injections, some of the RXP detected dorsal and lateral to the SCN previously interpreted as originating from the retinal ganglion cells may be the result of vascular transport of HRP. The presence of labelled cells near SCN after IV injections suggest a mechanism by which SCN neurons may affect or be affected by circulating blood constituents. (Supported by NIMH Grant MH 37877 to A.A.N.)

115.10 THE DEVELOPMENT OF CYTOCHROME OXIDASE ACTIVITY WITHIN THE SCN OF THE CAT. D. M. Murakami* and C. A. Fuller. Dept. of Animal Physiology, University of California, Davis, CA 95616.

Previously we have demonstrated the presence of a high metabolic region within the ventral suprachiasmatic nucleus (SCN) of the rat and cat (Fuller et al., Soc. Neurosci. Abstr. 10:503, 1984). This region of high activity in the cat was confined to those corinal sections containing the middle portion of the ventral SCN. Anterior and posterior portions of the SCN did not exhibit metabolic differences between dorsal and ventral regions. The dorsal region from the middle portion of the SCN and the anterior and posterior portions of the SCN exhibited significantly less metabolic activity than the surrounding medial and lateral hypothalamic (LH) areas.

In this study we have examined the development of metabolic activity within the SCN using the cytochrome oxidase (CyOX) method. Coronal sections through the SCN were taken from cats two weeks of age through adulthood. One series of sections was stained with thionin to reveal the nuclear boundaries. Alternate sections were stained for CyOX in order to reveal the metabolic activity within the SCN at the various stages of development. A microdensitometer was used to quantify the intensity of CyOX staining within the various regions of the SCN and for comparison in the lateral hypothalamus.

From 2 to 4 weeks of age there are no dorsal-ventral differences in CyOX staining within the SCN. However, between 4 and 6 weeks of age there is a dramatic increase in CyOX staining within the ventral SCN. This increase in metabolic activity is confined to the middle portion of the SCN, while anterior and posterior portions do not exhibit dorsal-ventral differences. From 2 to 6 weeks of age the anterior and posterior portions and the dorsal region from the middle portion of the SCN are equal in CyOX staining to the LH. However, after 6 weeks of age these areas of the SCN gradually decrease in CyOX staining until they stain significantly less intensely than the LH.

This study has shown that, within the middle portion of the SCN, the ventral region is a critical locus of high metabolic activity that matures dramatically between 4 and 6 weeks of age. This ventral SCN region is known to be the primary receiving area for afferent input, and the change in metabolic activity may reflect the maturation of these afferents. A decrease in metabolic activity in the other regions of the SCN appears to correlate with an extended period of neural development.

115.11 THE SUPRACHIASMATIC NUCLEUS OF THE ADULT MACAQUE: AN ULTRASTRUCTURAL ANALYSIS. S.L. Dewey, J.P. Card & R.Y. Moore. Departments of Neurology and Neurobiology and Behavior, SUNY @ Stony Brook, Stony Brook, New York, 11794.

The suprachiasmatic nucleus (SCN) of the rat and hamster (Moore, '83; Card and Moore, '84) is characterized by subdivisions which are distinguished by the differential termination of visual afferents and the location of immunohistochemically distinct neurons. Visual afferents arising from the retina and lateral geniculate nucleus terminate in the ventrolateral (VL) aspect of the nucleus, an area containing vasoactive intestinal polypeptide immunoreactive neurons. The dorsomedial (DM) SCN receives no direct visual input and contains vasopressin immunoreactive neurons. Ultrastructural studies of the rat SCN have shown that neurons in these subfields are also morphologically distinct.

The monkey SCN also contains subdivisions which can be distinguished using the same criteria established in rodents (Moore & Card, '82). In the present study the subdivisions of the macaque SCN were studied with transmission electron microscopy to determine if homologies also exist in the ultrastructural organization of the monkey and rodent SCN. As in the rat, this analysis revealed at least two morphologically distinct populations of neurons. The compact, retinal recipient portion of the SCN contains spherical to slightly oval neurons which exhibit multiple invaginations of the cell nucleus and an organelle rich cytoplasmic matrix. These cells have few axo-somatic synaptic contacts and are consistent in morphology with the neurons in the VL subdivision of the rat SCN. This portion of the nucleus is also distinguished by the presence of numerous axon terminals which contain lucent mitochondria, large numbers of lucent spherical vesicles and occasional dense core vesicles. These terminals synapse upon distal dendrites and spines and their morphology is consistent with that previously reported for retinal afferents in the monkey lateral geniculate nucleus and the SCN of other species. The second class of morphologically distinct neurons are confined primarily to the area of the SCN surrounding the compact cell mass. Cells in this subdivision are bipolar and are characterized by occasional fingerlike invaginations of the cell nucleus. Organelles are concentrated at the poles of the cell and are not nearly as numerous as in the first cell type. The morphology of this cell type is consistent with that reported for neurons in the DM aspect of the rat SCN.

Taken together, these data support previous conclusions regarding parcellation of the macaque SCN and emphasize the consistency in the organization of this nucleus in divergent mammalian species. Material was kindly provided by Dr. Anita Hendrickson, University of Washington Regional Primate Center. Supported by HIH grants NS-16304 and NS-19714.

115.12 MAGNOCELLULAR NEUROSECRETORY AXONS TERMINATING IN THE THIRD VENTRICLE OF THE GOLDEN HAMSTER BRAIN: ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION. J.A. Mitchell and J.P. Card. Department of Anatomy, Wayne State University, Detroit, Michigan 48201 and Department of Neurology, SUNY @ Stony Brook, Stony Brook, NY.

Several investigations have established that vasopressin is present in the cerebrospinal fluid (CSF) and that levels of the peptide exhibit circadian fluctuations which are entrained to the light-dark cycle. In the present study we have utilized light microscopic immunohistochemistry and transmission electron microscopic analysis to determine the location of neurons in the golden hamster brain which secrete vasopressin into the CSF and to define the neural circuitry which modulates its circadian fluctuation in the CSF. Vasopressin-containing neurons are concentrated primarily within the suprachiasmatic (SCN), paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. Consequently, we employed immunohistochemical localization of vasopressin to determine if neurons in any of these nuclei gave rise to processes which penetrated the ependymal lining to terminate in the ventricular lumen. Since the SCN is known to be the primary neural locus responsible for the control of circadian rhythmicity our initial efforts were concentrated on this nucleus. Systematic analysis of closely spaced sections through the SCN demonstrated large numbers of parvocellular vasopressin neurons in the dorsomedial aspect of the SCN. These cells gave rise to an extensive plexus of thin, varicose axons which arborized within the SCN and also projected dorsally to terminate at the ventral limit of the PVN. However, immunoreactive fibers arising from the SCN neurons never entered the ventricular lumen. This was also true of the PVN and SON; large numbers of immunoreactive cells and fibers were present in both nuclei, but processes never entered the ventricle in the vicinity of either cell group. In contrast, magnocellular axons in the median eminence (ME), which arise from neurons in the PVN and SON, were routinely observed entering the CSF through the floor of the third ventricle. Ultrastructural analysis of the ME confirmed the immunohistochemical observations. Large magnocellular axons containing massive accumulations of large, dense core vesicles penetrated the ependymal lining of the ventricle floor and terminated within the CSF. The SCN is known to project to both the PVN and SON and we therefore suggest that while magnocellular axons arising from neurons in one or both of these nuclei account for secretion of vasopressin into the CSF, the circadian release of the peptide into the CSF is controlled via the projection of the SCN to these nuclei. Supported by RR-05387 (JAM) and NS-19714 (JPC).

OPIATES, ENDORPHINS AND ENKEPHALINS: BIOCHEMICAL CHARACTERIZATION

116.1 PURIFICATION OF ENKEPHALINS AND ENKEPHALIN-CONTAINING PEPTIDES ON BIO-REX 70. S.P. Wilson. Department of Pharmacology, Duke University Medical Center, Durham, NC 27710.

During studies on enkephalins and enkephalin-containing peptides (ECPs) in adrenal medullary chromaffin cells, a technique for partial purification of the peptides before further analysis was required because a number of endogenous and exogenous substances interfere with the enkephalin radioreceptor assay employed. In addition, removal of contaminating peptides and other small molecules before analysis by high performance liquid chromatography was desired. Chromatography on the acrylic ion-exchange resin Bio-Rex 70 proved to be a convenient technique for the partial purification of ECPs.

Bio-Rex 70 was prepared by washing the resin sequentially with 3 M HCl, 3 MNaOH, and 3 M acetic acid and was finally resuspended in 0.1 M acetic acid. Columns of the resin (0.25 cm^3 bed) were prepared in Pasteur pipets plugged with a small amount of glass wool and were washed with 0.1 M acetic acid. The sample was applied to the column in 0.1 M acetic acid (0.5-5 ml) and the column was washed with two 1-ml aliquots of 0.1 M acetic acid. The peptides were eluted from the resin with 2 ml of 1 M acetic acid in methanol. This peptide-containing solution was evaporated to dryness and resuspended in a small volume of 1 M acetic acid. Recoveries of peptide standards, including leu-enkephalin, met-enkephalin (ME), ME sulfoxide, ME-arg^6, ME-arg^6-phe^7, and ME-arg^6-gly^7-leu^8 and of both small and large molecular weight ECPs derived from the adrenal medulla were typically greater than 90%. Losses of ECPs when low amounts of the peptides were applied to the columns could be prevented by preadsorbing 0.5 mg of insulin to the resin bed. Catecholamines and other substances such as the gradient density media sucrose and Renografin were not retained by the resin under the conditions employed. A significant removal of protein and other material with absorbance at 280 nm was also obtained when adrenal medullary extracts were chromatographed. Bio-Rex 70 was superior to other chromatographic media that have been employed for enkephalin purification including Amberlite XAD-2, Porapak Q and Sep-Pak C_{18} cartridges. Columns of Bio-Rex 70 are relatively inexpensive, especially when compared to products such as Sep-Pak cartridges. Hence, this chromatographic technique should prove useful where preliminary purification of peptides derived from proenkephalin A is required and may be adaptable to other peptides as well.

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116.2 METABOLIC PROFILING OF NEUROPEPTIDES BY USE OF RP-HPLC, RECEPTOR ASSAY, RIA, AND MASS SPECTROMETRY. D.M. Desiderio[1,2], F.S. Tanzer*[3], G. Eridland*[2], H. Onishi*[2], H. Takeshita*[2], C. Dass*[2], P. Tinsley*[2], J. Killmar*[2], and G. Wood*[4,5]. [1]Dept. of Neurology, [2]Charles B. Stout Neuroscience Mass Spectrometry Labortory, [3]Dept. of Biologic and Diagnostic Sciences, [4]Dept. of Orthopedics, Univ. Tenn. Center for the Health Sciences, Memphis, TN, 38163; [5]Campbell Clinic, Madison, Memphis, TN 38103.

This research aims towards developing a method to obtain the metabolic profile of endogenous peptides in a biologic matrix. This metabolic profile is used to determine in normal and stressed situations the relationship among several opioid peptidergic families (enkephalinergic, endorphinergic, dynorphinergic, and substance P-ergic) and the individual peptide constituents. These experimental techniques are used to examine peptide-rich fractions prepared from extracts of biologic tissues (brain, pituitary, tooth pulp) and fluids (CSF).

Gradient reversed phase-HPLC is used with acetonitrile as organic modifier and a volatile triethylamine formate buffer to separate individual peptides in a fast and facile manner with high resolution and high recovery. Ninety fractions are collected from the gradient. A canine limbic system (P_2 or synaptosome) preparation is used for the radioreceptor assay where several tritiated ligands are competitively displaced. Radiolabeled etorphin, dihydromorphine, ethylketoazocine, and substance P are used. Commercial RIA kits are used to measure met-enkephalin, leu-enkephalin, and substance P. Fast atom bombardment mass spectrometry is utilized as an objective analytical method because MS offers the highest level of molecular specificity. FAB-MS produces the protonated molecular ion of a peptide, a parameter useful for determining the presence of a particular peptide. However, because of the polymer problem (n amino acids could be linked into n! peptides), amino acid sequence-determining information must also be obtained by increasing the structural resolution of the MS method. Linked-field scanning in the B/E mode is useful to provide amino acid sequence-determining information and for use of quantification of the peptide utilizing an ^{18}O-peptide internal standard.

Results will be presented on the use of these methods for measuring the peptide content in brain regions, pituitary tumors, tooth pulp (controls and stressed), and cerebrospinal fluid (lower back pain patients) and for metabolic profiling of several peptidergic pathways. Work supported by NIH GM 26666.

116.3 RESERPINE-INDUCED ALTERATIONS IN PROENKEPHALIN PROCESSING: INCREASED AMIDATION. I. Lindberg, Dept. of Biochemistry, Louisiana State Univ. School of Medicine, 1100 Florida Ave., New Orleans, LA. 70119

Reserpine treatment of cultured bovine adrenal chromaffin cells has been shown to increase the production of low molecular weight met^5-enkephalin-immunoreactive peptides (Eiden et al, PNAS 81, 3439, 1984). We have used antisera directed toward six different portions of the proenkephalin molecule to examine the processing rates and patterns of proenkephalin-derived peptides in chromaffin cell cultures. Gel filtration was used to separate multiple molecular weight forms of immunoreactive peptides. Reserpine (10^{-6}M, three days) increased the production of not only low molecular weight met^5-enk, but also met^5-enk-arg^6-gly^7-leu^8, met^5-enk-arg^6-phe^7, and leu^5-enk in a parallel fashion. Immunoreactivity corresponding to intermediate-sized enkephalin-containing peptides (such as the 5.3 kDal peptide and Peptide B) was decreased, supporting the notion that increased processing of these intermediates occurs in the presence of reserpine. These results suggested that reserpine is able to increase the general activity of trypsin-like enzymes within the chromaffin granule. We therefore investigated the effect of reserpine treatment on the production of two amidated opioid peptides in order to ascertain whether activation of amidation also occurred in the presence of reserpine. Reserpine treatment produced profound effects on the levels of metorphamide and amidorphan; these peptides increased ten and five-fold, respectively, after three days of treatment. The effect on the amidated peptides was rapid, with a 70-80% increase occurring within the first 12 hours after exposure to reserpine. Only one molecular weight form of each amidated peptide was observed in control and treated cultures. The ratio of two Peptide E-derived peptides, leu^5-enk and metorphamide, decreased from 50 to 8 in response to reserpine treatment, reflecting a disproportionate increase in the content of metorphamide. These results indicate that activation of amidation occurs in the presence of reserpine, and suggest that reserpine may be capable of altering not only the extent but also the pattern of proenkephalin processing.

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116.4 **PROJECTION-SPECIFIC PROCESSING OF PROENKEPHALIN IN THE RAT CNS.** J.D. White, C.M. Gall[1] and J.F. McKelvy, Dept. of Neurobiology and Behavior, SUNY Stony Brook, NY 11794 and [1]Dept. of Anatomy, U.C. Irvine, CA 92717

Several recent studies have suggested that post-translational proteolytic processing of the precursor proteins to the opiate peptides occurs in a tissue-specific and, perhaps, a projection-specific manner. The implications of these findings are that the prohormones contain an array of potential biologically active peptides and that different neuron systems differentially use specific peptides to facilitate intercellular communication. The present studies were designed to directly test this hypothesis using dynamic measurements in three distinct, anatomically well-defined, enkephalin projections in the rat brain.

The biosynthesis and processing of proenkephalin was studied in vivo using our previously described methods (White et al., J. Neurosci., 4:1262), i.e. radiolabeled amino acid was delivered to the cell bodies of interest through stereotaxically implanted cannulae using an osmotic mini-pump delivery system. Radiolabeled peptides were harvested from the terminal fields and purified to constant radiochemical specific activity, in the presence of carrier peptides, through three sequential HPLC steps and two chemical modification steps. For these studies we chose to examine the biosynthesis of six Met-enkephalin containing peptides: Met^5-, Met^{5}Arg^{6}Gly^{7}Leu^8-, Met^{5}Arg^{6}Phe^7-enkephalin (MENK, MERGL, MERF), metorphamide, Peptide E and BAM 18 P. The three projection systems we studied were the caudate putamen to the globus pallidus, the paraventricular nucleus of the hypothalamus to the median eminence and the mossy fiber system of the granule cells of the hippocampus. In all cases 500 µCi of ^{35}S-Methionine was delivered over a 4 hour period followed by artificial extracellular fluid for an additional two hours to allow for synthesis, processing and transport of the peptides to the terminal field. To facilitate comparison of the data between animals and projection systems, the amount of each peptide was expressed as a ratio of radioactivity with the amount of radioactivity present in MERF for each animal arbitrarily set equal to one.

We were able to reach several conclusions regarding proenkephalin processing using this approach. (a) Neither metorphamide nor Peptide E contributed substantially to the pool of Met-enkephalin peptides in any of the three terminal fields. In all cases the amount of radioactivity present in these peptides was less than 10% of that present in MERF. (b)The ratio of MENK:MERGL:MERF was virtually identical in the hypothalamic and hippocampal projections, i.e. 3.4:0.5:1. (c) This ratio was significantly different in the striatal projection, i.e. 4.3:0.9:1. (d) Both the hypothalamic and hippocampal projections produced substantial amounts of BAM 18 P, roughly equal to the molar amount of MERGL, however, this peptide was virtually absent in the globus pallidus, i.e. less than 10% of MERF.

These data demonstrate that projection-specific processing of proenkephalin occurs in the rat CNS. Furthermore, the data demonstrate that the post-translational processing of proenkephalin is not solely directed by the location of paired basic amino acids. We are currently investigating the processing of proenkephalin in the mossy fiber system during hippocampal seizures in a model in which enkephalin biosynthesis dramatically increases to investigate the coordination of enkephalin synthesis and processing. Additionally, we are exploring the mechanisms by which this diversity of enkephalin-containing peptides is generated in these projection systems. Supported by NIH NS 20372 and NSF BNS 8111475 (JFM) and NSF BNS 8200319 (CMG).

116.5 AGONISTS INHIBITORY TO ADENYLATE CYCLASE STIMULATE PROENKEPHALIN SYNTHESIS IN NG-108 CELLS. Joan P. Schwartz. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

The neuroblastoma-glioma hybrid cell line NG-108 contains three receptors which are coupled to the adenylate cyclase (AC) in an inhibitory mode, the opiate, the α_2-adrenergic and the muscarinic receptors. Growth of the cells in the presence of an agonist for any one of these receptors has been shown to result in an initial inhibition of AC and decrease of cyclic AMP content, which is followed by an increase in AC activity such that the cyclic AMP content is restored to control levels. Recent reports have demonstrated that the cells contain enkephalin peptides (Glaser et al., Eur. J. Pharm. 65: 319, 1980) and that their content is regulated by cyclic AMP (Braas et al., J. Neurosci. 3: 1713, 1983). Using a cDNA probe for proenkephalin (PE), we have detected the PE mRNA in the cells and find that it has the same size, 1400 bases, as has been found in brain and adrenal. Growth of the cells in 10^{-7}M etorphine had no effect on the content of PE mRNA for the first three days: by day 5, the content had increased 2-3 fold. Morphine has the same effect and the action of both drugs is blocked by naloxone. The EC_{50} for etorphine is ~1 nM, similar to that for morphine. Although both etorphine and morphine can inhibit forskolin-stimulated AC 50% acutely, an effect which is also blocked by naloxone, neither opiate agonist changes the cyclic AMP content of the cells following a five-day treatment period. Exposure to either 10^{-6}M carbachol or 10^{-7}M clonidine inhibits forskolin-stimulated AC activity (over a 30 minute incubation) and leads to a 50-100% increase of PE mRNA after five days exposure. Forskolin ($5x10^{-6}$M) alone, which produces a 10-20 fold increase of cyclic AMP content, also increases PE mRNA 2-3 fold, a response which is not additive with that of etorphine. The results therefore suggest that PE synthesis in NG108 cells can be regulated by two different mechanisms, one involving cyclic AMP while the other, yet to be determined, is common to opiate, muscarinic and α_2-adrenergic receptor stimulation. The two mechanisms may converge prior to stimulation of PE mRNA content in NG-108 cells.

116.6 Ca^{++} CHANNEL ANTAGONISTS ALTER MORPHINE HYPERTHERMIA. N.P. Pillai* and D.H. Ross. Div. Molecular Pharmacology, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

The action of opiates on body temperature has been shown to depend on both dose and route of administration. Morphine (15 mg/kg, s.c.) produces hyperthermia (30 - 120 min) in unrestrained rats, while producing biphasic responses in restrained rats. Opiates in vitro and in vivo have been shown to alter Ca^{++} content, binding and transport, and Ca^{++}-dependent transmitter release from synaptosomes. These effects correlate well with analgesia tolerance and physical dependence. Temperature regulation has previously been shown to be affected by hypothalamic movement of Ca^{++} ions and it is believed that Ca^{++} ions may act to control the temperature set point in this specific brain region. Ca^{++} antagonists such as lanthanum and EGTA are known to block the effect of Ca^{++} in reversing opiate analgesia. We have therefore studied the effects of organic Ca^{++} channel antagonosts, the 1,4-dihydropyridines, on morphine hyperthermia and Ca^{++}-ATPase activity in brain regions and subcellular fractions. Morphine (15 mg/kg, s.c.) produced hyperthermia (+ 1.5°C) at 2 hrs. Ca^{++}-ATPase in synaptic membranes from whole brain was inhibited 24%. Morphine hyperthermia and Ca^{++}-ATPase inhibition was reversed by pretreatment with naloxone (2 - 5 mg/kg). Ca^{++}-ATPase activity was decreased in P_2 fractions from hypothalamus (18%) and cortex (20%) $P < 0.05$, but not in cerebellum. Nimodipine (1 mg/kg), verapamil (10 mg/kg) or diltiazem (5 mg/kg) reversed inhibition of Ca^{++}-ATPase, while nimodipine was most effective in reversing hyperthermia. The Ca^{++} channel agonist BAY K 8644 (3 mg/kg) produced hypothermia (-2.2°C) and increased Ca^{++}-ATPase activity (28%) in hypothalamus but not cortex or cerebellum. Nimodipine blocked the BAY K 8644 hypothermia and increase in Ca^{++}-ATPase. Naloxone (2 - 5 mg/kg) was ineffective in antagonism of BAY K 8644. These results suggest that activation of opiate receptors may alter thermoregulatory mechanisms by interferring with Ca^{++} transport in the hypothalamus. While opiate inhibition of ATPase was seen also in cortex, no effects were demonstrated in cerebellum. Specific changes in hypothalamic ATPase following BAY K 8644 and antagonism by nimodipine suggest that Ca^{++} movement in hypothalamus may be differently regulated by opiate and dihydropyridine receptors. The actions of opiates to alter temperature may be coupled to Ca^{++} transport.

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116.7 OPIOID TOLERANCE IN SYMPATHETIC PREGANGLIONIC NEURONS DUE TO LOSS OF ADENYLATE CYCLASE INHIBITION. Donald N. Franz and Lewis C. Miner* (SPON: J.H. Petajan). Department of Pharmacology, University of Utah, Salt Lake City, Utah 84132.

Chronic exposure of neuroblastoma X glioma hybrid cells (NG 108-15) to opioids produces tolerance to opioid-induced depression of adenylate cyclase (Klee et al., Life Sci. 16:1869, 1975). We have previously shown that both opioids and clonidine markedly depress the excitability of sympathetic preganglionic neurons (SPGNs) by respective actions on opioid and alpha-2 receptors (Science 215:1643, 1982). These depressions appear to be mediated by negative receptor coupling to adenylate cyclase because both opioids and clonidine prevent the marked increases in descending transmission to SPGNs that are produced by phosphodiesterase (PDE) inhibitors (Soc. Neurosci. Abstr. 9:797, 1983: 10:713, 1984). The present study was designed to assess the ability of SPGNs to become tolerant to opioids.

Sympathetic discharges, recorded from upper thoracic preganglionic rami, were evoked by stimulation (0.1 Hz) of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized spinal cats and were analyzed by signal averaging. Theophylline (50 mg/kg), IBMX (1 mg/kg), or RO 20-1724 (1 mg/kg) rapidly enhanced transmission to about 175% of control levels. Morphine (2 mg/kg) or methadone (1 mg/kg) rapidly depressed transmission by 60-90% and prevented the typical enhancement by the PDE inhibitors. Naloxone (20 ug/kg) then rapidly reversed the opioid depression and restored enhancement by the PDE inhibitors. A second series of experiments was conducted on cats pretreated with increasing doses of methadone HCl for 3 weeks; implanted Alzet minipumps delivered 5 mg/kg/day during the 3rd week. Plasma levels averaged 1.5 ug/ml on the final day. In these cats, 1 mg/kg of methadone produced only a transient 25% depression, but 1 mg/kg of IBMX rapidly enhanced transmission to the same level as in untreated cats (175%). Naloxone produced further enhancement. The depressant effect of clonidine (25 ug/kg) was unaltered by the tolerant state.

The markedly reduced depression of intraspinal transmission by methadone and its failure to prevent enhancement by IBMX after chronic methadone treatment indicates that SPGNs and their adenylate cyclase develop tolerance to opioids. The mechanism may involve an impaired ability of the negative regulatory proteins to couple opioid receptors to adenylate cyclase.

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116.8 THE DESIGN AND TESTING FOR ANALGESIA OF SOME ENKEPHALIN-LIKE COMPOUNDS IN THE RAT. M.A. Khaled* and J.M. Beaton, Neurosciences Program and Departments of Nutrition Sciences and Psychiatry, Univ. of Alabama at Birmingham, B'ham., AL 35294.

Although many opiate-like peptides have been isolated from either brain or pituitary gland, the enkephalins remain the smallest active peptides. These pentapeptides, H-Tyr-Gly-Gly-Phe-Met-OH(Met-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH(Leu-enkephalin) are only briefly active in the CNS after central administration. Structure activity relationship studies have shown that the Tyr moiety is the most critical one in the peptides and modifications to this amino acid result in decreased opiate like activity. Studies on dipeptides with Tyr at the N-terminus amino acid have also shown some activity. Horn and Rodgers (Nature, 260, 795, 1976) theorized that the amino group of Tyr and the hydroxyl of its side chain adopt a distance (ca. 7A) similar to one between the tyramine and hydroxyl groups in morphine. In order to verify this fact experimentally we synthesized some small molecules with tyrosine.

H-Tyr-D.Lys was synthesized first following the usual solution method for peptide synthesis. D.Lys was used in the hope of slowing enzymatic degradation. Next, two other compounds with Tyr covalently linked to long lipophilic hydrocarbon chains were synthesized. They are Tyr-$(CH_2)_6$-NH_2 and Tyr-$(CH_2)_{12}$-NH_2. The purity of the compounds was checked using thin layer chromatography and nuclear magnetic resonance.

Behavioral testing was carried out in groups of rats at several doses for each compound. The compounds were dissolved in distilled water and injected subcutaneously immediately prior to testing in a flinch-jump apparatus. Morphine was used for comparison. Five trials of an ascending, followed by a descending series of 0.75 sec foot shocks, delivered at 15 sec. intervals were carried out. The initial shock was 0.05 mA and was altered in 0.05 mA increments. The flinch and jump thresholds were noted. The trials were repeated at 10 minute intervals. The average jump thresholds, in mA, after 4 mg/kg, are shown below.

	Jump Threshold (mA)						
Time (Min)	0	10	20	30	40	50	60
Compounds							
Tyr-D.Lys	1.95	1.85	1.23	1.30	0.95	0.90	0.90
Tyr-$(CH_2)_6$-NH_2	1.80	1.50	1.80	1.00	0.90	0.90	0.85
Tyr-$(CH_2)_{12}$-NH_2	1.63	1.83	1.40	1.55	1.30	1.15	0.95
Morphine	1.45	1.50	2.50	2.50	2.25	2.50	2.25

These results indicate that all three of the synthesized compounds have analgesic properties after subcutaneous administration. Ongoing studies are examining the ability of naloxone to block these effects. Planned studies are to synthesize and test compounds in which the side chain of tyrosine is further constrained to maintain the distance between the amino group and the side chain hydroxyl as discussed above. (Supported in part by grants from NIH SO7-RR05349 and Alabama Consumer Research Fund).

116.9 MORPHINE AND OTHER OPIATES FROM BOVINE HYPOTHALAMUS AND ADRENAL. R.W. Barrett*, C.J. Weitz*, L.I. Lowney* and A. Goldstein. Addiction Research Foundation, Palo Alto, CA 94304.

We have recently reported the presence of six substances (Peaks 1-6) from bovine hypothalamus and adrenal, which are recognized by antisera to morphine. One of these (Peak 1) was identified as morphine by 500 MHz proton NMR after purification to homogeneity (Proc. Natl. Acad. Sci. USA, in press). Peaks 4 and 5 were not present in sufficient amount, but the other peaks have now been purified to homogeneity using polystyrene resin adsorption, anion and cation exchange, and reverse-phase HPLC. The criterion for purity is that both immunoreactive and UV-absorbing material is adsorbed to and recovered from a morphine antibody affinity resin but not a control immunoglobulin resin.

Peaks 2, 3 and 6 were inactive in the guinea pig ileum bioassay at doses (IR-morphine equivalents) 10 times higher than morphine. They are better recognized by an antiserum raised against a BSA-3-ethylamine-morphine conjugate ("937" antiserum) than by an antiserum raised against a BSA-N-carboxymethyl-normorphine conjugate. Titration experiments with "937" antibody affinity resin against a fixed amount of ir-equivalents revealed that Peaks 2 and 6 had higher affinity than morphine. Peak 3 and morphine had equivalent affinities.

Peaks 2, 3 and 6 are likely to be structurally related. Peak 6 is converted to Peaks 2 and 3 in NH_4OH. Since the isolation procedure included exposure to NH_4OH, it is not clear if Peaks 2 and 3 are present in tissue. However, the isolation procedure does not convert morphine or normorphine into other immunoreactive compounds.

Their HPLC behavior shows that Peaks 2, 3 and 6 are not any of the known major biosynthetic precursors or metabolites of morphine. Their structures are currently being determined.

116.10 LOCALIZATION OF NEUTRAL METALLOENDOPEPTIDASE (ENKEPHALINASE) ACTIVITY IN RAT BRAIN BY FLUORESCENT HISTOCHEMISTRY. Stephen A. Back and Charles Gorenstein. Department of Pharmacology, University of California, Irvine CA 92717.

We have histochemically localized neutral metalloendopeptidase (enkephalinase) in rat brain. Nitrosalicylaldehyde (NSA) was used to fluorescently localize the enzyme product resulting from cleavage of the substrate, glutaryl-Ala-Ala-Phe-4-methoxy-2-naphthylamide.

Rats were perfused with a low formaldehyde concentration which achieved maximal preservation of tissue morphology while retaining enzyme activity. Vibratome sections were incubated with 0.5 mM substrate and 0.5 mM NSA in the presence of microsomal leucine aminopeptidase at neutral pH. The yellow fluorescent precipitate formed in the tissue was observed using a Zeiss fluorescent microscope.

The enzyme was highly localized and displayed a unique pattern of distribution. Intense staining was observed in striate cortex with little or no staining in the adjacent globus pallidus. A number of other brain regions stained positively including the olfactory bulb, nucleus accumbens, anterior commissure, hippocampus, substantia nigra, and superior colliculus. The brain stem and spinal cord stained relatively lightly. No activity was detected in cerebellum. The pia and blood vessels throughout the brain showed considerable staining.

Following colchine injection into the lateral ventricle (100ug), fluorescent reaction product within cell bodies was found to be localized in punctate structures. That the staining pattern observed is due to "enkephalinase" is suggested by staining done in the presence of the enkephalinase inhibitor, thiorphan, at concentrations ranging from 3 nM to 30 nM. 30 nM thiorphan completely inhibited all staining throughout the brain. By contrast, the staining pattern obtained in the presence of the angiotensin converting enzyme inhibitor, captopril, did not appear different from control.

The distribution pattern of the enzyme and its selective response to thiorphan support a role for the enzyme in the metabolism of enkephalin. This technique provides a sensitive method to assess the extent to which the distribution of the enzyme correlates with the distribution of opiate peptides and opiate receptors in the brain and peripheral tissues.

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116.11 ANTIBODIES TO THE MU OPIATE RECEPTOR PURIFIED FROM RAT BRAIN.
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Active opiate receptors have been solubilized from rat neural membranes and purified approximately 5000-fold using a specific affinity column (Affigel 401 to which "hybromet", a newly synthesized opioid ligand with high affinity for the μ receptor, had been attached) and hydroxylapatite column chromatography. A potent and specific antibody to the purified μ receptor was produced in rabbits. The specificity of the antiserum was demonstrated by immunoprecipitation studies, as well as by the specific displacement of ^{3}H-opioid ligand binding. Immunological titration of opiate binding activity from rat brain showed that the antibody was able to effectively displace specific ^{3}H-etorphine and ^{3}H-dihydromorphine binding, but was ineffective against the binding of ^{3}H-ethylketocyclazocine, ^{3}H-D-Ala^{2}-D-Leu^{5}-enkephalin or ^{3}H-phencyclidine. The antibody was able to precipitate the M_r 94,000 component of the ^{125}I-labeled affinity purified receptor, suggesting the possibility that this subunit represents the μ opioid recognition component. By indirect immunofluorescence, the antibody was shown to specifically bind to the plasma membranes of NCB-20 neuroblastoma X chinese hamster brain hybrid cells and NC41A neuroblastoma cells, both of which revealed high affinity ^{3}H-etorphine and ^{3}H-dihydromorphine binding activity. The observed fluorescence in the neuroblastoma cells was prevented by pre-adsorption of the antibody with solubilized extracts of rat brain. These results indicate that the rabbit antibody to the purified receptor is μ-specific and will prove valuable in the precise localization of the μ receptor subtype in the central and peripheral nervous systems using immunohistochemical procedures.

116.12 BETA-ENDORPHIN AND DYNORPHIN A IMMUNOREACTIVITY IN THE PLASMA AND CSF OF RHESUS MONKEYS.
D.E. Gmerek*, M. Knobloch*, J.H. Woods and H. Akil. Department of Pharmacology and the Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0010.

The purpose of these studies is to detect changes in levels of endogenous opioid peptides in the plasma and CSF of rhesus monkeys associated with chronic administration of receptor-type selective opioid agonists. This report presents the results from preliminary studies. Radiommunassay of plasma and CSF samples proceeded as reported previously (Cahill et al., J. Clin. Endocrinol. Metab. 56: 992, 1983). Plasma and CSF were withdrawn from monkeys anesthetized with pentobarbital (28 mg/kg, i.v.). The acidified samples were extracted on Sep-Pak C18 cartridges. The samples were resuspended in 150 mM $NaPO_4$ buffer for beta-endorphin assays and MeOH:0.1 N HCl (1:1) for dynorphin assays. The antibodies used for the beta-endorphin assays recognize positions 17-27 of beta-endorphin (1-31). The antibodies used for the dynorphin assays recognize the COOH-terminus of dynorphin A (1-17) or the COOH-terminus of dynorphin A (1-8) (Dores et al., Neurosci. Abstr. 9: 586, 1983). The samples were incubated in buffer (150 mM $NaPO_4$, pH 8.2 or 50 mM $NaPO_4$ with 0.1 % Triton, pH 7.6) in the presence of antibody for a minumum of 24 hrs before addition of the trace (iodinated human beta-endorphin (1-31), dynorphin A (1-17), or dynorphin A (1-8), respectively). The assays were separated 24 hrs later by immunoprecipitation with goat antirabbit immunoglobulin G. Quantification was based on concurrently assayed standard curves of the appropriate cold ligand.

The amounts of beta-endorphin-like immunoreactivity in normal rhesus monkey plasma (n=5) of 26 ± 5 fmole/ml were in agreement with previous studies in our lab (Cahill, doctoral thesis, 1983). The amount of beta-endorphin-like immunoreactivity detected in normal rhesus CSF, which should reflect the peptides released from brain rather than pituitary, was 15 ± 4 fmoles/ml. Dynorphin A (1-17)-like immunoreactivity was detected in normal rhesus plasma (10-15 fmole/ml). Dynorphin A (1-8)-like immunoreactivity was also found in normal rhesus plasma in lesser amounts (approx. 3 fmole/ml). Dynorphin A (1-17) could not be detected consistantly in rat plasma. These results reflect the observations that rat posterior pituitary contains twice as much dynorphin A (1-8) as dynorphin A (1-17) (Dores et al., Neuropeptides 5: 501, 1985); whereas in rhesus, dynorphin A (1-17) is by far the dominant form (Dores, et al., unpublished observations). Supported by USPHS Grant 00254.

LIMBIC SYSTEM: DEVELOPMENT AND PLASTICITY I

117.1 THE MODULATION OF LONG TERM POTENTIATION BY DELTA-9-TETRAHYDRO-CANNABINOL IN THE IN VITRO RAT HIPPOCAMPAL SLICE.
Alexander V. Nowicky, Richard M. Vardaris, and Timothy J. Teyler, Neurobiology Program, Kent State Univ., and Northeastern Ohio Universities College of Medicine, ROOTSTOWN, OH 44272.

Delta-9-Tetrahydrocannabinol (THC), the major psychoactive cannabinoid in marihuana, causes an increase in neuronal excitability in the CA1 region of the in vitro Hippocampal slice,(Foy, et al, Brain Res. Bull., 1982, 8(4):341-45). The increase in excitability is seen with THC doses ranging from 10^{-12} to 10^{-10} M., and occurs within 15 min. after onset of drug incubation. Other work with the cannabinoids has shown that they cause membrane fluidity perturbations in synaptosomes, probably due to their high lipophilicity. However, doses necessary to obtain these effects are in excess of 10^{-6} M. Because the excitability effects in slices occurs at much lower doses, we have suggested that THC may be acting through specific receptors and/or the modulation of endogenous neurotransmitter systems. Recently, a THC binding study on brain tissue indicated the Hippocampus contained the highest level of THC binding, (Essman and Das, Fed. Proc., 1984, 43:951). We wondered whether THC might modulate Long Term Potentiation (LTP) in the CA1 region of the Hippocampus. LTP, which is a form of synaptic plasticity, is found in the Hippocampus, and has been suggested as a model of synaptic change underlying the mechanisms of memory.

Briefly, Hippocampal slices were obtained from adult, male, Long Evans rats by standard means, (Teyler, Brain Res. Bull., 1980, 5: 391-40). Evoked population responses were obtained from the stratum radiatum--CA1 pyramidal cell region of slices. Data collection involved recording 2 mV. population responses before, and 30 min. after control, 10 or 100 picomolar (pM) THC exchanged with the bathing media. Following the exchanges, LTP was induced by a tetanizing train of 260 Hz., .25sec., repeated 4 times. The stimulus intensity which evoked the original response was used to tetanize and then monitor the responses throughout the experiment. Data was collected to 100 min. post-tetanus. All response amplitudes were normalized to pretetanus baseline levels for analysis.

There were no significant differences between the control and 10 pM THC groups, with respect to early, post-tetanus LTP magnitude. Both groups showed an average of 200% increase over baseline levels at 15 min. However, whereas the control group exhibited the same magnitude increase of LTP at 100 min., the 10 pM THC group showed a significant decay to a magnitude of 50% increase over baseline. Thus the 10 pM THC treated sliced showed a significant decrease in the time constant of LTP. The 100 pM THC treated slices exhibited a more rapid decrease, such that at 100 min. LTP magnitude had decayed to 50% below baseline levels. We have shown that an acute pretreatment of slices with THC has no apparent effect on induction or magnitude, but significantly increases the rate of LTP decay.

117.2 NONLINEAR CHARACTERISTICS OF HIPPOCAMPAL PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION ARE DEPENDENT ON THE INTENSITY OF STIMULATION.
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Random impulse trains of electrical stimulation delivered to the perforant path (PP) effectively reveal nonlinear characteristics of synaptic transmission to the hippocampal dentate gyrus (DG) as measured by amplitude of the granule cell field potential population spike (Berger et al., Soc. Neurosci. Abstr., 1983). In the experiments reported here, we tested the effects of varying intensity of stimulation on nonlinear properties of PP-DG synapses while maintaining a constant mean frequency (λ) of stimulation. Varying stimulation intensity will change the number of presynaptic PP fibers and postsynaptic DG cells included in the system response.

Experiments were conducted using chronically implanted rabbits while animals were awake and mildly restrained. A computer generated random interval train (Poisson distribution, λ=500 msec, range-1/msec-1/5 sec) was used to determine the sequence of 4,064 single shock (0.1 msec duration) stimulations applied to the PP. Intensities used were those that produced 5%, 10%, 20%, 30%, 50%, and 100% of maximum spike amplitude; one 4,064 impulse train at one intensity was given each day.

First order kernels, which reflect the averaged population spike response to each impulse in the train, increased 300-400% as a function of intensity, reflecting the fact that average spike amplitude increased with increasing intensity. Second order kernels, which reflect the modulatory influence of a previous impulse on the activity evoked by the current stimulus whether or not other impulses occur between the two, also revealed significant changes as a function of intensity. The most prominent changes involved short-interval inhibition and longer-interval facilitation. For example, at 5% intensity inhibition to the second impulse of a pair of impulses was observed for inter-impulse-intervals up to approximately 40 msec. At 100%, inhibition lasted up to approximately 60 msec. Facilitation decreased in magnitude and occured at longer intervals as intensity was increased. At 5%, an average facilitation of 50-60% (at approximately 70 msec) was observed. At 100%, an average facilitation of 15-20% (at approximately 100 msec) was observed. Third order nonlinearities, which reflect the modulatory influence of any pair of preceding intervals on the activity evoked by the most current stimulus, also decreased as a function of intensity. Once intensity reached 30%, no more than a 10% deviation was observed in third order kernel values, whereas at 5% intensity, deviations as great as 60% were observed. In summary, hippocampal perforant path-dentate synaptic transmission becomes more linear as intensity is increased. Supported by The Whitaker Foundation.

117.3 NONLINEAR CHARACTERISTICS OF HIPPOCAMPAL PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION ARE DEPENDENT ON THE MEAN FREQUENCY OF STIMULATION. G.B. Robinson, J.L. Eriksson*, R.J. Sclabassi and T.W. Berger. Depts. of Psychology, Psychiatry and Neurosurgery, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We are utilizing random impulse train stimulation and nonlinear system analysis techniques to characterize synaptic transmission in the rabbit perforant path-granule cell (PP-GC) circuit (Berger et al., Soc. Neurosci. Abstr., 1983). Population spikes were recorded from the GC layer of awake rabbits with stimulation intensity adjusted to produce a response 10-15% of the maximum. Random interval trains (Poisson distribution) were presented at mean frequencies (λ) of 0.5, 1.0, 2.0, 3.3, 5.0, 6.7 and 10.0 Hz. Kernels were computed by cross-correlation of the random input with the amplitude of the PP-GC population spike.

Second order kernels, which represent the response to the second stimulation of an impulse pair embedded within the random train, exhibited nonlinearities that depended on λ. Briefly, increasing λ from 0.5 to 10.0 Hz had the following effects: 1) decreasing the magnitude of maximum facilitation (from 80% to 5%) and 2) increasing the duration of inhibitory interactions (from 35 ms at 0.5 Hz to 60 ms at 10.0 Hz). Regardless of λ, the second order kernels were approximately linear for interstimulus intervals greater than 300 ms. Third order kernels, which represent the response amplitude following the final impulse of a triplet of impulses embedded within the random train, also depended on λ. Generally, increasing λ resulted in a decrease of both the third order facilitative and inhibitory effects. That is, the third order kernel values approached zero. Regardless of λ, the largest facilitative effects (up to 70%) were observed when the third impulse occurred 20-40 ms after an impulse pair of less than 30 ms. Generally the largest inhibitory effects (up to 60%) occurred when the two intervals were approximately 40-70 ms. At 2.0 Hz, for example, if the third impulse occurred 50 ms after an impulse pair of 50 ms, then the response following that third impulse was approximately 60% smaller than would have been predicted from the linear summation of the responses to two 50 ms intervals. These results suggest that the output of the dentate gyrus GCs approaches linearity as their frequency of activation by the PP increases. Supported by The Whitaker Foundation and the Natural Sciences and Engineering Research Council of Canada.

117.4 **IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN SEPTAL NEURONS TRANSPLANTED TO THE RAT HIPPOCAMPUS.** K.J. Anderson, R.B. Gibbs, P.M. Salvaterra, and C.W. Cotman. Dept. of Psychobiology, Univ. California Irvine, Irvine CA 92717 and Div. of Neurosciences, City of Hope Research Institute, Duarte CA, 91010.

Septal neurons have been used extensively in transplant studies to replace cholinergic deficits when the intrinsic innervation has been lost or compromised. It is not known, however, whether these neurons form synapses with, or receive synapses from, the host. We have studied transplanted cholinergic neurons with electron microscopic immunocytochemical techniques using a monoclonal anitbody to choline acetyltransferase (ChAT). We demonstrate that transplanted ChAT positive neurons form asymmetric synapses with the host tissue and receive non-cholinergic synapses.

Ten days following a bilateral transection of the fimbria-fornix, adult rats received an injection of dissociated embryonic (E18) septum into the rostral hippocampus. Animals were allowed to survive for 30 days and killed by vascular perfusion. Sections from the entire hippocampal formation were processed for ChAT immunocytochemistry by the PAP method and then prepared for electron microscopy. At the light microscopic level, ChAT positive neurons from the transplants were identifiable by their large size (35-50 μm in diameter) and location in the hippocampus. Immunoreactive neurons were seen within the hippocampus itself and often formed clumps on the ventricular surface. Plastic embedded sections containing transplanted septal neurons were mounted and thin sections were cut on face for electron microscopy. At the electron microscopic level, ChAT positive neurons were identified by electron dense precipitate that associated with cytoplasmic organelles such as mitochondria, Golgi apparatus, microtubules and endoplasmic reticulum. These neurons were seen to receive axosomatic synapses from non-cholinergic axon terminals. Both asymmetric and symmetric synapses were observed terminating on immunoreactive neurons. ChAT positive dendritic processes arising from transplanted neurons were seen receiving non-immunoreactive synapses on both spines and shafts that were largely asymmetric. Myelinated axonal processes were observed arising from several ChAT positive neurons. Serial sections of immunoreactive axonal processes revealed that they made synaptic contacts with small dendritic processes and dendritic shafts of non-immunoreactive neurons. Axon terminals from ChAT positive neurons were round to oval in shape, contained small clear vesicles and formed asymmetric contacts.

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117.5 PARTIAL RECOVERY OF PERFORMANCE ON A DRL OPERANT SCHEDULE IN HIPPOCAMPAL-DAMAGED RATS GIVEN POST-LESION IMPLANTS OF EMBYRONIC HIPPOCAMPUS. Michael L. Woodruff, Ronald H. Baisden, Dennis L. Whittington, and Amy E. Benson*. Department of Anatomy, Laboratory for Neurobehavioral Science, Quillen-Dishner College of Medicine, East Tennessee State University, Johnson City, TN 37614.

Several published reports have indicated that implantation of fetal neural tissue into the site of a brain lesion in an adult animal can produce some recovery of function on "cognitive" tasks. For example, spatial alternation deficits usually observed to follow destruction of the medial frontal cortex in rats were attenuated by implantation of embryonic frontal cortical tissue into the lesion site in the adult brain (Labbe et al. Science 221, 1983), and Dunnett et al. (Brain Res. 251, 1982) found that implants of fetal septal tissue into the cavity produced by a fimbria-fornix lesion significantly reduced the deficit in performance of rewarded alternation usually produced in the rat by such lesions. However, the possibility that behavioral recovery might be produced by implantation of fetal hippocampal tissue into the site of a hippocampal lesion produced in an adult animal has not yet been tested. We now report that partial recovery of function on one behavioral measure can be produced by such an implant. Ten rats served as unoperated controls and ten as cortically damaged controls, while ten rats were given bilateral aspiration lesions of the hippocampus. The final ten rats were given the same type of lesions, but received implants of fetal hippocampus. The embryonic anlage of the hippocampus was dissected from embryos removed from ketamine-anesthetized mothers 13 days after conception. Care was taken to free the neural tissue from all connective elements. It was then transferred to a Hams F10 nutrient medium before being placed into the lesion cavity of the host. Six months were allowed for recovery after which all rats were food deprived and tested on a DRL 20 sec operant schedule for 50 days. Bilateral hippocampal damage is known to produce deficits in the ability of rats to perform efficiently on this task. Histological evaluation conducted after behavioral testing indicated that the implants had indeed developed and resembled those described by Kromer et al. (J. Comp. Neurol. 218, 1983). Analysis of the behavioral data from the present experiment revealed that rats receiving implants of fetal hippocampus were significantly better at the DRL task than the rats receiving only hippocampal lesions. However, both groups were inferior to the control groups. The enduring deficit usually produced in performance of the DRL task by hippocampal lesions can, then, apparently be attenuated, but not completely reversed, by development of embryonic tissue within the lesion cavity.

117.6 **TRANSPLANTATION OF SEPTAL NEURONS MAINTAINED IN LONG-TERM CULTURES** Robert B. Gibbs, Sarah Pixley and Carl W. Cotman Department of Psychobiology, U. C. Irvine, Irvine, CA. 92717

Postnatal CNS tissues do not readily survive transplantation to the brain. In the present study, we examined whether embryonic central neurons could be maintained in culture for a long period of time and then successfully transplanted to the adult CNS. Cells transplanted from culture were compared with non-cultured cells taken at comparable developmental stages.

Adult, male Sprague Dawley rats (~200 g) received bilateral transections of the fimbria to remove the native septal cholinergic input to the hippocampus. Ten days later, transplants of; (1) 18 day embryonic septal cells (E18) maintained in dissociated cell culture with 10% FCS for 1 week (n=10), 2 weeks (n=13), or 4 weeks (n=13); (2) non-cultured E18 septal cells in suspension (n=10); or (3) solid septal grafts from postnatal day 4 (P4) (n=7) or postnatal day 13 (P13) (n=7) neonates, were injected unilaterally into the host hippocampus. All grafts were examined 30 days after transplantation.

Transplants of embryonic septal cells contained many acetylcholinesterase (AChE) -positive neurons which innervated the host hippocampus in a pattern comparable to that of the native cholinergic input. A similar pattern of innervation was observed using transplants of 1-week septal cultures and P4 septal tissues; however the P4 grafts had considerably fewer surviving cells. In contrast, there was no evidence of transplant survival in grafts from 2-week or 4-week septal cultures or from P13 neonates.

To control for the trauma of removing the cells from culture, aliquots of cell suspensions prepared from 2-week and 4-week septal cultures were replated under conditions identical to those used above. All primary and secondary cultures contained cells which bound antibodies against neurofilament proteins and which stained for AChE within 1-2 weeks after plating.

We conclude that embryonic septal neurons maintained for a short period in culture can be successfully transplanted to the adult CNS, but that prolonged culturing under our conditions is incompatible with transplantation. The ability of cultured cells to survive in the brain parallels the survival of grafts of neonatal tissues taken at comparable developmental stages.

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117.7 IMMUNOCYTOCHEMISTRY OF DEVELOPMENT IN THE OLFACTORY CORTEX OF POSTNATAL RATS. R.E Westenbroek[*1], L.E. Westrum[1], A. Hendrickson[2], M.C. Beinfeld[*3], and J.Y. Wu[4]. Depts. of Biological Structure[1], Neurological Surgery[1], and Ophthalmology[2], Univ. of Washington, Seattle, WA 98195 and Dept. of Pharmacology[3], St. Louis Univ., St. Louis, MO 63108 and Dept. of Physiology,[4] Pennsylvania State Univ., Hershey, PA 17033.

Although conventional microscopic studies have provided considerable information about the organization and development of the olfactory cortex (area 51), the immunocytochemistry of this area of cortex is generally lacking. Cholecystokinin (CCK), a neuropeptide, and gamma aminobutyric acid (GABA), a neurotransmitter, have been shown to be present in the olfactory cortex. Using anti-CCK serum prepared by M.C. Beinfeld and anti-GAD (glutamic acic decarboxylase), prepared by J.Y. Wu, and the indirect peroxidase anti-peroxidase technique of Sternberger, the immunoreactivity of the olfactory cortex during development is being investigated. Light microscopic (LM) studies of this cortical region at several known "critical periods" between postnatal (PN) day 0 (day of birth) and PN 30 have revealed distinct changes in the immunoreactivity of cells, fibers, and terminal puncta. Anti-CCK reactivity shows labelled cells present especially in layer II in both subareas 51a and 51b at PN 0. The relative numbers of labelled cells appears to double by PN 9, remains the same at PN 13 but increases dramatically by PN 21. Little or no change occurs thereafter. CCK-cells are also present in small numbers in layer Ib and in layer III in the younger animals. Labelled CCK-fibers are virtually absent from the lateral olfactory tract (LOT) until PN 13 after which they appear to double by PN 21, especially in sublayer Ib. Terminal puncta are rare at PN 0-3, gradually increases between PN 6 and PN 13 and thereafter are densely distributed within layer II with fewer found in the deeper part of layer I and in III. LM studies of anti-GAD reactivity reveal that labelled cells are seen as early as PN 2 in layer Ib and in layer III. GAD-positive terminal puncta are also present at PN 2 and are basically of uniform size being most numerous in the superficial part of Ia and the deeper part of Ib. They are extremely rare in layer II. This pattern of distribution persists through PN 13. By PN 30 terminal puncta are large and densely distributed subadjacent to the LOT and in layer Ia but decrease in size and numbers in deeper Ib, while remaining rare in layer II. The results show that there are complementary patterns of cellular and terminal immunoreactivity for the two antisera and that the "critical periods" of immunoreactivity are different during development for each of them. Also, the presence of labelled cells suggest as origins for the fibers and terminals, nearby, possibly intracortical cells.

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117.8 NEUROGENESIS IN THE SUBNUCLEI OF THE INTERPEDUNCULAR NUCLEUS (IPN) AND IN THE MEDIAL HABENULA (MH) OF RAT. N.J. Lenn and S.A. Bayer, Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908 and Dept. of Biology, Indiana-Purdue Univ., Indianapolis, IN 46223.

Development of the habenulo-interpeduncular system is of interest because of its adult structure, including 7 subnuclei of IPN, localization of synapses of different types and MH sites of origin within the subnuclei, and different localization of neurochemicals amongst the subnuclei. To further investigate the mechanisms controlling these features, the birthdays of IPN and MH neurons were reinvestigated. Autoradiographs from groups of rats whose mothers had been injected with ^{3}H-thymidine on embryonic days (E) 12+13, 13+14, ... 18+19 were studied. Counts of 30,000 labeled and unlabeled cells from the 7 subnuclei of IPN at 4 uniform coronal levels of the nucleus were expressed as % labeled cells for each 24 hr interval. All IPN neurons were formed on E12-16. Birthdays varied for the subnuclei of IPN, with the earliest being those of the serotonin-containing apical subnucleus and the latest being those of the rostral subnucleus ($p<.001$; sign test). Rostral lateral and lateral subnuclei were approximately simultaneous with apical. The intermediate and central subnuclei, related to each other in a number of ways, had simultaneous birthdays earlier than the rostral and later than the other subnuclei. Neurons at the caudal levels of the central, intermediate and lateral subnuclei were formed latest, and at their middle levels earliest ($p<.021$ to $.0001$).

In these autoradiographs, MH consisted of 3 regions. Medial and lateral regions of equal size occupy the rostral ½ of MH, and medial ventral and lateral ventral portions caudal to the exit of the fasciculus retroflexus. The dorsal region is only present in the caudal ½ of MH. Neurons in these regions undergo their final mitoses on E15-16, 16-17 and 17-18, respectively.

It is possible to relate the subdivisions of MH and IPN, the neuronal birthdays of these subdivisions, and the localization of neurochemicals. The dorsal region of MH contains substance P immunoreactive cell bodies which have early neuronal birthdays, as do the neurons of their target, the lateral subnucleus of IPN. The cholinergic cell bodies in MH are located in the lateral and medial portions of MH. The birthdays of these MH cells are in the middle to late part of the range of MH birthdays, E16-18. They project to the intermediate and central subnuclei where they terminate as crest and S synapses on cells which have birthdays in the middle and late part of the range for IPN. For the opiate related regions, there is less complete data.

These findings provide additional support for the validity of the subnuclei as currently delineated. They further suggest mechanisms of development which involve early interactions between specific groups of MH neurons and specific groups of IPN neurons.

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METABOLIC STUDIES

118.1 ANOXIA WITH AND WITHOUT CALCIUM ON HIPPOCAMPAL SLICE MACROMOLECULAR SYNTHESIS. K.M. Raley*, and P. Lipton, Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

We are using an in vitro hippocampal slice preparation to investigate the irreversible brain damage caused by a brief period of anoxia. Two questions are being asked: (i) is the irreversible damage seen with anoxia due to a disruption of macromolecular synthesis--namely protein and RNA synthesis?; ii) what might be the role of calcium in mediating such effects on synthesis?

To answer these questions, we incubated transverse slices of hippocampus from ~90 day old rats in a 95%O_2-5%CO_2-buffered modified Ringers solution at 36°C. Experimental slices were exposed to 10' of anoxia(95%N_2-5%CO_2) and allowed to recover for 30' or 3 hr in reoxygenated buffer. To test the involvement of calcium, some slices were placed in a Ca-free buffer during and 5' following the anoxic period. 30' of ^{14}C-lysine(0.1mCi/ml) and ^{3}H-uridine (1mCi/ml) incorporations into PCA insoluble material were used to measure protein and RNA synthesis.

10' of anoxia caused a decrease (to 47-51% of control) in the incorporation of ^{14}C-lysine into protein both 30' and 3 hr post-anoxia.

When slices were placed in Ca-free medium during the anoxia and allowed to recover for 30', protein synthesis was 69% of control rates. In contrast, when slices were allowed to recover for 3 hr, protein synthesis was not inhibited at all. These results suggest that the long-term damage to protein synthesis involves a Ca influx during or immediately following the anoxia. In support of this, it was found that there is an approximate 20% increase in net Ca influx into hippocampal cells during 10' of anoxia. Anoxia did not inhibit ^{14}C-lysine incorporation into tRNA.

Anoxia affected the incorporation of ^{3}H-uridine into RNA somewhat differently. 30' following anoxia, RNA synthesis rates were the same as those found in control slices. However, following 3 hr recovery, RNA synthesis rates were 42% of control rates. These results suggest that the damage to RNA synthesis progresses with time.

When slices were exposed to Ca-free medium during anoxia and allowed to recover for 3 hr, RNA synthesis rates were 42% of control. However, slices exposed to a Ca-free medium for 20' and allowed to "recover" in normal buffer for 3 hr exhibited a decrease in RNA synthesis to 45% of control levels.

These data support the following conclusions:1) 10' anoxia inhibits protein and RNA synthesis 3 hr post-anoxia, in a Ca-dependent manner; 2) the Ca-dependent inhibition develops with time.

We are currently investigating the following questions:1) How is Ca exerting its damaging effect on protein and RNA synthesis during anoxia? 2) Is the inhibition of protein synthesis a global effect or does it involve a specific group of proteins?

118.2 GAMMA-HYDROXYBUTYRATE EFFECTS ON CEREBRAL SEROTONIN METABOLISM AND TURNOVER. Y. Ueki*, B. Djuricic*, K. Kumami* and M. Spatz (SPON: H.G. Wagner). LNNS, NINCDS, NIH, Bethesda, MD 20205

Gamma-hydroxybutyrate (GHB), the naturally occurring central nervous system depressant affects various cerebral metabolic pathways in normal and pathological conditions. In ischemia, GHB has a beneficial effect as a preventive and therapeutic agent. Preischemic GHB treatment of gerbils reduces the development and progression of edema, spares the energy metabolism, stabilizes the 5-HT metabolism as well as increases the survival rate of the affected animals. On the other hand, the late post-ischemic GHB treatment has no significant effect on the energy metabolism but decreases the progression of edema, reduces the increased BBB permeability and the mortality rate of the gerbils. Since the improvement in the recovery of the ischemic GHB treated animals could not be directly related to the carbohydrate and energy metabolism, we investigated the influence of postischemic GHB treatment on the metabolism of 5-HT which has been implicated as one of the main factors involved in development and progression of ischemic edema.

Bilateral cerebral ischemia was induced in gerbils by clipping of common carotid arteries for 15 min. only or with clip release for 4 hours. GHB (500 mg/kg b.w.) was injected intravenously 1 hour prior to the termination of the experimental period (4 hrs. after clip release). For the 5-HT turnover studies p-chlorphenylalanine [(PCPA) 300 mg/kg b.w.] were given intraperitoneally just before removal of the clips. Similarly treated sham operated animals served as controls. The gerbils were sacrificed by microwave irradiation and the acid-soluble metabolites were extracted from each dissected brain structure with perchloric acid. Tryptophan, 5-HT and 5-hydroxyindole acetic acid (5-HIAA) were determined by high pressure liquid chromatography (HPLC) using electrochemical detection.

The overall responsiveness of the 5-HT metabolism and turnover to GHB shows regional differences in the brain of sham operated gerbils. Particularly, GHB decreases the 5-HT turnover in the hippocampus while increases 5-HT turnover in the striatum and does not affect the cortex. On the other hand, GHB reverses the observed increased turnover time and decreased turnover rate (decreased 5-HT release and synthesis) at 4 hours reflow after the ischemic insult in the cortex hippocampus and partly in the striatum. However, 5-HIAA accumulation is unaffected by GHB treatment.

The results indicate that GHB effects on the 5-HT metabolism of sham operated controls is distinctly different from those seen in the brains of gerbils recovering from ischemia. Moreover, these findings suggest that the reported beneficial manifestations of the postischemic GHB treatment may be related to 5-HT synthesis which appears to be enhanced in the treated as compared to the untreated ischemic animals.

118.3 DIFFERENTIAL DISTRIBUTION OF LYSOSOMAL ENZYMES IN THE BRAIN AND INCREASED ACTIVITY OF DPP II AFTER CHRONIC EXPOSURE TO LEUPEPTIN. M.Kessler, G.Ivy, C.Gorenstein, M.Baudry and G.Lynch. CNLM and Dept. of Pharmacology, University of California, Irvine CA 92717.

Several of us have reported that the thiol protease inhibitor leupeptin and the general lysosomal enzyme inhibitor chloroquine cause the accumulation of ceroid-lipofuscin in brain cells (Ivy et al., Science 226:985,1984). This led us to propose that the lipofuscin which accumulates during aging and in certain disease states results from defects in lysosomal enzymes. We thus decided 1) to examine the brain distribution of different lysosomal enzymes and 2) to see if leupeptin treatment caused any changes in enzyme activity. We have employed histochemical techniques and computerized densitometry to compare the distribution of dipeptidyl aminopeptidase II (DPP II) and acid phosphatase (AP) in control and leupeptin treated animals.

Six rats each were implanted with miniosmotic pumps filled with saline (0.9%) or leupeptin (40 mg/ml) and 6 rats were untreated.The rats were perfused with buffered formalin. Sections were cut on a Vibratome at 50 μm and processed histochemically for DPP II or AP.

First, we have extended the findings of Gorenstein et al.(J.Neurosci., 1:1096, 1981) on the differential brain distribution of these two lysosomal enzymes. While both DPP II and AP are often co-localized, the relative distribution of the enzymes among neuronal populations differs. In addition, AP is commonly found in glia whereas DPP II is not. Computerized color contrast enhancement showed that DPP II staining is most dense in cerebellar granule and Purkinje cells, hippocampal regions CA3 and CA4, dentate gyrus granule cells and layer V of neocortex. AP is most concentrated in cerebellar granule cells, brainstem and layer VI of neocortex. We did not find any changes in the relative distribution of the enzymes after leupeptin or saline treatment.

Second, selected cell populations were analyzed for staining density in leupeptin and saline treated and untreated rats. Leupeptin treatment caused an increase in DPP II staining in hippocampal areas CA3 and CA4, in dentate gyrus granule cells and in deep cerebellar nuclei by a mean of approximately 20% over saline treated rats. AP levels were not affected by leupeptin treatment. Saline treatment caused a small decrease in both DPP II and AP levels. Studies are in progress to determine the significance of this effect.

In summary, we have found that lysosomal enzymes are not homogeneously distributed throughout the brain and that levels of DPP II but not AP are affected by leupeptin treatment. It is interesting to speculate that the differential distribution of lysosomal enzymes may underlie various pathological phenomena which are restricted to certain brain regions. Supported by NIA AG 00538 (G.L.).

118.4 PHOSPHOLIPID METABOLISM DURING NORMAL AND DYSTROPHIC AVIAN PECTORAL MUSCLE CELL DIFFERENTIATION IN CULTURE. E.L. Hogan and K.C. Leskawa* (SPON: L.D. Middaugh). Department of Neurology, The Medical University of South Carolina, Charleston, SC 29425

The biosynthesis of phospholipids during the differentiation of normal and dystrophic muscle cells (i.e., myogenesis) was studied in vitro using [32P] added to the culture medium. The course of myogenesis was divided into several phases: (1) a replicating stage, when single myoblasts actively divide, (2) a stationary phase brought about by the addition of EDTA to the medium, when myoblasts exit the cell cycle and align in parallel arrays, (3) an initial fusion stage, when opposing muscle cell membranes fuse together, (4) a late fusion stage, when over 70% of the total cell nuclei are contained within multinucleated, contractile myotubules, and (5) a final phase where functional myotubules are isolated by the addition of cytosine arabinoside to the culture medium. At each phase, 160 uCi [32P] was added to the medium and cultures incubated at 37o C for 3 hr. After extracting and partitioning, labeled phospholipids were separated by two-dimensional thin-layer chromatography, exposing the plates to HCl vapors between solvents to cleave the alk-1-enyl bonds. Chromatograms were visualized by autoradiography and spots comigrating with standards were scraped from the support and radioactivity determined by liquid scintillation spectrometry.

Incorporation of label into plasmalogens (ethanolamine or choline) did not vary during myotubule formation, and no differences were observed between normal and dystrophic cultures at any stages of differentiation, which is in disagreement with in vivo studies (Kester & Privitera, 1984, Biochim. Biophys. Acta, 778:112).

A sharp decrease in phosphoinositide labeled was observed as myoblasts first achieved cell contact and recognition. PI labeling continued to decline slowly as myotubules developed. Others have suggested that phosphoinositides act to block fusion (Wakelam & Pette, 1984, Ciba Found. Symp., 103:100), and a decrease in membrane PI content may promote proper conditions for myoblast membrane fusion to occur. More dramatic was the labeling of phosphatidylcholine during myogenesis -- from 18.4% of the total labeled phospholipids in myoblasts to 48.6% of the total myotubule phospholipid. The gradual increase in PC labeling during differentiation roughly corresponded to the shape of a cell fusion curve. This increase in PC labeling may reflect development of the sarcoplasmic reticulm during the differentiation of muscle fibers, since PC is known to be enriched in the sarcoplasmic reticulum, where it is essential for ATPase activity and Ca++ uptake (Martonosi, 1963, Biochem. Biophys. Res. Comm., 13:273).

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118.5 PHORBOL ESTERS STIMULATE PHOSPHATIDYLCHOLINE METABOLISM IN NG108-15 CELLS. M. Liscovitch*, A. Freese*, J. K. Blusztajn* and R.J.Wurtman. (SPON: J. D. Milner). Department of Applied Biological Sciences. Massachusetts Institute of Technology, Cambridge, MA 02139.

The effects of tumor-promoting phorbol esters on phosphatidylcholine (PtdCho) metabolism were investigated in the NG108-15 neuroblastoma x glioma hybrid cell line. After one hour of incubation of 12-O-tetradecanoylphorbol-13-acetate (TPA, 100 nM) with cells that were prelabelled with [^{3}H]choline for 24 hours, the release of radioactivity into the medium was stimulated by 158±24% (Mean±S.D., N=9). This effect was dose-dependent (ED_{50}=10 nM) and was observable as early as 5 min after exposure to TPA. The TPA-induced release of radioactivity into the medium was accompanied by a small and variable increase in the radioactivity associated with cellular, acid-soluble choline metabolites (mainly [^{3}H]phosphorylcholine) and a corresponding decrease in [^{3}H]PtdCho. 4β-phorbol-12,13-didecanoate (PDD), another biologically active phorbol ester, had a similar effect, while its non-tumor-promoting isomer 4α-PDD had no effect. When NG108-15 cells were labelled with [^{3}H]choline in the presence of either TPA or PDD (at 100 nM), the incorporation of [^{3}H]choline to PtdCho was stimulated by 180±33% (N=4). In a pulse-chase study cells were labelled with [^{3}H]choline for 1 hour and then chased for up to 8 hours in growth medium that did not contain radiolabelled choline, in the absence or presence of phorbol esters. PDD (100 nM) enhanced the transfer of radioactivity from [^{3}H]phosphorylcholine to [^{3}H]PtdCho, causing a 30% increase in [^{3}H]PtdCho and a corresponding decrease in [^{3}H]phosphorylcholine after 2 hours of chase. However, no difference between control and PDD-treated cells was noted after 8 hours of chase. As phorbol esters were shown to activate the Ca^{2+}-, phospholipid -dependent protein kinase C, we have also investigated the effects of a Ca^{2+} ionophore on PtdCho metabolism. A23187 (at concentrations up to 2 μM) did not significantly affect the release of radioactivity from [^{3}H]choline-labelled cells nor did it affect the incorporation of [^{3}H]choline to the phospholipid fraction. In addition, A23187 did not affect the response of the cells to PDD (100 nM) at any of the concentrations tested. Under pulse-chase conditions, however, A23187 stimulated the accumulation of intracellular [^{3}H]glycerophosphocholine and [^{3}H]choline by 200 to 300%, in a time-dependent manner.

These findings show that phorbol esters can cause a rapid stimulation of PtdCho metabolism in a neuronal cell line and may suggest the possible involvement of protein kinase C in the regulation of PtdCho metabolism. Increased PtdCho degradation induced by the phorbol esters may be due to stimulation of phospholipase C activity, which may also trigger the enhanced incorporation of choline into this phospholipid.

118.6 HYPERGLYCEMIA INHIBITS THE DEVELOPMENT OF ALTERATIONS IN BRAIN MITOCHONDRIAL FUNCTION DURING ANOXIA. K.R. Wagner and R.E. Myers. Research Service, VA Medical Center, Cincinnati, Ohio 45220 and Department of Neurology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Exposure of hyperglycemic animals to anoxia/ischemia causes severe brain injury. In contrast, saline infused animals identically exposed remain brain intact. Thus, we hypothesized that alterations in brain mitochondria occurring during anoxia in hyperglycemic animals would importantly influence development of injury. We tested this hypothesis by exposing normo- (5 mM) and hyperglycemic (50 mM) cats to anoxia by respiring them with 100% nitrogen. Brain mitochondria were isolated after 8 minutes of anoxia as described by Clark and Nicklaus (1970). Mitochondria from saline infused cats showed 30-50% inhibition of state 3 respiration with NAD-linked substrates glutamate or pyruvate (both plus malate). Addition of the uncoupler FCCP maximally stimulated respiration, indicating that alteration of adenine nucleotide translocase or inner membrane ATPase, not inhibition of substrate transport or oxidation was present. State 3 respiration with succinate (plus rotenone) was not inhibited. Mitochondrial respiratory control ratios were decreased while ADP/O ratios were unchanged. In contrast, hyperglycemic cats showed no such inhibition of state 3 respiration with NAD- or FAD-linked substrates. No differences were present between saline and glucose infused controls. At the end of exposure, hyperglycemic cats showed significantly higher brain tissue lactate concentrations but similar reductions in ATP and phosphocreatine versus saline infused cats. Thus, decreased energy state alone did not account for inhibition of state 3 respiration in saline infused cats. In conclusion, hyperglycemia inhibits the development of dysfunction of mitochondrial inner membrane properties during anoxia. Since hyperglycemic cats develop brain edema, severe neurologic injury and altered brain mitochondrial function by 5 hours after exposure (Soc. Neurosci. Abs. 10:120, 1984), processes taking place following reoxygenation are responsible for the development of mitochondrial dysfunction and brain tissue injury in hyperglycemic cats exposed to anoxia. Furthermore, since saline infused animals will recover neurologic function and show no or minimal brain pathology, brain mitochondrial dysfunction which develops during anoxia in saline infused cats is reversible following reoxygenation. (Supported by Veterans Administration Medical Research Service.)

118.7 AMINO ACID TRANSPORT IN ASTROCYTE CULTURES. E. Stöcklin*, S.C. Quay*, Y-L. Lee* and L.F. Eng. Dept. Path., Stanford Univ. Sch. of Med. and Veterans Admin. Med. Cen., Palo Alto, CA 94304.

In other systems (Quay and Oxender, In: Biological Regulation and Development, R.F. Goldberg, ed., Plenum Press, New York, 1980, pp. 413-436) the study of amino acid transport in mammalian tissue cultures has provided insight into the process of cell differentiation and the response of the cell to injury, proliferation, and senesence. We report here the study of amino acid transport in rat astrocytes grown on Millipore filters. Secondary astrocyte cultures were prepared from newborn rat brains according to McCarthy and deVellis (J. Cell Biol. 85:890-902, 1980). One ml aliquots of primary astrocytes (5 x 10^5 cells per ml) were plated on 13 mm Millipore filters, placed in 24 well culture plates and maintained in serum supplemented medium until the cells were confluent (5-10 days). Some filters were immunocytochemically stained for glial fibrillary acidic protein and vimentin, some were analyzed for total protein and the remaining were used for transport studies.

The filter discs containing astrocytes (typically 70 µg protein/disc) were washed free of serum-containing medium and incubated with tritium-containing α-amino isobutyric acid, a non-metabolizable substrate for the mammalian amino acid transport system designated the A system (Christensen H.N.: Biological Transport, Benjamin, Inc., 1975, pp. 435-437). The uptake was time dependent, reaching a steady state in 6-10 min. The concentration dependence was examined and indicated a single independent uptake system with a Km of 4.55 mM and a V_{max} of 48 nmoles/min/mg protein. Finally, the system exhibited its typical sodium-ion dependence, since substitution of choline for sodium lead to a dramatic loss in uptake capacity. With the fundamental parameters of this system established, we are now exploring the effect of physiological or pathological manipulation of the cell culture on the transport system.

Supported by the Veterans Administration, NIH grant NS 11632, and the Swiss National Science Foundation.

118.8 CA UPTAKE DURING ANOXIA IN THE RAT HIPPOCAMPAL SLICE. Peter Lipton, Cynthia Hurtenbach, and Ira S. Kass (Spon: D. Gilboe), Dept. of Physiology, University of Wisconsin, Madison, WI.

Irreversible anoxic damage to hippocampal slices depends on extracellular calcium. Thus, prolonged (at least 3 hrs) inhibition of synaptic transmission following 10 minutes of anoxia is prevented if the buffer during anoxia contains 10 mM Mg/OmMCa (Kass & Lipton, J. Physiol., 1982); a similarly induced inhibition of protein and RNA synthesis is prevented if the buffer during anoxia contains OmMCa (Raley & Lipton, these meetings). These results suggest that an anoxic induced increase in Ca uptake may be causing irreversible damage to these processes. Prolonged inhibition of transmission is more sensitive to anoxia in CA1 than in the dentate granule cell (DG) region.

Ca uptake was measured by incubating hippocampal slices with 45-Ca (10µCi/ml) for different periods of time and measuring intracellular accumulation.

Anoxia increased 45-Ca accumulation in both DG and CA1. The increased accumulation was about twice as great in CA1 as in DG and, via a kinetic analysis of uptake, appeared to result from a decrease in unidirectional efflux and not an increase in unidirectional influx. The analysis showed that ten minutes of anoxia should increase exchangeable Ca in CA1 by 30% and in DG by 15%.

The presence of 2 mM Cobalt during anoxia prevents transmission damage. It did not prevent the increase in Ca accumulation during anoxia at all in DG and inhibited it by only 35% in CA1. This is consistent with the conclusion that inhibition of efflux is the major cause of excess Ca accumulation during anoxia.

Pre-incubation with creatine strongly protects the tissue against anoxic transmission damage, maintaining ATP and K/Na levels during anoxia. This pre-incubation reduces the anoxic increase in Ca influx by about 50% in both CA1 and DG.

Ouabain, at a dose which increases cell Na/K more than anoxia, does not increase Ca uptake. This suggests that decreased Na gradient driven Ca efflux does not account for the anoxic increase in Ca uptake.

Thus, 1) there is an anoxia-associated increase in Ca uptake which is larger in CA1 than in DG; CA1 is more sensitive to anoxic transmission damage than is DG; 2) Treatments which strongly protect against anoxic transmission damage (Cobalt & Creatine) also strongly attenuate net Ca influx during anoxia. These data are consistent with the conclusion that increased Ca influx during anoxia participates in irreversible anoxic damage. The increase appears largely due to an inhibition of the efflux Ca-ATPase but, in CA1, is partially due to an increase in influx through depolarization-sensitive channels.

118.9 SIGNIFICANT INTRACELLULAR ZINC ACCUMULATION IN CARDIAC AND SKELETAL MUSCLES OF DYSTROPHIC HAMSTERS WITH HYPERTROPHIC CARDIOMYOPATHY. A.J. Crawford and S.K. Bhattacharya. Surgical Research Laboratory, University of Tennessee Medical Center, Memphis, TN 38163.

In previous investigations, we have quantitatively established the presence of excessive intracellular calcium accumulation in BIO-14.6 strain dystrophic hamsters (DH) and C57BL/6J-dy^{2J} strain dystrophic mice, in patients with Duchenne muscular dystrophy (DMD), in patients with other neuromuscular disorders, and in prenecrotic muscle from male human fetuses at risk of DMD (Neurology,34:1436, 1984). The tissue zinc (Zn) content in muscular dystrophy (MD), however, has not been extensively characterized. Recently, zinc has been shown to play important roles in protein synthesis, cardiac malfunctions, cellular necrosis, collagen cross-linking, and wound healing. In a study of striated muscle, Zn has also been shown to act much like UO_2^{2+} on the membranes by prolonging the action potentials and muscle contraction times. Since all of these process are intricately associated with the dystrophic lesions, we measured the Zn content in cardiac and skeletal muscles of 7-month-old male DH and age and sex matched BIO-F1B normal hamsters (NH). The atomic absorption spectrophotometric technique of Bhattacharya et al. (Analytical Letters,17:1567,1984) was employed for Zn determinations in the nitric acid extracts of the tissues.

Zinc Contents in mg/kg Fat-Free-Dry Tissue (Mean ± SEM)

Group(N)	Myocardium	Diaphragm	Rectus Femoris
NH (10)	81.17±3.25	125.16±3.39	72.14±5.14
DH (8)	105.20±15.30	153.25±7.25	131.11±12.84
Significance	p<0.002	p<0.005	p<0.001

The tissue Zn contents of the myocardium, diaphragm, and rectus femoris were significantly elevated in DH compared to NH. This excessive intracellular Zn accumulation is physiochemically compatible with the increased protein synthesis in the diaphragm and heart, the significant ventricular hypertrophy, the characteristic electrocardiographic and mechanophysiologic abnormalities, and the histological changes observed in DH. These observations suggest a possible physiologic role of Zn in MD and perhaps implicate an accelerated effort by the cellular system(s) to repair the tissues damaged by the dystrophic processes.

[Supported in part by a grant from the Muscular Dystrophy Association of America.]

118.10 CHANGES IN THE RATE OF OXIDO-REDUCTION OF THE NADH IN THE RESPIRATORY CHAIN IN TOAD RETINA INDUCED BY CADMIUM IONS. Carlos Rodríguez-Estrada. Cátedra de Fisiología, I.M.E., Facultad de Medicina, U.C.V., Caracas, Venezuela.

In a recent review G.P.Cooper et.el. (Cellular and Molecular Neurotoxicology, Raven Press,N.Y.1984) postulated an anabolic effect of heavy metals as a partial explanation to the released of neurotransmitters by the impairment of energy production. In this work it has been studied the oxido-reduction change of nicotinamide adenine dinucleotide (NADH) of mitochondrial respiratory chain. Fluorometric determinations were performed in several preparation (13) as previously described. Each isolated preparation was placed in a gas circulated chamber at a constant temperature (298K). A full reduction of NADH was obtained after a period of anoxia, i.e. 5 minutes of circulating moistened nitrogen. Subsequently this gas (nitrogen) was replaced by oxygen. A 5 minute period in oxygen was allowed before a drop of Ringer solution with 10 micro-Mole of cadmium chloride was placed on top of the preparation. Then a second period of anoxia was repeated followed by oxygen. The rate of change of oxidation was measured timing it from the largest reduction to half oxidation of NADH ($t_{1/2on}$). The mean value after cadmiun ions was 7.679 seconds (SD =3.735, SEM =1.078) and the control, (i.e. preparation Cd ions-free) was 3.228 seconds (SD= 1.549, SEM= 0.447), P less than 0.001 (Student test =7.1; Underwood,B.J. et.al. Elementary Statistics) was obtained. These results indicated an action of cadmium ions on the respiratory chain, and supports the postulated anabolic effect as a partial explanation of the effect of heavy metals to the released of neurotransmitters.

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118.11 EFFECT OF INTRACEREBROVENTRICULAR INSULIN ON METABOLIC SUBSTRATES OF HYPOTHALAMIC-LESIONED RATS. E.C. Lotter and L.L. Bernardis. Univ. of Rochester, Sch. of Med., Rochester, NY 14610 and Vet. Administration Medical Center, Buffalo, NY 14215.

Recently, we have reported that insulin infused into the cerebrospinal fluid (CSF) of normal and ventromedial hypothalamic lesioned rats (VMNL) resulted in a significant decrease of plasma epinephrine (E) and norepinephrine (NE) with no significant change in plasma insulin or glucose. The present study, extends the previous findings and examines plasma glycerol, free fatty acids, triglycerides and plasma protein of VMNL rats.

Weanling male Wistar rats (n = 10) received bilateral electrolytic lesions of the VMN; sham-operated animals served as controls. At maturity, free-moving unanesthetized VMNL rats (832 ± 25 g) and controls (420 ± 11 g) were infused with artificial rat CSF or CSF plus rat insulin (100 μU/15 μl) into the left lateral ventricle for 1 h.

Infusion of CSF alone had no reliable effect on plasma E and NE or glucose. Infusion of insulin resulted in a significant decrease of E and NE ($p < 0.05$). No change in plasma glucose was observed, thus replicating our previous findings. Mean baseline plasma glycerol (VMNL: 8.5 ± 1.78; C: 4.7 ± .28 mg%); free fatty acid (VMNL: 1438 ± 537; C: 890 ± 49.5 μM/L); triglycerides (VMNL: 136.9 ± 68; C: 32.9 ± 6.2 mg/dl) were significantly different between the VMNL rats and their controls. No significant difference in plasma protein was observed between the two groups.

These data suggest that CSF insulin infusions may have a direct effect on plasma E and NE of normal and obese rats and may account for the observed reduction of food intake and body weight.

FUNCTIONS OF GLIA II

119.1 SYNERGISTIC ACTION OF FIBROBLAST GROWTH FACTOR AND DEXAMETHASONE ON ASTROGLIAL CELL MITOGENESIS. Douglas A. Kniss and Richard W. Burry, Dept. of Anatomy and Neuroscience Research Laboratory, The Ohio State University, College of Medicine, Columbus, OH 43210.

Astroglial cell proliferation is an important component of CNS ontogeny. In addition, marked astroglial hyperplasia is a common feature of CNS trauma. We have used a primary cell culture model in an effort to understand the putative signal molecules governing glial cell cycle activity.

Primary astrocyte cultures were prepared from 2-d rat cerebellum. After 9-10 d in vitro cells were subcultured into 24-well plates at $1x10^5$ cells per well with 10% fetal calf serum (FCS). Glial cells were growth arrested with 0.25% FCS and, after 2-4 d growth in low serum, were stimulated with test substances and labeled with ^{3}H-TdR for 24 h. DNA synthesis was assessed by liquid scintillation counting of trichloroacetic acid-insoluble radioactivity to serve as a measure of cell proliferation.

Initial experiments tested several peptide growth factors (Epidermal Growth Factor, Fibroblast Growth Factor, Platelet-derived Growth Factor, and insulin) for their ability to stimulate DNA synthesis in serum-free medium. Only bovine brain FGF induced a significant enhancement of astroglial cell DNA synthesis. Half-maximal stimulation occurred at 2-3 ng FGF per ml.

Previously, we reported that dexamethasone (DEX) arrested the growth of astrocytes cultured in FCS-containing medium (Kniss and Burry, Soc. for Neurosci. Abstr. 10 (1984) 764). In another experiment, we examined whether similar growth arrest could occur when DEX was presented to cells stimulated by FGF (a brain tissue-derived mitogen). When DEX and FGF were presented simultaneously to growth arrested astrocytes there was a potent synergistic effect on DNA synthesis. That is, cells were stimulated almost 2-fold over levels seen in FGF alone or in FGF plus 5% FCS. Moreover, DEX alone caused a 50% growth arrest in serum-stimulated astrocytes. These results suggest that FGF-induced mitogenesis is potentiated by DEX, while serum-stimulated mitogenesis is inhibited by DEX.

FGF, PDGF, and EGF were generously provided by D. Gospodarowicz, W.J. Pledger, and S. Cohen, respectively. (Supported by NS-19961 (RWB), The Spinal Cord Injury Research Center, NS-10165 (RWB), and Sigma Xi (DAK), and a Graduate Alumni Research Award from The Ohio State University Graduate School (DAK).

119.2 PROLIFERATION AND DIFFERENTIATION OF ASTROCYTES IN RESPONSE TO NEURAL TRAUMA. B.T. Faddis and V.K. Vijayan. Dept. of Human Anatomy, School of Medicine, Univ. of Calif., Davis, CA 95616.

By contributing to glial scar formation, astrocyte proliferation may play a key role in both the functional recovery of damaged neural systems and the successful integration of neural transplants with host tissue. Previous studies of neural trauma using pulse injections of ^{3}H-thymidine have shown labelling of 1-2% of GFA-reactive astrocytes in the rat cortex (Dev. Biol., 72:381, 1979). Combining autoradiography and GFA immunohistochemistry with plastic embedding, we examined trauma-induced proliferation of astrocytes in the cortex and hippocampal neuropil of the rat brain to determine: (1) whether a greater magnitude of astrocyte proliferation could be demonstrated with repetitive intralesion injections of ^{3}H-thymidine, and (2) whether the morphologically distinct stages of astrocyte differentiation observed in vitro (J. Neuroimmunol., 2:235, 1982) could be identified in vivo during the course of astrocyte proliferation.

Adult, male Sprague-Dawley rats received unilateral needle wounds through cortex and hippocampus (N=4). They received daily intralesion injections of 10.0 μCi ^{3}H-thymidine at 24,48,72, and 96 hours postlesion. Control rats (N=6) did not receive lesions or thymidine. Rats were sacrificed by vascular perfusion with buffered 4% paraformaldehyde one hour after the last injection. Seventy μm slices through the lesion were stained immunohistochemically for GFAP, flat embedded in methacrylate, and sectioned at 1.0 μm for autoradiographic processing.

In control rats, astrocytes showed a regional difference in their numerical densities. Density in the cortex (2.2 per oil immersion field) was less than that of the hippocampal neuropil (3.0 per field) ($P < .02$). Repetitive injections of thymidine resulted in labelling of 40% of astrocytes in both cortex and hippocampus at three days postlesion. Although the hippocampal astrocyte population showed no increase over control numbers, those of the cortex demonstrated an increase of 200% ($P < .01$). Several stages of astrocyte differentiation were apparent in "stabbed" tissues. An astroblast stage with little or no GFA staining and immature processes characterized the earliest form detected. A ring of GFA-staining cytoplasm then appeared around the nucleus followed by vacuolation of the cytoplasm which lead to the formation of thicker processes and development of the mature astrocyte.

We have shown that substantial astrocyte proliferation occurs within three days of neural trauma. Reactive astrocyte proliferation is associated with astrocyte differentiation similar to what has been shown in culture. These studies suggest that neural trauma will prove to be an ideal model for the in vivo testing of various factors known to affect astrocyte behavior in culture.

119.3 POST-ISCHEMIC CHANGES IN PERINEURONAL GLIA. C.K. Petito, Department of Pathology, New York Hospital-Cornell Medical Center, New York, N.Y. 10021.

The response of perineuronal (PN) glial cells to cerebral ischemia and to the presence of ischemic neuronal necrosis was studied by electron microscopy (EM) and by quantitative estimates of cell size and number. Ischemia was produced in rats by permanent occlusion of the vertebral arteries and 30 minute occlusion of the common carotid arteries. This insult produces severe and consistent ischemic neuronal damage in the dorsolateral striatum (Stroke 10:267-72, 1979) and is associated with early proliferative changes in astrocytes. Animals were sacrificed by perfusion-fixation following post-ischemic (P-I) intervals of 3, 15, 30, 120, and 180 min and pieces of dorsolateral striatum were prepared for EM . Dark oligodendrocytes (oligos) represented 35.5% of control PN glia and showed no significant post-ischemic changes in cell size or morphology although a relative increase (65% of all PN glia) was noted at 180 min. Medium oligos represented 55% of control PN glia and showed a significant mild and persistant increase in cell size by 3 min after ischemia and a marked decrease in number to 6.5% of all PN glia at 180 min. Astrocytes represented 11% of control PN glia. A significant increase in size occurred at 120 min and an increase in number to 23% of all PN glia at 180 min; morphologic changes included mitochondrial alterations as previously reported (Ann Neurol 11:510-18, 1982) and microtubule proliferation; intermediate filaments were present but sparse. Transitional PN glia appeared first at 15 min P-I and showed characteristic EM features of oligos as well as of astrocytes (dispersed nuclear chromatin) or microglia (myelin figures and lipid inclusions). Well-differentiated microglia were seen at 180 min, contained lipid inclusions, myelin figures, and long wavy endoplasmic reticulin cisterns, and represented 8.5% of all PN glia. These results suggest that the PN glia identified by EM criteria as medium oligos are transformed into reactive astrocytes or into microglia in areas of ischemic neuronal necrosis. The current data is insufficient to determine if medium oligos seen in controls actually represent oligos, intermediate glia, or true astrocytes and microglia unidentifiable as such by EM criteria alone.

119.4 ASTROCYTIC REACTION IN CULTURE FOLLOWING ISCHEMIA. W.J.Goldberg J.R.Connor and J.J. Bernstein. Lab. CNS Injury and Regeneration, Veterans Administration Medical Center, Washington, DC. 20422 and Depts. of Physiology and Neurosurgery, George Washington University School of Medicine, Washington, DC. 20037.

Reactive astrocytes are observed in response to injury to the CNS. Such cells are morphologically characterized by fibrillogenesis and an increase in the density of staining reaction with anti-GFAP. We report here the appearance of astrocytes with altered morphologies and increased reaction with anti-GFAP in primary cultures of rat spinal cord following 4 hours of ischemia and 24 hours recovery. Dissociated cultures of spinal cord cells from day 14 gestation rat embryos were maintained for 3 weeks in nutrient medium supplemented with a survival factor isolated from horse serum (Kaufman and Barrett, Science 220:1394,1983). Ischemia was induced by removal of oxygen and glucose from the culture medium (Goldberg et al. Neurosci. Abstr. 9:855,1983) for a period of 4 hours. 24 hours after the return to normal culture medium the cells were fixed in 3.7% buffered formaldehyde and stained immunohistochemically for GFAP (GFAP antiserum provided by Dr. L.F. Eng, VA, Palo Alto) using the peroxidase-antiperoxidase reaction for visualization. In control cultures the majority of GFAP-positive cells had an unreactive nucleus and 2 major processes leaving from the soma which divided into many fine processes at the terminus. Following ischemia 2 new classes of staining reaction were observed. The predominant reaction was more intense than that observed in control cultures and the astrocytes had an altered morphology. The soma became more prominent and now had many short, broad processes which were less branched than those observed in control cultures. Some astrocytes were observed which had increased reaction product density but not the morphological alterations. In some regions of the ischemic cultures normal and reactive glial cells were observed in the same photographic field. In experiments involving shorter periods of ischemia these intensely staining astrocytes were not observed. When spinal cord cultures were subjected to a similar period of anoxia (removal of oxygen from the culture medium and surrounding atmosphere) no increase in the density of reaction product with anti-GFAP was observed but the morphological alterations previosly described were wide-spread. The appearance of intensely GFAP-positive astrocytes with altered morphology seems to be related to the extent of the damage suffered by the culture, as measured by loss of neurons and neuronal processes, and resembled reactive astrocytes observed in vivo following CNS insult.
Supported by the Veterans Administration.

119.5 MORPHOLOGICAL CHARACTERIZATION OF REACTIVE ASTROCYTES IN EXPLANT CULTURES OF GLIAL SCARS. Patricia A. Trimmer. Department of Neurological Surgery, School of Medicine, University of Virginia, Charlottesville, VA 22908.

Astrocytes become reactive and form a glial scar in response to a variety of pathological conditions which induce axonal degeneration in the CNS. A number of questions on the subject of reactive astrocytes and their role in axonal sprouting and regeneration in the CNS remain unanswered. Glial scars derived from rat optic nerve were used in this study to establish and characterize the morphology of reactive astrocytes in vitro. Glial scars were formed in 5-7 day-old rat pups by unilateral enucleation. The unoperated control optic nerves and glial scars were excised 14 days later using sterile techniques. The dural sheath was removed and the tissues were cut into 1-2 mm segments. The explants were grown on poly-D-lysine coated glass chamber slides in media consisting of Eagle's basal metabolic medium with Earle's salts supplemented with 10% fetal calf serum, 0.1% glutamine and 0.6% glucose. During the first week in culture, both polygonal and process-bearing cells grew out of the glial scar and optic nerve explants. The outgrowth of cells from the explant proper slowed down and after about 2 weeks appeared to stop. Examination of explants processed for electron microscopy revealed that the glial scars were composed of a homogeneous population of hypertrophied astrocytes whose cell bodies and processes contained large numbers of densely-packed intermediate filaments. Other prominent features of these reactive astrocytes were irregularly-shaped euchromatic nuclei, microtubules, and wide-bore cisternae of rough endoplasmic reticulum. The ultrastructural morphology of reactive astrocytes in culture closely resembled the morphology of reactive astrocytes in vivo. However, reactive astrocytes in vitro did not contain the large quantities of glycogen normally found in reactive astrocytes in vivo. Explants of normal optic nerve underwent degeneration in culture. In general, astrocytes in the central portion of optic nerve explants also became reactive and formed a glial scar. These observations illustrate that reactive astrocytes can be maintained in culture and that cultured reactive astrocytes exhibit ultrastructural features comparable to reactive astrocytes in vivo. Furthermore, astrocytes in normal optic nerves which degenerate in vitro can become reactive and form a glial scar. These findings suggest that explant cultures of rat optic nerve and glial scar can be useful models for future studies of the causes, characteristics and consequences of the reaction of astrocytes to CNS injury.
Supported by NIH Training Grant NS07199.

119.6 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NORMAL AND REACTIVE GLIA IN THE ADULT FROG SPINAL CORD. F.J. Liuzzi and R.H. Miller*, Dept. of Anatomy, Case Western Reserve Univ., Cleveland, Oh. 44106

Recent studies (Sah and Frank, J. Neurosci. 4:2784, 1984 and Liuzzi and Lasek, J. Comp. Neurol. 232:456, 1985) have shown that dorsal root axons regenerate into the spinal cord of adult frogs. This suggests that the amphibian glial response does not impede axonal regeneration. In this study we used immunohistochemical techniques to characterize the glia of the normal frog spinal cord and to study their response to axonal degeneration within the cord subsequent to dorsal root section.

Previous studies have shown that the adult frog spinal cord contains only one morphological type of astrocyte (Sasaki and Mannen, J. Comp. Neurol. 198:13, 1981). These radial glial cells have their somata within the gray matter and have long processes that extend through the white matter to the cord surface where their endfeet form the glia limitans. In addition, Dahl and Bignami (Brain Res. 61:279, 1973) have demonstrated GFAP positive elements in the adult frog spinal cord. However, they did not describe the detailed distribution of the protein in the gray and white matters.

In transverse sections of normal adult frog spinal cord, we have found that GFAP immunofluorescence reveals radially oriented processes within the white matter. There are, however, no GFAP positive cell bodies within this region. By contrast, within the gray matter, the processes of the radial glial cells were either weakly GFAP positive or negative. Similar patterns of immunofluorescence were obtained with vimentin antibody.

Following dorsal root section, the intensity of GFAP and vimentin immunofluorescence increased dramatically in the white and gray matters. Moreover, within the dorsal funiculus, there appeared to be a breakdown of the radial orientation.

These results suggest that in the normal frog spinal cord, both GFAP and vimentin appear to be preferentially localized within those regions of the radial glial processes that extend through the white matter. In response to axonal degeneration, the radial glial cells become reactive showing increased GFAP and vimentin immunofluorescence. Changes in the glia may contribute to the support of axonal regeneration in the adult frog spinal cord.

119.7 **Interleukin like molecules in normal and injured brain.**
M. Nieto-Sampedro, K.G. Chandy*, M. Berman*, C.I .Sandborg*, C.W. Cotman, S. Gupta* and G.J. Friou*. Dept. of Psychobiology and Dept. of Immunology, University of California, Irvine, CA 92717.

The mitogenic activity of extracts of rat brain on purified astrocytes in culture increased up to 3-fold in the tissue surrounding a brain injury. Maximal activity occurred 6 days postlesion and was still twice the basal level 20 days later.

Rat brain also contained molecules capable of promoting thymidine incorporation into phytohemoagglutinin stimulated mouse thymocytes. Such IL-1 like activity increased about 10-fold following brain damage, and its increase postlesion paralleled or slightly preceeded that of astrocyte mitogens. Brain "IL-1" behaved in a Sephacryl S-200 column as authentic IL-1 did, giving peaks at apparent molecular weights of 30,000, 15,000 and 5,000. The most abundant species in brain was that of 15,000 molecular weight. Monoclonal antibodies to human IL-1 inhibited 60 to 70 % of the brain mitogenic activity on astrocytes and thymocytes and more than 90% that on fibroblasts.

Purified brain astrocytes in culture secreted in their growth medium at least 5-times as much IL-1 like activity as similar numbers of peripheral monocytes. However, in the case of astrocytes the most abundant species was that of mol.wt. 5,000 (about 70%) followed by mol.wt. 15,000 (ca. 30%). The high molecular weight species was barely detectable.

Brain injury caused a 7-10 fold increase in the activity of a mitogen promoting thymidine incorporation in IL-2-specific CTLL cells. In a hplc sizing column, this mitogen had an apparent molecular weight of 23,000 whereas under identical conditions murine IL-2 had a mol.wt.of 15,000.

Brain interleukin-like molecules could play a regulatory role in glia proliferation and hypertrophy after CNS injury. In normal brain, IL-1 may control hypothalamic pituicyte swelling, thereby contributing to thermoregulation and regulation of water intake.

119.8 IMMUNE RESPONSES IN BRAIN. L.L.Y. Chun* and A. Rao*. (SPON:K.J. Sweadner). Dept. Neurology, Massachusetts General Hospital, Boston, MA 02114 and Div. of Immunopathology, Dana Farber Cancer Institute, Boston, MA 02115.

The central nervous system (CNS) has classically been thought to be outside the surveillance of the immune system because it is protected by the blood brain barrier and lacks lymphatic drainage. Recent evidence, however, suggests that endogenous brain cells may play a role in the generation of immune responses in the CNS. Two aspects of these responses which appear to mediated by astrocytes are reported here. In these studies we dissociated tissue from early postnatal mouse brain and prepared cultures which were at least 90-95% astrocytes as judged by immunofluorescent staining with antibodies to glial fibrillary acidic protein (GFAP).

We studied the ability of these dissociated cells to present antigen to T helper lymphocytes. T cell clones specific for ovalbumin or p-azobenzenearsonate co-cultured with astrocytes exhibited increased proliferation as measured by ^{3}H-thymidine incorporation. This effect was antigen specific and major histocompatibility restricted (MHC). Similar results were obtained when T cell activation was measured by interleukin-2 production. In double-label immunofluorescent experiments with antibodies to GFAP and I-region associated antigens (Ia), we found that at least a subpopulation of astrocytes activated by T cell clones expressed Ia, whereas astrocytes cultured alone did not. These data suggest that one role the astrocyte may play in brain immune responses is that of an antigen presenting accessory cell.

We have also studied the ability of astrocytes to mediate target cell lysis in vitro. Co-cultures of astrocytes and T helper cell clones exhibited the ability to kill P815 tumor cells. The ability of the astrocytes to be activated to cytotoxicity was antigen specific and MHC restricted. In addition, cells from several CNS areas were competent, whereas cells obtained from several different ganglia in the peripheral nervous system were not. It is not yet known whether the ability to present antigen and the ability to mediate target cell lysis reside in the same cell.

The finding that astrocytes can actively participate in immune responses may have important implications where there is acute injury to the brain or in certain neurologic diseases such as multiple sclerosis, where there is evidence of lymphocyte infiltration into the CNS. Whether these mechanisms also play a role in other types of brain pathogenesis is unclear.

119.9 GLIA RESPOND TO SYSTEMIC IMMUNE DISEASE. L. Lonsberry* and W.S.T. Griffin. Univ. of Tx. Hlth. Sci. Ctr., Dallas, Tx. 75235.

Graft-vs-host-disease (GVHD) is a systemic immune disease characterized by high serum titers of factors produced by activated lymphocytes (Grebe, S. & Streilein, W., Adv Immunol., 22:119, 1976), increased cortisol levels (Hoot, G., et al., Transplant., 35:478, 1983), increased gamma interferon (IFN-) levels (Zawatsky, R., et al., Can Fed Biol Sci., 22:415, 1979), and lymphocytic infiltration of many organs, but not brain. Lymphocytic infiltration is associated with increases in class II antigens, suggested infiltration targets (Suitters, A. & Lampert, I., Transplant., 38:194, 1984). In vitro, either cortisol or soluble factors from activated lymphocytes stimulates astrocyte proliferation, RNA synthesis, and elaboration of growth-factor-like products (Fontana, A., J Neurosci Res., 8:443, 1982), and IFN- induces class II antigens (Ia) (Wong, G., et al., Nature, 310:688, 1984). We have investigated whether or not experimentally-induced GVHD 1) mimics in vitro responses to immune factors and cortisol, i.e., proliferation of glial-fibrillary-acidic-protein (GFAP) immunoreactive astrocytes and 2) induces Ia expression in the absence of infiltrating lymphocytes. Brain tissue was collected from FI rats with GVHD induced on the day of birth by intravenous injection of DA rat lymph node cells, as well as from rats with adult-onset GVHD. Uninjected littermates were controls. One-half of each brain was fixed in Bouin's, embedded in paraffin, and stained with antibody to GFAP using a peroxidase-anti-peroxidase technique, while the other half was mounted in OCT (Miles) and frozen at -70C. After sectioning, frozen tissue was acetone fixed and immunoreacted with antibodies to GFAP and Ia. There was an age-related, progressive increase in GFAP immunoreactivity in rats with neonatally-induced disease compared to their littermates. Moreover, rats with adult-onset GVHD had a similar increase in GFAP immunoreactivity, signifying that the blood brain barrier does not exclude GVHD-induced, astrocyte stimulating factors. Compared to controls, there were more GFAP-immunoreactive cells and more immunoreactive product in individual astrocytes. This gliosis was not associated with an induction of Ia, suggesting that other facets of GVHD, such as increased titers of immune factors and/or alterations in hormone levels are responsible for the GVHD-induced gliosis in neonatal as well as adult rats. The lack of Ia induction in the brain during GVHD may be related to the absence of lymphocytic infiltration in brain. This non-cell mediated gliosis is reminiscent of that in Alzheimer's and other dementing disorders, suggesting immune involvement in neuropathologies. We propose GVHD as a model for studying factors generated during the immune response and their varied effects on the brain. This work was supported by AI 14663 and AG 05537.

119.10 INHIBITION OF ASTROCYTE VOLUME CONTROL BY CIRCULATING FACTORS ASSOCIATED WITH REYE'S SYNDROME. J.E. Olson, R. Sankar, and D. Holtzman. Dept. Psych. and Neurol., Tulane Univ. Sch. of Medicine, New Orleans, LA 70112

Reye's Syndrome is an acute childhood illness with severe intracellular brain edema. The brain pathology is characterized by large, swollen astrocyte cell bodies and foot processes with little apparent change in extracellular volume (Partin et al., J Neuropath Exp Neurol 34:425-444, 1975). Serum factors; including elevated levels of short chain fatty acids such as octanoate, prolonged elevation of NH_4^+, and the presence of salicylates; have been implicated in the pathogenesis of brain edema in Reye's Syndrome. To investigate potential mechanisms of cellular brain edema, we have measured the effects of Reye's Syndrome serum factors on energy metabolism and volume control in primary cultured rat cerebral astrocytes.

Astrocytes suspended in hypo-osmotic medium swell initially and then shrink toward the volume of cells maintained in iso-osmotic medium. This regulatory volume decrease is energy-dependent and tightly coupled to (Na,K)-ATPase activity (Olson et al., Biophys J 47:475a, 1985). The astrocyte regulatory volume decrease is completely inhibited with 1-4 mM octanoate, concentrations observed in the serum of Reye's Syndrome patients. Very high NH_4^+ (15 mM) or salicylate (10 mM) concentrations do not affect the astrocyte regulatory volume decrease. At these concentrations, octanoate, but not salicylate, inhibit basal oxygen consumption in astrocytes. Salicylate, more than octanoate, increases respiration in cells previously exposed to oligomycin (an inhibitor of oxidative phosphorylation). Octanoate (0.4 mM) also reduced by 30% the component of astrocyte oxygen consumption inhibited by ouabain, suggesting a direct effect on (Na,K)-ATPase activity. Thus, in Reye's Syndrome, elevated serum levels of octanoate, but not NH_4^+ or salicylate, could produce cellular brain edema by interfering with astrocyte volume control. This effect is not related to uncoupling of mitochondrial electron transport, but may be a result of direct inhibition of astrocyte (Na,K)-ATPase.

This research was supported by the Robert Katz Medical Research Foundation and a Tulane University Biomedical Research Support Grant.

119.11 REVERSIBILITY AND SODIUM INDEPENDENCE OF MONENSIN-INDUCED GOLGI SWELLING. A.F. Boyne, P. Yarowsky, R. Wierwille* and N. Brookes. Dept. of Pharmacol. & Exp. Therap., Univ. of Md. Sch. of Med., Balt. MD 21201.

The sodium-selective ionophore, monensin, is well known to cause swelling of Golgi cisternae and block of normal Golgi function in several cell types. This effect is generally thought to result from sodium loading of the cytoplasm. We have shown that in mouse cerebral astrocytes monensin causes a large, rapidly reversible increase in glucose use, measured by tritiated 2-deoxy-glucose uptake, in addition to causing marked Golgi swelling and vacuole formation. Whereas monensin-stimulated glucose utilization was abolished in a Na-free solution, the Golgi effect was still apparent.

Cell cultures of mouse cerebral astrocytes were exposed to 5 μM monensin in Tris-buffered balanced salts solution (BSS) at 35°C for 20 min. The cultures were prepared for EM study by quick freezing or by aldehyde fixation. Golgi swelling increased in the cis-trans direction across the stack, with spherical vacuoles appearing to arise from the trans cisternum. A similar pattern was present in cultures exposed to monensin in Na-free (choline substituted) solution. Astrocyte Na-loading by 20 min exposure to zero K^+ caused relatively selective swelling of the trans Golgi cisternum and small vacuole formation.

Morphometric analysis of aldehyde fixed cultures showed that the effects of 5 μM monensin and zero K^+ solution were additive, together producing 10 ± 0.9% (SE, 25 cells) vacuolization, as a fraction of the total non-nuclear cytoplasmic area. Morphologic recovery was rapid on returning these cultures to normal BSS at 35°C. By 10 min after washing, vacuoles occupied 7 ± 0.8% (7 cells) and by 20 min they had reverted to the control level of 2.4 ± 0.4% (16 cells).

Thus, monensin-induced swelling of astrocyte Golgi is at least partially independent of Na-loading. The rapidity with which the swelling reverses on washing suggests that the ionophore moves readily in and out of cell membranes. It further suggests an ability to penetrate directly to the Golgi. Swelling may then occur by exchange of Golgi protons (which are not osmotically active) for cytoplasmic Na. Further active H^+ pumping and passive Cl^- entry would give rise to osmotically active salt accumulations, and visible swelling.
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119.12 Ca-CONTROLLED, REVERSIBLE STRUCTURAL TRANSITION IN MYELIN

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The myelin in goldfish spinal cord, originally compact, swells spontaneously when excised cord is kept in physiological saline at 23°C without any fixation. As studied by X-ray diffraction, this myelin initially has a 150 Å repeating distance (from one major dense line to the next), but it gradually converts to a slightly swollen, although equally regular, form having a 175 Å repeat. No intermediate repeat is detected (A. E. Blaurock, J. L. Yale and B. I. Roots, Trans. Am. Soc. Neurochem. 16, 183 [1985]).

We now report that Ca is required in the saline for swelling to occur in goldfish spinal-cord myelin and that, after an initial swelling phase, swelling can be reversed by removing Ca. First, about half the myelin converts to the swollen form after one day in the normal saline, but none of the swollen myelin is detected after a day in saline lacking Ca. Second, 6.5 mM NaCN in the saline speeds up the rate of swelling about four-fold, but there is no swelling if Ca is omitted from the saline containing NaCN. Other agents that accelerate swelling when added to the normal saline (colchicine, β lumi-colchicine, A23187, high pH) also fail to have any effect in the absence of Ca. Third, when a NaCN-treated cord is treated subsequently with Ca-free and NaCN-free saline containing 10 mM EDTA, about half of all the myelin converts back to the normal, compact form; however, if NaCN is present in the Ca-free saline, there is no recovery and more of the compact myelin swells. Fourth, myelin first swollen in saline without NaCN also tends to recover in Ca-free saline, but to a lesser extent than after swelling in saline containing NaCN. Regarding specificity for Ca, only a trace of the myelin swells when Ba or Sr is substituted for Ca in the saline. A similar cycle of swelling and recovery has been induced in human oculomotor-nerve myelin but not in human optic-nerve myelin.

These results demonstrate that some kinds of myelin are bistable. They are consistent with the hypothesis that in some cases active metabolism maintains normal, compact myelin by keeping down Ca concentration within the sheath. Although the swelling here is pathological, it would appear possible for a limited region of a normal internode to swell due to a local influx of Ca *in vivo*, as a precursor to large-scale opening of the interbilayer spaces, e.g. in Schmidt-Lantermann clefts. Finally, our results suggest a new basis for myelin pathology similar to the failure of heart muscle cells due to excessive free Ca inside them.

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DEVELOPMENTAL DISORDERS I

120.1 TRISOMY 16 IN MOUSE AS A MODEL OF DOWN'S SYNDROME IN HUMANS: ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS MAINTAINED IN CULTURE. C. Orozco* C.J. Epstein* S. Smith* and S.I. Rapoport (SPON: Ch. Edwards). Lab. of Neurosciences, NIA. Bethesda MD 20205 and Dept. of Pediatrics. School of Medicine. Univ. of Cal. San Francisco.

Alterations in the electrical membrane properties of neurons have been postulated to cause mental retardation in subjects with Down syndrome (DS)(Scott, et al. 1982. Dev Brain Res. 2: 257-270). Trisomy 16 in mice is considered as to be a model of trisomy 21 in humans (Epstein et al. 1984. In: Research Perspectives in Cytogenetics. Sparks & de la Cruz Eds. Univ. Park Press. Baltimore). The purpose of this study was to characterize the electrical membrane properties of normal and trisomic neurons from mice embryos to correlate with the alterations seen in human neurons.

The experimental embryos were obtained by breeding normal females C57BL/6N with heterozygous males with double Robertsonian translocation from the strains 32Lub/2H or 9Rma/32Lub. 10% of the embryos are trisomic and have 2 metacentric chromosomes. The rest of the embryos with normal numbers of chromosomes and only 1 metacentric chromosome, were taken as controls. Dorsal root ganglia neurons taken from 12-14 days old embryos were dissociated and maintained in culture up to 3-4 weeks. A bridge amplifier (WPI model M-707) was used for intracellular recording and stimulation with single micro-electrodes filled with 3M KCl. The pipet resistance varied from 40 to 120 megaohms.

Control neurons showed a resting membrane potential (Vm) -48.2 ± 1.1 mV (S.E.M.)(n=59). They could conduct spontaneous and evoked action potentials whose amplitude and duration were 84.4± 2.1 mV (n=20) and 3.0± 0.1 msec (n=24). The magnitude of the after-hyperpolarizing potential was 13.4± 1.0 mV (n=24). The maximal rate of rise of the action potential was 120.0± 1.5 V/sec meanwhile the time required for repolarization from the peak of the spike to 25% of the spike height was 3.9± 0.2 msec (n=15). The threshold of excitation at 50 msec of duration pulse was 1.8±0.3 nA (n=20). These values are similar to published values for normal cells (Ransom et al. 1977. J. Neurophysiol. 40: 1132-1150) and have showed that the translocation of one chromosome had no effect in the neuronal electrical membrane properties.

In 29 trisomic neurons Vm was similar to that in the controls, -48.4± 2.9 mV. Action potentials were also similar. Differences were found in the duration of the repolarization; however, due to the small number of cells sampled (n=7) additional experiments are required to support the suggestion that the trisomic cells posses altered electrical membrane properties. One clear difference was a lower survival of the trisomic cells in culture.

120.2 DEVELOPMENT OF CAUDAL AND SCIATIC NERVE CONDUCTION IN THE TWITCHER MOUSE AND HETEROZYGOUS AND NORMAL LITTERMATES. S. D. Lavine,* K. E. Burrell* and Ch. E. Olmstead.(Spon: G. C. Galbraith) UCLA School of Medicine, Mental Retardation Research Center Group at Lanterman State Hospital, Pomona, Ca. 91769.

The murine mutant "twitcher"(C57Bl/6J-twi) is an enzymatically authentic model for human globoid cell (Krabbe's) leukodystrophy (Suzuki & Suzuki, Am. J. Pathol, 1984, 111). This recessively transmitted genetic deficiency is for galactosylceramidase. We have previously (Soc. Neurosci. Abstr., 1983, 1984) described the neurological and neurobehavioral development of the mutant twitcher and its littermates. In addition to the progressive debilitation of the affected animal we have described some subtle differences between the normal and the heterozygote during development that suggest that this mutant may also be an excellent model for heterozygote risk for subtle neurological disease during life span development. The studies described here were carried out to develop a better understanding of the functional pathophysiology of this demyelinating disease in both the homozygously affected and heterozygous carriers.

Enzyme Assays. Between 5 and 8 days of age, lengths (<0.5cm) of tail were clipped and galactosylceramidase was determined by an assay adapted from Suzuki (Meth. Enzymol., 1978, 50). Tail and sciatic nerve conduction velocities (NCV) were performed under Nembutal anesthesia with the mouse immobilized on a temperature controlled (38 °C) platform.. Tail stimulation and recording was via needle electrodes inserted into the tip of the tail and at two points along the tail, respectively. For the sciatic the nerve was surgically exposed and elevated on silver hook electrodes in a warmed pool of mineral oil. Stimulation was to the pad of the foot through needle electrodes. Results of in vitro recordings from excised, desheathed sciatic nerve will also be reported.

The three genotypes were clearly different on the basis of tail NCV by 21 days of age with Normal > Heterozygote > Affected. The tail NCV of the affected mouse decreased from a median of 11.2 m/s at 21 days to 6.8 m/s at 36 days and was not elicitable at 42 despite the fact that a tail pinch still elicited a behavioral response. The sciatic showed a similar pattern in the affected animal showing a high of 12.0 m/s at 21 days decreasing to 6.5 m/s at 30 to 35 days of age and it was unmeasurable by 42 days. Generally this decline was accompanied by severe, observable, edema, but a hind limb withdrawal response remained. The tail NCV difference between the normals and the heterozygotes(22.5 versus 13.2 m/s) was clearly present at 21 days and both groups showed a consistent increase in velocity with aging. They were still significantly different (26.8 m/s vs 17.7 m/s) after 50 days of age. Similar findings will be reported for the sciatic nerve.

120.3 **SLOWED LOCOMOTOR RECOVERY FOLLOWING SCIATIC NERVE CRUSH IN MICE HETEROZYGOUS FOR GALACTOSYLCERAMIDASE DEFICIENCY (C57bl/6j-twi "Twitcher")** Ch. E. Olmstead. UCLA School of Medicine, Mental Retardation Research Center Group at Lanterman State Hospital, Pomona, Ca. 91769

The murine mutant "twitcher"(C57Bl/6J-twi) is an enzymatically authentic model for human globoid cell (Krabbe's) leukodystrophy (Suzuki & Suzuki, Am.J. Pathol,1984,111). This recessively transmitted genetic deficiency is for galactosylceramidase. We have recently developed evidence that this mutant may also be an excellent model for heterozygote risk for subtle neurological disease during life span development. The studies described here were carried out to develop a better understanding of the functional pathophysiology of this demyelinating disease in both the homozygously affected and heterozygous carriers.

Enzyme Assays. Between 5 and 8 days of age, lengths (<0.5cm) of tail were clipped and galactosylceramidase was determined by an assay adapted from Suzuki (Meth. Enzymol.,1978,50) Affected homozygotes(twi/twi), heterozygous carriers and normal animals were readily identified and were assigned to groups based on sex and genotype.

Sixty to 80 day old heterozygous and normal mice received crush or sham lesions of the left sciatic nerve under Nembutal anesthesia. Locomotor recovery was evaluated by a modification (Sugarman & Olmstead, Soc. Neurosci. Abstr., 1983) of the footprint technique of de Medinaceli, et al. (Exp. Neurol. 1982) and Hruska, et al. (Life Sci. 1979). Myelin turnover was estimated by the 24hr uptake into sciatic, spinal cord and brain of i.p. ^{35}S(1mCi ^{35}S $NaSO_4$/100g).

All of the mice with crushed sciatics showed significant disruption of the left leg gait that persisted until 8 to 12 days post-lesion. After that time the normal animals and a few of the heterozygotes showed a progression to complete recovery by thirty days. Significantly fewer heterozygotes showed complete recovery by 30 days post-lesion and those that did recover did so at slower rates than the normal controls. In addition there were subtle differences between the normals and heterozygotes on selected gait parameters during the recovery process. When studied at 40 days post-lesion the heterozygotes showed greater incorporation of ^{35}S into both the crushed and normal nerves when compared with the homozygous normals. This is consistent with other data from this laboratory demonstrating that during development myelin turnover remains high in heterozygotes. These data present both functional and biochemical evidence for slowed recovery of function in mice heterozygous for a demyelinating disease and suggest that carriers for such disease might be at increased risk for subtle neurological disease.

120.4 SPATIAL ABILITY IN A MURINE MODEL OF KRABBE'S DISEASE. R. N. Shull and C. E. Olmstead, UCLA Mental Retardation Research Center Group at Lanterman State Hospital, Pomona, CA 91769.

In the recessively transmitted Krabbe leukodystrophy, evidence indicates that adult carriers perform abnormally on some intelligence subtests, including spatial cognition (Christomanou et al., Hum. Genet. 1981, 58). The twitcher mouse (C57BL/6J-twi) is an anatomically and enzymatically correct model of this disease (Suzuki & Suzuki, Am. J. Pathol. 1983, 111). In order to test for higher-order behavioral abnormalities in heterozygotic murine carriers, two groups of normals and carriers (one per sex/4-6 months of age) were tested in an eight-arm radial maze similar to that described by Pick and Yanai (Int. J. Neurosci. 1983, 21). Genotype determinations were made on the basis of galactosylceramidase activity levels as determined by homogenized tail assays (Olmstead, Soc. Neurosci. Abstr. 1984, 8). Subjects were food deprived to 80% body weight and run one trial per day for 3-4 weeks. No significant genetic differences were found in either group regarding choice accuracy as measured by the number of non-repeated arm entries made during the first eight choices in the food baited maze. Both groups showed a rapid decline over trials in time spent in each arm while also showing a marked tendency to enter the arm closest to the last arm previously entered in a sequential pattern, although several times the subjects did alter this pattern to enter previously missed arms. In groups of aged animals (>20 months) this sequential pattern has not been seen, regardless of sex or genotype. There was also a definite bias in the female carriers for clockwise maze rotation/arm entry with counterclockwise movement seen in the female normals, a situation opposite to that seen in the male group. None of the groups showed significant performance deficits when tested in the dark under red-light conditions. In the female group, both atropine sulfate (0.5-4.0 mg/kg) and scopolamine HBR (0.5-2.0 mg/kg) produced a dose-dependent reduction in choice accuracy, with a larger reduction in number of pellets eaten, and a dose-dependent increase in time spent in each arm. Similar results were seen in the male group with scopolamine (0.5-4.0 mg/kg) alone. Scopolamine methyl bromide (4.0 mg/kg) produced similar results, although choice accuracy was somewhat less reduced Physostigmine salicylate (.05-0.4 mg/kg) in the male group did not produce any apparent decrease in choice accuracy but did, at the higher doses, increase time spent in each arm while reducing the number of pellets eaten. These results would indicate that simple choice accuracy as measured in a radial maze may not be adequately sensitive, at least under these conditions, to determine higher-order behavioral abnormalities in a genetically compromised murine CNS and that mice in such an environment may use different sensory cues from those of other mammalian species to negotiate the maze effectively.

120.5 DISPROPORTIONS IN NUMBER OF AFFERENT AND TARGET NEURONS FOLLOWING TEMPORAL EFFECT OF METHYLAZOXYMETHANOL ACETATE. S. Chen and D.E. Hillman, Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Methylazoxymethanol acetate (MAM) is ideal to selectively reduce the number of specific types of neurons undergoing active cell division at the time of administration. A single 20 mg/kg dose of MAM was injected peritoneally into pregnant rats at gestational day (G) 11 through 21 or subcutaneously into neonatal rats from postnatal (P) day 0 through day 5. All liters were limited to 8 pups and sacrificed at 60 days of age. The brains were first dissected into 4 parts: brainstem, cerebellum, olfactory bulbs, and cerebrum together with the thalamus and tectum. The hippocampus was dissected from the cerebral mass following separation from the thalamus and tectum. The parts were weighed, and light microscopy done on all regions to determine the cellular distribution.

Weight reduction for each brain region over the developmental period supports the contention that prenatal injections can differentially reduce the number of specific types of macroneurons while postnatal injection, in rat, affects only specific groups of microneurons.

Following G13 or 14 injections, the cerebellum was moderately decreased in weight and had a decrease in the number of Purkinje cells. For G13 to 17 (especially G15) injections, there was clearly a large deficit in the number of pyramidal cells of cerebrum and hippocampus. This was accompanied by a deficit in mitral cells of the olfactory bulb. Postnatal administration produced size reductions in the cerebellum, hippocampus and olfactory bulb due to the effect on granule cell proliferation in these three regions. These were most severe on day P0 and gradually improved until P5. The size of olfactory bulbs was reduced more than was the cerebellum. The hippocampus had only a mild change. The brainstem weight was virtually unaffected at any of these times.

Histological examination of the pre- and postnatally injected preparations revealed hypoplasia in the cerebrum, hippocampus, olfactory bulbs and cerebellum. The most severely affected hippocampus occurred prenatally with occasional disruption of the CA^{1-3} layer. The olfactory bulbs from P0 had very small glomeruli and mitral cells were in multilayers. Ectopic neurons in the cerebella were as described by others. MAM injection coinciding with the birthdate of neurons altered the proportion of Purkinje cells and granule cells of the cerebellum, mitral cells and the granule cells of the olfactory bulb, or pyramidal cells (entorhinal and CA^{1-3}) and the granule cells of the hippocampus. [Supported by USPHS grant NS-13742 from NINCDS]

120.6 **EARLY POSTNATAL EXPOSURE TO PHENYLACETATE INCREASES RESISTANCE TO EXTINCTION IN ADULTHOOD.** A.Rabe, Y.H. Loo*, P.Wang*, and R. Fersko*. NY State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, NY, 10314.

Phenylacetate (PA), a major metabolite of phenylalanine, may be the primary cause of brain dysfunction associated with sustained hyperphenylalanemia. This hypothesis has found support in biochemical (Loo et al., Life Sci, 1980, 27, 1283), morphological (Robain et al., Acta Neuropathol., 1983, 61, 313) and behavioral (Fulton et al., Life Sci, 1980, 27, 1271) studies. In a series of experiments we sought to determine the nature of the behavioral changes resulting from early postnatal exposure to PA. Two aspects of these experiments are described here: responding on operant timing schedules and during subsequent extinction.

The offspring of Sprague-Dawley rats were injected with PA twice daily. The injections were given subcutaneously in the following doses as umol/g body wt: days 2-3, 2.5, days 4-16, 3.0, and days 17-22, 3.5. The control litters were injected with corresponding volumes of saline. As adults (70-120 d), the rats were deprived of water for 22.5 hr daily and shaped to press a lever for water reward on a continous reinforcement schedule. Subsequently, the animals received extended training (at the rate of 30 min/day) on one of three operant schedules; extinction, or responding in the absence of reinforcement, concluded each experiment. (1) Control (n=8) and PA-treated (n=8) groups were trained for 24 d on a fixed interval schedule with a 60 sec delay. There were no differences between the groups in either the total responses or in the distribution of responses. During 60 min of extinction, the PA animals made more median responses (297) than the controls (243). This difference was not statistically significant. However, the PA-treated rats had a significantly ($p<.05$, two- tailed U-test) smaller (56) drop in responses from reinforced to unreinforced responding than the controls (102). (2) Other control and PA-treated groups (n=6 in each) were trained for 24 d on a differential reinforcement for low rates (DRL) schedule with a 12 sec delay, then shifted to DRL 20 sec for 6 d. There were no significant differences between the two groups in total responses, the reinforcements received, and the percent of rewarded responses. During 90 min of extinction, however, the PA group emitted significantly ($p<.04$) more responses (376) than the controls (261). (3) A third pair of control (n=8) and PA groups (n=16) was trained on DRL 20 sec for 24 d and then shifted to DRL 30 sec for 9 d. There were no differences between the two groups. During 100 min of extinction, the PA rats again made significantly ($p<.02$) more responses (282) than the controls (196). (Supported in part by NIH grants 1 R01 16153 and 06843).

120.7 DEFECTS OF THE GLIAL SLING IN MOUSE FETUSES WITH HEREDITARY ABSENCE OF CORPUS CALLOSUM. D. Wahlsten, Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

About 20% of adult BALB/c inbred mice have serious deficiency or total absence of the corpus callosum (CC), whereas mice of the inbred C57BL/6J strain and the hybrid B6D2F2/J cross never show defects of CC. The defect seen in adult BALB/c mice seems to be specific to the CC, because the hippocampal and anterior commissures are of normal size in all BALB/c adults.

Prenatal development of the CC was examined using H & E staining in normal C57BL/6J and B6D2F2/J fetuses ranging in age from 16.0 to 18.0 days ($\pm$ 2 hr.) from conception, and comparisons were made with BALB/c fetuses matched for overall degree of morphological maturity or developmental age determined from whole body size. Principal findings were as follows.

1. Growth of the anterior commissure relative to whole body size was similar in all three strains.

2. Growth of the hippocampal commissure of BALB/c fetuses was mildly retarded in comparison with C57 and F2 fetuses, but that structure eventually reached normal size in BALB/c adults.

3. Growth of the corpus callosum of BALB/c mice was very retarded compared to the other strains. Every C57 and F2 fetus which weighed more than 0.46 gm (less than 16.0 days developmental age) had some CC axons crossing midplane, whereas no BALB/c fetus less than 0.60 gm had a CC at midplane, which amounted to retardation by at least 1 day.

4. At 18.0 days chronological age when BALB/c body weights averaged 0.79 gm, there were still many BALB/c fetuses with no CC at midplane (34 of 102 with no CC), but at 19.0 days only 7 of 103 BALB/c fetuses lacked a definite CC ($z=4.6$, p 0.0001).

5. In all BALB/c fetuses with no CC at 18.0 days, the glial sling (Silver, et al., J. Comp. Neurol., 1982, 210, 10-29) was either absent or grossly deficient. The sling was also defective in many BALB/c fetuses with a small CC at 18.0 days.

6. The frequency of an abnormal glial sling in 18.0-day BALB/c fetuses was similar to the frequency of abnormal shape or deficient size of CC in adult BALB/c mice.

These results indicate that axons of the CC in BALB/c mice are sometimes able to traverse the region between the cerebral hemispheres in the absence of a normal glial sling. However, when this occurs, evidently the shape of the CC in the adult mice is quite unusual, being shorter but thicker at midplane than in C57 or F2 mice.

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120.8 BRAIN ANOMALIES AND AUTOIMMUNITY IN MICE. G.F. Sherman, P.O. Behan*, and A.M. Galaburda. Neurological Unit, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215; Southern General Hospital, Glasgow, Scotland.

The NZB strain of autoimmune mice exhibits cerebrocortical anomalies of developmental origin that are similar in type to those seen in human dyslexic individuals (Sherman et al., Soc. Neurosci. Abs., 9:939, 1983; Galaburda et al., Ann. Neurol., in press). Furthermore, non-righthandedness, dyslexia, and autoimmunity have been shown to cluster together (Geschwind and Behan, PNAS, 79:5097, 1982). Because the anatomical findings in a single mouse strain (NZB) cannot answer the general question of whether autoimmunity and developmental neuropathology are linked, we examined the brains of several other strains of immune-defective mice.

The mice were perfused, and their brains removed, embedded in celloidin, sectioned at 30μ, and every tenth section stained for Nissl substance. The inbred autoimmune strains examined were additional NZB (n=84), as well as BXSB (n=68), and MRL (n=45). All three strains are considered models of human lupus erythematosus, although the disease in NZB and BXSB (characterized by B-cell lymphoproliferation) differs from that of MRL (T-cell proliferation). We also studied the following crossbred strains: BXSB/NZB (n=46), MRL/NZB (n=20), MRL/BXSB (n=12), NZB/NZW (n=209), BXSB/NZW (n=71), and MRL/NZW (n=21), and the inbred strains NZW and C57Bl/6, and the CFW outbred strain. The mice ranged in age from 10-90 days and the data were collapsed over these ages, as no age differences were present.

Twenty-six percent of the NZB, 32% of the BXSB, and 20% of the BXSB/NZB and MRL/NZB had cortical anomalies consisting of ectopic clusters of neurons in layer I of the cerebral cortex. The MRL, NZW, and the remaining crosses had low rates of abnormality (less than 5%) and no anomalies were seen in the CFW controls. Eight females of the 64 C57Bl/6 had anomalies. Interestingly, there is evidence indicating that there are immunological abnormalities in this strain (Shirai and Mellors, PNAS, 68:1412, 1971).

The localization of anomalies differed significantly in the two strains that had the highest incidence of ectopias (NZB and BXSB): Anomalies were present mostly in the somatosensory cortex in NZB and in the frontal and motor cortices in BXSB ($X^2=17.27$, $df=2$, $p < 0.001$). When asymmetry of location was examined over all strains, it was seen that anomalies were usually present in the left frontal and motor cortices and in the right somatosensory cortex ($X^2=5.42$, $df=1$, $p<0.02$).

Thus, this study shows that brain anomalies are present in high numbers in at least two pure strains of autoimmune mice (B-proliferative NZB and BXSB) and in some crossbreeds. They are not, however, seen in large numbers in the T-proliferative MRL strain. The anomalies differ in architectonic localization and hemisphere involved among the strains. (Supported by NIH grants 14018 and 19819).

120.9 VISUAL PROSTHESES: PRELIMINARY REPORT OF USE BY INFANT MACAQUES. B. J. Sonnier, E. R. Strelow,* D. H. Warren* and A. H. Riesen. Dept. of Psychology, Univ. of California, Riverside, CA 92521.

We are examining the behavioral and neural development of infant stumptail macaques (M. Arctoides) reared from birth to three months with electronic sonar sensory substitution devices to assess correlations between spatial-motor behavior and auditory, motor and visual cortical development. Groups of four animals are reared as follows: 1) experimental group without vision but with the Trisensor Aid (TSA) in continuous use (the TSA is an advanced experimental version of a blind travel aid, the Sonicguide), control groups without vision but with 2) silent, dummy versions of the device, or 3) with a sound-making but non-functional device, and 4) normal, colony-reared animals.

We are exploring the advantages that the TSA provides for blind infant monkeys in early development. Behavioral observations and tests are being made throughout the three-month period, including cage observations and tests of auditory perception and locomotor behavior. After 90 days of in the experimental conditions all animals are sacrificed, and Golgi-Cox and computerized morphometric (Bioquant II) techniques are used to examine cortical development.

Preliminary behavioral results show that the first experimental animal was able to discriminate objects using the TSA: It showed a marked drop in general motor activity in its home cage when the device was turned off for 24 hrs and recovery of activity levels when the device was turned back on. A control animal showed markedly less motor activity overall. Neuroanatomical analyses are currently in progress for an experimental and two normally-reared control animals. Although cell orientation patterns may change, we expect to find few numerical differences in primary auditory and motor cortex in the three animals as the experimental animal engaged in as much or more motor activity as the normally-reared ones. However, we expect to see differences in association or secondary motor and auditory cortex. Finally, if visual cortex is in some sense a "spatial cortex," then the visual cortex of an animal reared with a working device should develop more normally than in an animal reared with control devices.

This research addresses questions which are important to blind human infants but which are difficult to answer with human subjects. Behavioral and cortical changes after prolonged early use of a sensory prosthesis could be of considerable importance to their eventual use by humans. The TSA is a potentially important research tool, for it allows the study of novel questions of intermodal organization via the sensory substitution paradigm. Correlations of behavioral and neuroanatomical evidence are necessary to understand the role of sensory information in the ontogeny of development.

121.1 RESPONSES OF POPULATIONS OF DSCT NEURONS TO ANKLE MOVEMENT. C.E.Osborn and R.E. Poppele, Lab. of Neurophysiol.,Univ. of Minnesota, Minneapolis, MN 55455.

The wide convergence of muscle afferents onto DSCT neurons reported earlier (Osborn & Poppele,Neurosci.Abs.10:744) suggested that the DSCT provides information on parameters of limb movement, rather than the state of single muscles. From the eariler study it was also likely that this information was encoded in the relative occurrence of a small number of distinct types of response within the total DSCT population. We have now recorded the behavior of 127 DSCT units in cats during randomly generated ankle flexions and extensions in an intact and otherwise immobile hindlimb. A population response for DSCT was derived from the average of the recorded changes in discharge probability in single units. Significant responses were found in 60% of the units; 43% of the units responded to extension, 53% to flexion and 37% to both (Fig. 1). The responses had a long time course (ave. >50 ms) which did not follow the time course of the imposed movement. There were 4 types of response: excitatory (E), inhibitory (I; Fig. 2A), and two types of mixed responses (Fig. 2B). One type of mixed response, EI, was excitatory for the first 20 ms after the stimulus, but was inhibitory after 30 ms. The other type (IE) was nearly the mirror image of the EI response. The proportions of the E and I responses did not change with the direction of the movement (38% and 21% respectively), but the proportions of the mixed responses did. IE responses were twice as common as EI during extension (26% vs. 13%), but the opposite was true for flexion (13% vs. 26%). Individual units often changed their response when the movement direction changed, but this also was more prominent among units with mixed responses. 92% of the units with EI or IE response to extension had a different type of response during flexion, compared to only 42% of the units with E or I responses.

FIG. 1

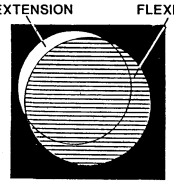
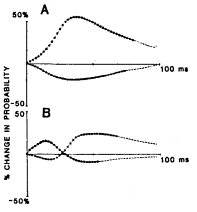


FIG. 2



121.2 ENHANCEMENT OF TONIC VIBRATION AND STRETCH REFLEXES IN THE DECEREBRATE CAT BY SEROTONIN. J. S. Carp and W. Z. Rymer. Dept. of Physiology, Northwestern Univ., Chicago, IL 60611.

In the tonic vibration reflex (TVR), high frequency longitudinal muscle vibration elicits a massive discharge of primary spindle afferents and an increase in electromyographic activity (EMG) and force. Upon cessation of vibration, afferent discharge rapidly returns to the prestimulus level, but motor output subsides over a much slower time course, such that force and EMG may take several seconds to reach the previbration levels. While this apparent discrepancy between sensory input and motor output has previously been attributed to afterdischarge of spinal interneuronal networks, it has recently become apparent that spinal motoneurons might contribute directly to this phenomenon. Hounsgaard et al. (Exp. Brain Res. 55:391, 1984) have described a serotonin (5HT) dependent property of spinal motoneurons in which brief depolarizing current injections into these neurons resulted in a prolonged depolarization and repetitive discharge which long outlasted the duration of the current input. While this behavior may be a significant determinant of motoneuronal function on the cellular level, its role in regulating motor output across the motoneuron pool is as yet unclear. In order to assess the functional significance of this intrinsically mediated behavior of spinal neurons, we have evaluated the effects of pharmacological manipulations of 5HT on segmental reflex mechanisms.

Force, EMG and primary spindle afferent discharge rate recorded during the TVR and the stretch reflex in unanesthetized decerebrate cats were compared before and after administration of the 5HT reuptake blocker fluoxetine (FLU, n=6), the 5HT precursor 5-hydroxytryptophan (5HTP, n=3) and the 5HT receptor antagonist methysergide (MS, n=4). The degree to which motor output continued to be elevated above control levels one second after cessation of vibration was increased by FLU and 5HTP to approximately 230% and 160% of control, respectively, and was reversed by subsequent administration of MS. Administration of MS alone decreased the postvibration motor output to approximately 50% of control. In addition, both mean dynamic and static stretch reflex stiffnesses were increased by FLU and 5HTP to approximately 150% and 200% of control, respectively, and were reversed by subsequent administration of MS. MS alone decreased mean stretch reflex stiffness to approximately 75% of control. These powerful effects on the tonic vibration reflex and the stretch reflex could not be explained by drug-induced alterations in primary afferent discharge. These data support the hypothesis that a 5HT dependent intrinsically mediated behavior of spinal neurons is an important factor in determining motor output.

121.3 SUPRASPINAL CONDITIONING OF SHORT-LATENCY CUTANEOUS PATHWAYS TO LUMBAR MOTONEURONS. J.W. Fleshman, P. Rudomin and R.E. Burke. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Supraspinal control of pathways for low threshold cutaneous input to spinal α-motoneurons was examined using a conditioning-testing paradigm and intracellular recording techniques in six barbiturate-anesthetized cats. A monopolar stimulating electrode was placed in the red nucleus (RN) and, in two experiments, a second bipolar electrode was placed in the medullary pyramidal tract (PT). A special effort was made to study the short latency excitatory pathway from the superficial peroneal nerve (SP) to flexor digitorum longus (FDL) motoneurons described by Fleshman et al. (Exp. Brain Res. 54: 133, 1984).

Conditioning stimuli to either RN or PT (typically 4 pulses, 50-100 μA, 500 Hz, 10-30 ms C-T interval) increased the amplitude and decreased the latency of EPSPs in FDL cells produced by single pulse stimulation of low threshold (<3xT) SP afferents. The mean onset latency of the earliest EPSP, measured from the negative peak of the first afferent volley at the dorsal root entry zone, decreased from 1.96 to 1.56 ms (n=12). Due to the confluence of early excitatory and later inhibitory components, amplitude changes could not be measured, though both components were clearly larger. The late IPSPs also decreased in latency. In a mixed sample of other extensor and flexor motoneurons with initial depolarizing responses to SP stimulation, conditioning stimulation decreased segmental latencies from 2.24 to 1.77 ms (n=15).

The finding that supraspinally conditioned EPSPs from low threshold cutaneous afferents in some hindlimb motoneurons may have segmental latencies in the 1.5-2.0 ms range raises a question as to the synaptic linkage of this pathway. Similar latencies in group Ib and group II pathways have been taken as evidence for a disynaptic linkage. Illert et al. (Exp. Brain Res. 26: 521, 1976) have described a supraspinally facilitated disynaptic cutaneous pathway to forelimb motoneurons with latencies in the same range. Our results suggest that some cutaneous excitatory reflex pathways in the cat hindlimb may also have a disynaptic component. The identity of the premotor interneurons in this pathway and their target motor nuclei requires further study.

121.4 SPECIFIC AND UNSPECIFIC MECHANISMS INVOLVED IN THE GENERATION OF PAD OF CUTANEOUS FIBERS. I. Jiménez*, M. Solodkin* and P. Rudomin (SPON: F.J. Alvarez-Leefmans). Dept. Physiol. & Biophys. C. Res. and Adv. Studies IPN, México, 14 D.F. México.

In a recent publication (J. Neurophys. 52 (1984): 921-940) we have showed that tetanic stimulation of group I fibers from the biceps posterior and semitendinosus (PBSt) nerve produces presynaptic depolarization (PAD) of group Ia muscle afferent fibers ending in the intermediate nucleus of the lumbo-sacral spinal cord, without significantly increasing the potassium concentration ([K+]). On the other hand, tetanic stimulation of the sural (SU) nerve increased the [K+], particularly in the dorsal horn, and could depolarize neighboring Ia afferent fibers but not fibers ending in the intermediate nucleus. It is therefore possible that unspecific mechanisms are more important in the generation of PAD of cutaneous than of Ia fibers. The aim of this study was to relate the negative DC potential shifts (which are proportional to changes in [K+]) with the changes in the PAD of single cutaneous fibers. The experiments were made in cats anesthetized with pentobarbital (35 mg/kg), paralyzed and artificially respired. PAD of single fibers was inferred from changes in their activation threshold. DC shifts were recorded intraspinally with the same microelectrode used for excitability testing. In 9 SU fibers, a single 2 xT stimulus to the superficial peroneus (SP) or to the SU nerve produced large threshold decrements (mean 83.1±10.7 % and 83.0±9.1 %, respectively) without significantly changing the DC potentials. On the other hand, graded stimulation of the SP nerve (1-10 xT) with high frecuency trains (100 Hz for 10-20 sec) decreased the threshold of SU fibers and produced a parallel increase of the negative DC shifts (correl. coef.=0.92, n=5). Graded stimulation of the SU nerve produced a dual effect. With low stimulus strenghts (1.2-2.5 xT) the fiber threshold was decreased without significant changes in the DC potentials. However, with stronger stimuli (>4 xT) the threshold decrements were highly correlated with DC potential shifts. Stimulation of the brain stem reticular formation reduced the activation threshold of 12 cutaneous fibers (mean 86.1±9.7 %) located in the dorsal horn without producing noticeable DC potential shifts at the site of excitability testing. It is suggested that the PAD of low-threshold cutaneous fibers produced by cutaneous inputs is due to both specific and unspecific mechanisms, whereas the PAD produced by reticulo-spinal inputs is predominantly due to activation of specific neuronal pathways in which the first order interneurons appear to be located in the intermediate nucleus or in more ventral regions of the spinal cord.

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121.5 **PATTERNS OF PRIMARY AFFERENT DEPOLARIZATION AND HYPERPOLARIZATION OF GROUP Ia AND Ib FIBERS PRODUCED BY SEGMENTAL AND DESCENDING INPUTS.** P. Rudomin, M. Solodkin* and I. Jimenez*. Dept. Physiol. & Biophys. CINVESTAV, Mexico 14 D.F. Mexico.

It is generally accepted that stimulation of cutaneous nerves produces primary afferent depolarization (PAD) of Ib fiber terminals, and inhibits the PAD of Ia fibers. These PAD response patterns were used as operational criteria to differentiate Ia from Ib fibers and it was concluded that stimulation of reticulo-spinal, rubro-spinal and cortico-spinal pathways have the same actions as cutaneous inputs on Ia and Ib fibers. However, in some group I fibers stimulation of the brain stem reticular formation produced PAD although cutaneous volleys had inhibitory actions. We assumed that these were Ia fibers in which the brain stem stimulation activated, in addition, descending pathways with excitatory actions (J. Neurophysiol 50 (1983): 743-769). However, contrary to the accepted views, they could be Ib fibers in which the PAD was inhibited by cutaneous inputs. Experiments were made on cats anesthetized with pentobarbital (35 mg/kg), paralyzed and under artificial respiration. Changes in membrane polarization of afferent fibers were inferred from alterations in the activation threshold of single group I fibers from the posterior biceps and semitendinosus (PBSt) nerve where Ia and Ib fibers have different peripheral thresholds and conduction velocities. Twenty four out of 44 single group I PBSt fibers ending in the intermediate nucleus were depolarized by stimulation of group I afferents from the PBSt or deep peroneous nerves but not by cutaneous or by reticulo-spinal inputs, both of which inhibited the PAD elicited either by group I muscle volleys or by vestibulo-spinal stimulation. The conduction velocities of these fibers (Type A) varied between 78 and 118 m/sec (mean + SD = 100.5 + 13.6 m/sec) and their electrical thresholds in the periphery between 1.01 and 1.56xT (1.23 + 0.17xT), which are in the Ia fiber range. The other 20 fibers were depolarized by all the tested descending inputs. Cutaneous volleys produced PAD in 6 of these fibers (Type B) and inhibited the PAD in 14 fibers (Type C). Fibers with type B and C PAD response patterns had overlapping conduction velocities and electrical thresholds in the peripheral nerve, which varied between 68 and 90 m/sec and between 1.47 and 2.16xT, respectively. The mean conduction velocities and peripheral thresholds of the Type B and C fibers were 76.2 + 4 m/sec and 1.8 + 0.15xT and 77 + 5.3 m /sec and 1.75 + 0.19xT, respectively, clearly in the Ib range. These results indicate that cutaneous fibers have a dual action in the pathways mediating the PAD of Ib fibers. They produce PAD in one set of fibers and inhibit the PAD in another set.

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121.6 ANALYSIS OF STEADY-STATE SYNAPTIC CURRENTS GENERATED IN CAT MOTONEURONS BY HOMONYMOUS IA AFFERENTS. C.J. Heckman* and Marc D. Binder. Dept. of Physiology & Biophysics, Univ. of Wa. Sch. of Med., Seattle, WA 98195.

Although it is known that the amplitudes of aggregate homonymous Ia EPSPs vary over an approximately 10-fold range within the cat triceps surae motoneuron pools, recent anatomical analyses indicate that all of these motoneurons generally receive about the same number of synaptic contacts from homonymous Ia afferents (Lev-Tov et al. J. Neurophys. 50: 1983). If one assumes that all Ia synapses have equivalent efficacies and that they are all "physiologically active", then the total Ia synaptic current should be equal in all motoneurons and the observed variation in EPSP amplitude should be largely attributable to differences in the intrinsic properties of the motoneurons (e.g. membrane surface area, membrane resistance, etc.), which together determine input resistance. Alternatively, it is possible that not all Ia synapses on all motoneurons are active (Henneman et al. J.Physiol. 352: 1984) or that Ia synapses display a range of efficacies and thus, the variation in Ia EPSP amplitudes within a motoneuron pool may be produced in part by differences in Ia synaptic current. To address this issue, we have devised a simple technique to measure synaptic current that is based on the established equivalence of synaptic and injected currents with respect to the generation of repetitive firing in cat motoneurons (Schwindt and Calvin. Brain Res. 59: 1973). Steady-state, homonymous Ia EPSPs were generated in cat MG motoneurons by subjecting the triceps surae muscles (lateral gastrocnemius-soleus nerve cut) to longitudinal vibration (150 μ, 200 Hz) for 1-2 sec. We then determined how much current the Ia afferents generated in the somata of the same cells by increasing the magnitude of 1 sec current pulses passed through the microelectrode until they produced a membrane depolarization equal to that observed during the vibration. In our initial results (n=30) steady-state homonymous Ia EPSPs had a 10-fold range in amplitude (0.5-6.7 mV) that was very strongly correlated with motoneuron input resistance ($r=0.89; p<.001$) which varied over a 5-fold range (0.4-2.2 MΩ). In addition, however, we have found that the EPSP amplitude was also systematically related to Ia synaptic current ($r=.77; p<.001$) which varied over a 4-fold range (0.8-3.1 nA). Taken together, these results suggest two conclusions: 1. that the variation in the amplitudes of homonymous Ia EPSPs observed within a pool of motoneurons must be ascribed both to differences in the intrinsic properties of the motoneurons and to the magnitude of the Ia synaptic currents generated in them; and 2. that Ia synaptic current must covary with one or more motoneuronal intrinsic properties. (Supported by NSF grant BNS82-06223)

121.7 VARIANCE OF SINGLE FIBER EPSPs REVEALS LONG LATENCY CONNECTIONS TO HOMONYMOUS MOTONEURONS. L.M. Mendell & W.F. Collins III, Dept. of Neurobiol. & Beh., SUNY, Stony Brook, NY 11794 & Dept. ObGyn, Yale Sch. of Med., New Haven, CT 06510

The variance of intracellularly recorded membrane potential measured in repeated trials during accumulation of an averaged EPSP has been shown to increase in conjunction with the EPSP (Rudomin et al., J. Neurophysiol. 38; Jack et al., J. Physiol. 321). This increase in variance during the EPSP has been attributed to the fluctuation in EPSP amplitude associated with non uniform release of transmitter. Single fiber EPSPs and the associated variance increases were studied in medial gastrocnemius (MG) motoneurons of deeply anesthetized cats using electrical stimulation of MG Ia fibers impaled in dorsal roots (Honig et al., J. Neurophysiol. 49). Standard stimulation conditions consisted of 400-1000 trials at 18 Hz. In 12 out of 25 cases the rising phase and initial fall of the variance trace was similar to that of the mean voltage trace although variance trace peaked before the mean in 6/12. At 5 connections with relatively small EPSPs, no increase in variance was observed. In others (8 out of 25) the variance trace was clearly different from the mean voltage trace reaching its peak earlier (6/8) and/or falling more quickly (8/8) to baseline values. At 6 connections, clearcut secondary components were superimposed on the falling phase of the variance trace which was associated with a deflection in the mean voltage trace. These secondary components could have latencies 2-3 ms longer than that of the initial response. Examination of single sweeps revealed that these responses could be intermittent in contrast to the short latency ones which fluctuated in amplitude but were present in all trials. The presence and amplitude of these long latency variance components was highly dependent on the frequency of stimulation usually disappearing at rates 100Hz. In contrast, the short latency increases in variance were virtually unchanged even when mean EPSP amplitude was induced to change (e.g., during high frequency burst stimulation (Collins et al., J. Neurophysiol. 52). The finding that peak of the variance trace often occurs during the rising phase of the mean voltage trace suggests either that latency fluctuations of EPSPs (Cope & Mendell, J. Neurophysiol., 47) may play an important role or that more distal synapses contribute less to the variance than proximal ones. The finding of frequency sensitive, long latency components suggests the possibility that polysynaptic pathways to homonymous motoneurons can be activated under these conditions (single fiber/barbiturate anesthesia) which may be of considerable importance in normal function. Supported by NIH NS 16996 and NS 14899 (LMM) and NS 21875 and a grant from ALSSA (WFC).

121.8 **POTENTIATION OF COMPOSITE EPSPs IN MOTONEURONS.** B.M. Davis, R.E. Druzinsky, L.M. Mendell. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY, 11794.

In studies of potentiation of motoneuron EPSPs produced by stimulation of single Ia fibers with repeated short high frequency bursts (32 shocks, 167Hz), we observed that the maximal amount of potentiation occurred within 200ms after the burst, and that a relationship existed between motoneuron rheobase and maximal potentiation. Furthermore, we found the greatest amount of potentiation at connections with small EPSPs (typically large rheobase motoneurons) while the duration of potentiation was greatest at connections with large EPSPs (small rheobase motoneurons) (Davis et al., Soc. Neurosci. Abstr. 10:742, 1984). These results suggested that the properties of potentiation was determined by the motoneuron. Here, we have examined potentiation of composite EPSPs to see if the amount and duration of potentiation are correlated with motoneuron rheobase when a large population of afferents is activated. We have also compared potentiation produced by short high frequency bursts to that produced by long high frequency trains used by others (Luscher et al., J. Neurophysiol, 49:269; Lev Tov et al., J. Neurophysiol. 50:379).

Recordings were made from triceps surae motoneurons in Nembutal anesthetized cats. Rheobase was determined using depolarizing current injections of 50ms duration. The heteronymous muscle nerve was then stimulated (3 times Group Ia threshold) at 18Hz to evoke a "control EPSP". Each connection was tested repetitively (every 2s) with a 32 shock burst (167Hz) followed by a single interburst test shock delivered 50-1250ms after the burst (64 or 128 trials). Finally, the EPSP produced by 1.8Hz stimulation (32 or 64 trials) was recorded. The nerve was then stimulated at 500Hz for 20s followed by a 10s pause; an EPSP produced by 1.8Hz stimulation was again recorded (this EPSP was averaged during the approximate period of maximal potentiation).

The maximal amount of potentiation produced by short bursts was seen 50-150ms after the burst. Potentiation then decayed slowly and at 2sec. often remained elevated above control values. The amount of potentiation observed 100ms after the burst was positively correlated with motoneuron rheobase ($p<.05$). The duration of potentiation was inversely correlated with motoneuron rheobase ($p<.05$). These results are very similar to that obtained with single fiber burst stimulation and suggest that even during activation of a large number of afferents, the parameters of potentiation are determined by factors intrinsic to the motoneuron. Polysynaptic EPSPs and IPSPs were reduced or abolished following short bursts at times when the monosynaptic EPSP was potentiated.

Of the 15 connections at which potentiation was elicited using both the short repetitive burst and the 500Hz train, the amount of maximal potentiation produced by both techniques was similar (1.68±.04, short burst; 1.68±.08, 500Hz). However, potentiation produced by the 500Hz train was accompanied by a large shift in latency (mean shift 126± 17μs (SEM)) compared to the potentiated EPSP elicted by repetitive bursting (Δlatency 26±6μs). The potentiated EPSPs in both paradigms exhibited changes (both positive and negative) in their rise time (10-90%), but there was no systematic relation between the amount of potentiation and the change in rise time. The independence between magnitude of potentiation and changes in latency and rise time suggests that the mechanisms involved in the shifts in latency and time to peak are not crucial for potentiation. Supported by NS16996 (LMM) and NS07319(BMD).

121.9 FACTORS INFLUENCING THE SHAPE AND AMPLITUDE OF EPSPs EVOKED IN MOTONEURONS BY IMPULSES IN SPINDLE AFFERENT FIBERS, Elwood Henneman, Hans-R. Luscher and H. Peter Clamann, Institute of Physiology, University of Zurich, CH 8057, Zurich, Switzerland.

A recent study (Clamann et al., J. Physiol. 358: 483,1985) of the projections of large numbers of spindle-afferent fibers to homonymous motoneurons has revealed that the sizes of the motoneurons, the diameters of the afferent fibers and the numbers and locations of the synaptic boutons they form on the individual motoneurons, all influence functional connectivity in these monosynaptic reflex arcs. Data from the experiments cited were analyzed to determine how these same factors affect the shape and amplitude of the "single fiber" EPSPs used to establish functional connectivity.

Five dorsal rootlets, each shown to contain 1-5 active spindle afferents, were placed in continuity on pairs of silver electrodes. An expanded version of spike-triggered averaging (STA) permitted us to average out the EPSPs evoked by the impulses in each active fiber from the other synaptic noise recorded in the same motoneuron. From 6 to 11 single-fiber EPSPs were elicited in a single motoneuron and 6 to 24 motoneurons were studied in each experiment. Rise-time, 1/2 width, and amplitude of each EPSP were measured and an analysis based on the Rall model was used to determine the location of the synaptic boutons on the soma and dendrites of the motoneurons. A total of 317 EPSPs was analyzed.

The mean amplitude of all EPSPs studied was 76.5μV. 162 EPSPs (51%) had shapes consistent with the predictions of the Rall model. For such EPSPs the electrotonic distance from the soma to the site of EPSP generation could be calculated. Putative somatic EPSPs had a mean amplitude of 84.5μV while EPSPs whose shape index suggested a site of generation >0.6 space constants from the soma had a mean amplitude of 106.8μV. The difference is highly significant. EPSPs which did not have shape indices consistent with the assumptions of the Rall model were of two types: "wide" EPSPs, whose 1/2 width was greater than would be predicted from the rise time, and "narrow" EPSPs, whose 1/2 width was too short. Many wide EPSPs were obviously composite, having abrupt changes or breaks in slope of either the rising or falling phases, or double peaks. Mean amplitude of wide EPSPs was 92.1μV, significantly larger than average. It is likely that such EPSPs are produced at a greater than average number of boutons and at more than one locus. Narrow EPSPs averaged 32.8μV in amplitude. Small EPSPs were elicited by impulses in fibers of any size; large EPSPs were evoked only by impulses in large fibers. Thus, both pre-synaptic and post-synaptic factors determine the shape and amplitude of EPSPs. (H.-R. L. was supported by a grant from the Swiss National Science Foundation. H.P.C., on leave from Virginia Commonwealth Univ., was supported by a grant from the A.D. Williams Fund; E.H. received support from the National Multiple Sclerosis Society and the National Institutes of Health.)

121.10 **PRIMATE MONOSYNAPTIC REFLEXES AND THEIR POST-TETANIC POTENTIATION. E. Vander Schaaf* and J. R. Wolpaw (SPON: R. A. Waniewski). Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201; and Departments of Neurology and Anatomy, Albany Medical College, Albany, NY 12208.**

In preparation for studies investigating possible segmental substrates of long-term adaptive plasticity in primate spinal stretch reflexes and H reflexes (Wolpaw et al., this vol.), we are studying monosynaptic reflexes to dorsal root stimulation in anesthetized monkeys before and after spinal cord transection. The goal is to determine reflex amplitude, stability over time, bilateral symmetry, and inter-animal variability before and after post-tetanic potentiation and before and in the hours following spinal cord transection.

Monkeys (Macaca nemestrina) are anesthetized throughout with pentobarbital (and sacrificed by overdose at the conclusion of recording). A recording cuff is placed around each posterior tibial nerve at the knee. The cauda equina is exposed from L3 to S2. L5-S2 dorsal roots are identified and cut. Each ventral root from L5 to S2 is stimulated supramaximally and the posterior tibial nerve response measured. The sum of these ventral root responses gives the maximum motoneuron response against which the reflexes to proximal dorsal root stimulation are measured. The combined L5-S2 proximal dorsal roots are stimulated supramaximally at 0.5 Hz before and after potentiation (500 Hz for 20 sec). Data are collected hourly or more frequently on both right and left sides.

In the initial two animals pre-potentiation reflexes measured repeatedly on right and left sides over 4-6 hours averaged 16.0% (±6.2% SD) of maximum. Reflexes increased to 49.0% (±6.2% SD) at 15-20 msec after potentiation. They declined to pre-potentiation amplitude over the next 5 min. In one animal a second laminectomy was performed at T8 and the cord cooled and then transected. Pre- and post-potentiation reflexes did not change significantly over the subsequent 6 hours.

On the basis of these limited data, pre- and post-potentiation monosynaptic reflexes are stable over time and symmetrical in pentobarbital-anesthetized animals. If further data confirm these results, these reflexes should provide means of assessing the persistence and location of substrates underlying adaptive plasticity in stretch reflexes and H reflexes.

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121.11 RECURRENT INHIBITION OF NECK MOTONEURONS IN THE CAT. E.E. Brink I. Suzuki*. The Rockefeller University, New York, N.Y. 10021.

Within the spinal cord, a powerful means of controlling motor outflow is provided by the recurrent actions of motor axons on homonymous and other motoneurons. The extent to which this mechanism operates within the neck segments of the spinal cord, for muscles involved in head movements, is controversial: reported as rare in one study (Rapoport, J. Physiol. 289: 311-327, 1979) but as frequent in unidentified dorsal neck motoneurons in another (Jankowska and Odutola, Brain Res. 194: 65-78, 1980). Indeed, it has been suggested that segmental mechanisms may be fairly unimportant for head movements; their role being supplanted by supraspinal centers (Rapoport, ibid). As important to this question of the role of segmental mechanisms in control of head movements, the occurrence of recurrent inhibition in the neck segments has been re-investigated.

Intracellular recordings were made in chloralose-anesthetized cats from motoneurons identified by their antidromic activation on stimulation of muscle nerves; dorsal roots were cut from C2-C5. The nerves stimulated were: C3,C4 nerves to Biventer cervicis; C3 Complexus; C3, C4 Splenius; C3, C4 Levator scapulae ventralis; Occipitoscapularis.

Recurrent inhibition was frequently found. Of 61 motoneurons, recurrent inhibitory postsynaptic potentials (ripsps) of 100 μV (criterion level) to 2.2 mV were seen in the majority: 22/25 Biv, 4/4 Compl, 6/9 Spl, 6/12 LSV, 9/11 Ocspl. Ripsps could be evoked in Biv, Compl, Spl, and Ocspl neurons from the motor axons to any of these muscles, but typically not from those to LSV. Frequency of occurrence and amplitude of the ripsps were greater when evoked from homonymous motor axons, synergist, or those of the same segmental level as the recorded neuron. Ripsps in LSV motoneurons were evoked from Spl motor axons. Central latencies of the ripsps ranged from 1.0 to 2.6 msec, the earliest compatible with disynaptic transmission. Additionally, interneurons (Renshaw cells) excited by stimulation of motor axons were frequently encountered: a number received convergent excitation from several nerves of the same segmental level. Earliest latencies indicated monosynaptic transmission. The interneurons typically responded with a burst of action potentials. Correspondingly, ripsps in motoneurons sometimes displayed distinct ripples, i.e. successive waves of inhibition. In all these features, the recurrent inhibition, and its organization, in the neck segments of the spinal cord resembles that described elsewhere. It is concluded that recurrent inhibition is a conspicuous feature of spinal cord organization within the neck segments, and, as such, may be an important mechanism for control of head movements. Supported in part by grants from NSF (BNS-8315908) and NIH (NS02619).

121.12 ESTIMATES OF BETA LOOP GAIN. J.L. Schotland*, G. Stuart*, and W.Z. Rymer. Neuroscience Program and Department of Physiology, Northwestern University Medical School, Chicago, IL 60611

Skeletofusimotor (beta) neurons are motoneurons that innervate both intrafusal and extrafusal muscle fibers. Since beta motoneurons receive excitatory feedback from the same muscle spindle afferents that they innervate, they constitute part of a positive feedback loop. The gain of this positive feedback loop has been estimated previously to be on the order of 0.4 by observing the reduction in spindle length sensitivity following interruption of the loop by transection of the dorsal roots (Grill and Rymer, Exp. Brain Res., 1985).

The point of the present series of experiments was to provide an independent measure of the gain of the beta loop. We estimated the loop gain in the decerebrate cat by recording the tonic vibration reflex (TVR) in a muscle and one of its close synergists before and after local anesthetic induced nerve block of the synergist muscle nerve. The local anesthetic blocks gamma efferent fibers to the muscle spindles. Since beta action on spindle afferents is severely attenuated when gamma fibers are not active (Grill and Rymer, Abstr. Soc. Neuro., 1983), the nerve block effectively opens the beta loop.

Medial gastrocnemius (MG) was vibrated longitudinally at 160 Hz with an amplitude of 75 microns, generating a substantial TVR in the MG and a smaller TVR in its close synergist, lateral gastrocnemius (LG). A nerve cuff on LG was then saturated with 0.02% lidocaine, thereby opening the beta loop, and the TVR repeated. A comparison of LG force production in open and closed loop configurations revealed differences of on the order of 10 - 30%, which imply a beta loop gain of approximately 0.3.

This measure of beta loop gain is potentially confounded by the effects of series compliance on primary afferent discharge rates. A large series compliance would tend to diminish the length changes seen by the intrafusal fibers, decreasing Ia discharge rates, and in this way decreasing the beta loop gain. Experiments are in progress to estimate the series compliance, as well as to directly measure the discharge rates of individual primary afferents of the synergist muscle, LG.

Supported by NIH PO1-NS17489 and RO1-NS21180.

121.13 Membrane Potential and Input Resistance of Motoneurons in the Awake and Halothane-Anesthetized Cat. J.F. Whitney* & L.L. Glenn* (Spon: C.J. Smith), Department of Physiology, Ohio College of Podiatric Medicine, University Circle, Cleveland, Ohio 44106

General anesthetics are known to influence motoneuron function. Intracellular studies come almost exclusively from anesthetized or spinally-transected preparations due to the technical difficulty of recording from awake animals. It was therefore of interest to compare the properties of spinal neurons before and during the administration of general anesthetics in an intact animal.

We report preliminary results on 5 motoneurons tested during and immediately prior to deep halothane anesthesia. Frontal ECoG, M. cleidotrapezius EMG, and respiration were monitored to assess the depth of anesthesia. Tibial n., common peroneal n., and ventral root electrodes were used for the identification of motoneurons. Only cells with a membrane potential in excess of -50mV were studied. The input resistance of motoneurons was measured during voltage clamping, and taken from the amplitude of microelectrode current required to produce a 10 - 15 mV change using a single-electrode voltage clamp. Impalements with input resistances in excess of 4 MΩ were presumed to be dendritic and discarded. Measurements of membrane potential and input resistance were taken during wakefulness and during deep anesthesia as indicated by periods of silent EMG and cortical slow-waves. The table shows that little change occurred in membrane potential, however, anesthesia lowered input resistance by 21%

INPUT RESISTANCE (MΩ)		MEMBRANE POTENTIAL (mV)	
Awake	Anesthetized	Awake	Anesthetized
1.99 + 0.15	1.58 + 0.16	-53.24 + 0.80	-54.60 + 1.23

These data were surprising because we presumed that anesthesia would decrease spontaneous synaptic activity which would lead to an increase in input resistance. However, there is a potential source of synaptic input from dorsal horn cells that show little spontaneous activity until halothane is administered.[1]

1. Collins, J. G. Brain Research, 322 (1984) 301-304.

121.14 BISTABLE MEMBRANE POTENTIAL IN SPINAL MOTONEURONS OF AWAKE CATS. L.L. Glenn, J.F. Whitney, J.S. Rewitzer, and J.A. Salamone (Spon: J.S. Baizer), Dept. of Physiology, Ohio College of Podiatric Medicine, University Circle, Cleveland, OH 44106

The membrane potential of some motoneurons in decerebrate and anesthetized cats have been shown to be bistable, in the sense that depolarizations over 10 mV from resting level longer than 30 ms can lock the membrane potential above the discharge threshold such that spontaneous firing occurs in the absence of synaptic drive or depolarizing current pulses (1,2). The present study was undertaken to estimate the proportion of spinal motoneurons that have a bistable membrane potential in awake, intact cats.

Spinal motoneurons of chronically-prepared cats were impaled and antidromically identified by stimulating ventral roots L7 and S1. A single-electrode multiplexing current clamp was used to pass 10-30 nA depolarizing pulses to determine if the membrane potential was bistable, as indicated by the triggering of sustained discharges or depolarizations that outlasted the microelectrode current pulse by a factor of 10 or more.

In a population of 34 motoneurons, 18 were bistable (53%). Depolarizing pulses, and not hyperpolarizing, were used to identify bistability, so the estimate should be regarded as a minimum. The spike mechanism deteriorated spontaneously in 11 motoneurons, showing a mean difference of 5.4 mV in the two stable membrane potential regions in these cells. We conclude that at least half of spinal motoneurons in unanesthetized, awake cats are bistable and thus motoneuron bistability is an important factor in the neural control of movement.

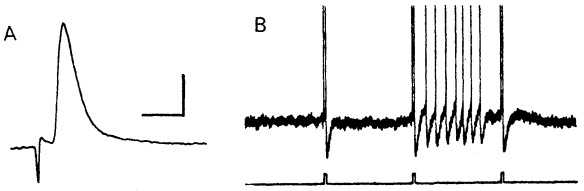


Fig. 1. A: Action potential of an S1 motoneuron. B: Discharge train 800 ms in duration evoked by 10 nA, 30 ms current pulse. Calib: 10 mV, 1 ms in A; 5 mV, 500 ms in B.

1. Housgaard, J. et al. Exp. Brain Res. 55: 391-394, 1984
2. Schwindt, P.C. & Crill, W.E. J. Neurophysiol. 43: 1700, 1980

121.15 RELATIONS BETWEEN CROSS-CORRELOGRAMS AND POSTSYNAPTIC POTENTIALS IN MOTONEURONS: EFFECTS OF SYNAPTIC NOISE. E.E. Fetz, B. Bishop and A. Reyes, Dept of Physiology & Biophysics and Primate Ctr., Univ. of Washington, Seattle, WA 98195

In motoneurons excitatory postsynaptic potentials (EPSP's) produce a change in firing probability that is given by the cross-correlation histogram of motoneuron firing aligned with the source of the EPSP. The transform between the shape of an EPSP (e(t)) and the associated motoneuron firing rate in the cross-correlogram (f(t)) is relatively simple for large EPSP's in negligible synaptic noise. Threshold-crossing models (1,2) predict (3) and empirical tests confirm (3) that the motoneuron firing rate is given by the expression: $f(t) = fo + (fo/\dot{V})(de/dt)$
where fo = baseline firing rate and $\dot{V}$ is the rate of closure between membrane potential ramp and threshold. (This expression is valid so long as the EPSP does not decay more rapidly than $\dot{V}$.)

For small EPSP's in the presence of synaptic noise with comparable amplitude, the correlogram peaks are generally wider than the EPSP derivative (3-6). While the EPSP derivative may still account for much of the correlogram peak, subtracting a function proportional to the derivative (appropriately scaled and shifted in time) from the correlogram peak typically leaves a remainder term. Under certain conditions, this remainder term may be approximated by the EPSP itself (4,6), but often it is briefer than the EPSP (3,5).

To investigate how correlogram peaks are related to the EPSP shape and noise parameters, we used a computer model to simulate the threshold crossing process. EPSP's and noise are superimposed on a motoneuron membrane potential which approaches threshold with a rate $\dot{V}$. The model allows the shape of the EPSP to be arbitrarily defined or to assume the shape of empirically measured EPSP's. Similarly, the synaptic noise can be mathematically defined (Gaussian, Markov, random walk) or can follow the course of intracellularly recorded membrane fluctuations. Preliminary simulations with mathematical functions confirm that the correlogram remainder term increases with the size of the synaptic noise, and typically decays more rapidly than the EPSP. Simulations using experimentally recorded single-fiber Ia EPSP's have replicated the corresponding correlogram peaks obtained with cat motoneurons (5), suggesting that this model incorporates the essential features of the process.

1. Knox, Biophys J 14:567; 2. Ashby & Zilm, Exp Brain Res 47:33; 3. Fetz & Gustafsson, J Physiol 341:387; 4. Kirkwook, J Neurosci Meth 1:107; 5. Cope, Matsumura & Fetz, Soc Neurosci Abs 8:448; 6. Gustafsson & McCrea, J Physiol 347:431.

121.16 STRUCTURAL DIFFERENCES BETWEEN DENDRITIC TREES OF CAT TRICEPS SURAE ALPHA-MOTONEURONS THAT INNERVATE FAST AND SLOW TWITCH MUSCLE UNITS. R.E. Burke, S. Cullheim*, J.W. Fleshman, and L.L.Glenn, Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

We have reported (Cullheim et al., Neurosci.Abstr. 9:430, 1983) on the spatial distribution of dendrites of 7 type-identified cat alpha-motoneurons. Further analysis of this material has disclosed systematic differences in dendritic branching structure and topology in cells that innervate fast twitch (F, including types FF and FR) versus slow twitch (S) muscle units. The average number of dendrites was similar for F and S motoneurons but the stem (zero order) dendrites tended to be thinner in S versus F cells (Burke et al., J.Comp.Neurol. 209:17, 1982). However, the difference between mean dendritic branch diameters in F and S cells disappeared beyond 300 μm from the soma, largely because the ratio of daughter to parent branch diameters at individual branch points was, on average, larger in S cell dendrites as compared to type F (mean $[\text{sum } d_{dau}^{3/2}] / d_{par}^{3/2}$ was 1.18 versus 1.10, respectively). For individual dendrites, the stem diameter (d_{stem}), membrane area (A_d), and number of terminations (T_d) were systematically interrelated but the correlations differed in F and S cells. The correlations between d_{stem} and A_d were best fit by a power function ($y=ax^b$), where b=1.74 for 44 F dendrites and b=1.05 for 35 S dendrites ($p<0.01$). The slopes of linear correlations between d_{stem} and T_d also were significantly different in F versus S dendrites (3.08 vs 1.38, respectively: $p<0.01$). Individual type S dendrites had fewer terminations than F dendrites (mean T_d was 10.6 versus 17.1, respectively) and branch order of terminating branches was, on average, lower in S cells (4.8 vs 6.2 in type F). Both observations show that type S dendrites were less profusely branched than type F. This difference in branching occurred between 200 and 1000 μm from the cell soma. Within this path distance range, the incidence of branching was relatively constant in S dendrites (about 10 branch points per 100 μm path distance from the soma), while it peaked at about 27 branch points per 100 μm shell (at 700 μm from the soma) in type F cells. A 'vertex analysis' of last order branching points (Berry and Flynn, Proc.Roy.Soc.B 221:321,1984) indicated that terminal branches tended to be more symmetrical in S cell dendrites than in F motoneurons. The branching topology of both F and S motoneuron dendrites was most compatible with a 'growth rule' indicating that branching occurs at growing terminals rather than along the shafts of existing dendrites (ibid., see also Van Pelt and Verwer, Bull.Math.Biol. 45:269, 1983). Thus, the anatomical differences between F and S dendrites probably arise as a consequence of intrinsic developmental differences.

121.17 DISTRIBUTION OF FLEXOR CARPI ULNARIS MOTOR NUCLEI IN THE MONKEY SPINAL CORD. E.M. Schmidt and J.S. McIntosh, Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20205

We have recently shown (McIntosh et al., Anatom. Rec., 211: 403, 1985) that the flexor carpi ulnaris (FCU) is a bipinnate muscle in monkey composed of a predominately slow humeral head and a predominately fast ulnar head. This suggest that the two heads of FCU possibly subserve different functions and might be innervated from separate motor nuclei in the spinal cord.

To investigate this question, 3 Macaca fascicularis female monkeys (2.4 to 3.7 Kg) were injected with lectin-conjugated HRP. Using sodium pentobarbital anesthesia and sterile operating conditions the nerves leading to the humeral head of FCU in the left arm and the ulnar head of FCU in the right arm were exposed and injected with 3.5 to 6 ul of a 10% HRP solution. The incisions were closed, antibiotics administered and the animal allowed to survive for 96 hours. The animals were then deeply anesthetized and perfused with saline followed by 5% gluteraldehyde. Spinal segments C6 through T2 were sectioned horizontally at 50 or 75 um on a vibratome. The sections were processed with tetramethylbenzidine and counter stained with neutral red. The location and projected area of every labeled cell was determined with a computer coupled microscope.

The number of labeled cells in each animal after injection of the nerve to the humeral head of FCU was 346, 572, and 594 while injection of the nerve to the ulnar head labeled 376, 222, and 475 cells respectively. Estimates were made on the number of labeled alpha and gamma motoneurons by considering all cells with projected areas of less than 1300 um^2 as being gamma motoneurons. The ratio of gammas to alphas ranged from 0.21 to 0.37. There was no significant difference in the average size of alpha motoneurons in the two heads of FCU. The labeled cells were mainly located in spinal segments C8 and T1 with a few cells in T2. The peak density of labeled cells was in the C8 segment near the T1 border. There was no difference in the longitudinal location of labeled cells for the two heads of FCU. Likewise, there was no difference seen in the distribution of labeled cells in the grey matter for the two heads of FCU. The labeled cells of both heads of FCU exhibted some evidence of two subdivisions within the gray matter, similar to the findings of Iawamto, et al. (Neurosci. Letters, 20:25, 1980) for the cat. The majority of labeled cells were in a column located just dorsolateral to the center of the ventral horn. The second group was located dorsolateral to the major group at the border of gray and white matter.

From these preliminary findings no appreciable differences exist between the motoneurons innervating the two heads of FCU. If differences in function do exist in the two heads of FCU, as indicated by the different proportion of fiber types, they are produced by overlapping populations of motoneurons.

121.18 TOPOGRAPHY OF AFFERENTS ENTERING THE SPINAL CORD FROM CAT NEUROMUSCULAR COMPARTMENTS. O.I. Weeks* and A.W. English (Spon. Robert Holland) Emory University, Atlanta, GA 30322.

Studies of the compartmentalized cat lateral gastrocnemius muscle (LG) have indicated that its motor units and muscle afferents are partitioned peripherally (English and Weeks, Exp. Brain Res. 56:361-368, 1984) and that the synaptic organization of muscle spindle group Ia afferents onto motoneurons is similarly arranged centrally (Vanden Noven et al., Soc. Neurosci. Abstr. 10:329, 1984). Recent work from this laboratory has shown that the motoneurons innervating different LG neuromuscular compartments are topographically organized within the LG motor nucleus (Weeks and English, J. Comp. Neurol. 235, 1985). However, the result from a similar analysis of LG afferents (Weeks and English, Anat. Rec. 211:211A, 1985) demonstrate that dorsal root ganglion cells (DRG's) from any compartment are widely dispersed across the L_6-S_1 ganglia. The present study was undertaken to determine whether the entry point of afferents from different LG compartments to the spinal cord is topographically organized in a manner similar to that of the LG motoneurons.

In pentobarbitol anesthetized cats, spinal segments L_6-S_1 were isolated in continuity and used to record the compound potential elicited by suprathreshold stimulation of the lateral gastrocnemius-soleus (LG-S) muscle nerve. By noting the most rostral and caudal positions of active rootlets, the extent of the LG-S dorsal root entry zone was delineated. Individual fine dorsal root filaments were then dissected free and used to record the response to stimulation of the LG-S nerve and the nerve branches which supply different LG compartments. The muscle nerve branches were stimulated at different strengths to elicit afferent responses in different size classes. By carefully noting the entry point of each filament, the relative entry point of LG afferents belonging to different compartments was determined. Preliminary results are supportive of the hypothesis that the entry point of LG compartment afferents is topographically organized. Afferents of both large and small size classes from proximal compartments enter the spinal cord at the rostral end of the LG entry point with a higher probability than afferents from more distal compartments. Thus LG compartment afferents enter the spinal cord with greater frequency at positions where motoneurons innervating the same compartment would be encountered. It is concluded that at least some of the central partitioning of LG afferents onto motoneurons may be related to topographic considerations. Supported by NS 07137 from the USPHS.

121.19 PEPTIDERGIC INPUTS TO HUMAN AND MONKEY SPINAL MOTONEURON POOLS. S.E. Kapadia* and N.C. de Lanerolle, (Spon: E.E. Manuelidis) Sections of Anatomy and Neurosurgery, Yale Univ. Sch. Med., New Haven, CT. 06510.

The distribution of neuropeptides in motoneuron pools of the human and monkey spinal cord was examined by light and electron microscopic immunocytochemical techniques. In the human, motoneuron pools were abundantly supplied by thyrotropin releasing hormone-(TRH), Angiotensin II-(ANG), Substance P-(SP) and metenkephalin-(ENK) like immunoreactive fibers and terminals. TRH, ANG and SP processes were the most abundant, and were often seen closely apposed to motoneuron somata and outlined their dendrites. In earlier work we showed that the same motoneuron could be innervated by more than one peptidergic system (Neuroscience, 6:713-723, 1981). In the monkey ventral horn too TRH, ANG, SP, ENK and cholecystokinin (CCK) immunoreactive fibers and terminals were found among almost all motoneuron pools, the first four being the most abundant. Fibers and terminals immunoreactive for somatostatin (SOM), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and prolactin (PRL) were also present in the ventral horn but in much smaller numbers. However, SOM, CCK, and ENK immunoreactive elements were found in greater density in some motoneuron groups such as the phrenic nucleus and Onuf's nucleus. Neurotensin-(NT) like immunoreactivity was found only in cervical motoneuron pools. The medial group of motoneurons in comparison to the lateral groups seem to have a greater innervation by the several neurochemical systems.

An electron microscopic examination of elements immunoreactive for TRH, ANG, SP and ENK in the thoracic ventral horn revealed that such immunoreactivity was mainly located in two types of synaptic terminals--those containing small, round agranular vesicles ('S'terminals) and those with an additional population of large dense-core vesicles ('G' terminals). These terminals formed asymmetrical synapses, most commonly with somata and large dendrites, and less commonly with small dendrites.

These studies point to several features of the neurochemical innervation of motoneurons. (i) Some peptidergic neuron systems (TRH, ANG, SP, ENK) innervate all motoneuron groups, but there may be differences in the degree of innervation of different motoneuron pools even by these peptidergic systems. (ii) Some peptidergic systems (SOM, CCK, NT) appear to selectively innervate only some groups of motoneurons. (iii) The same motoneuron may receive multiple neurochemical inputs. These features taken in conjunction with the facts that monoaminergic systems also strongly innervate motoneurons, and some neuropeptides may be co-exist with monoamines in nerve terminals point to a complex level of neurochemical modulation of motoneuronal activity.

(Supported by ALSSOA grant to N.C. de L)

121.20 EXCITATORY EFFECTS OF NOREPINEPHRINE ON LATERAL HORN CELLS OF NEONATAL RAT SPINAL CORD SLICES. Z. G. Jiang* R. C. Ma*and N. J. Dun. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were made from lateral horn cells including antidromically identified sympathetic preganglionic neurons (SPN's) situated in thin transverse sections of thoracolumbar spinal cord removed from 8-15 days old rats and the effects of norepinephrine (NE) on these neurons were investigated. Superfusion of NE (1-50 μM) caused dose-dependent depolarizations in 56 of the 71 lateral horn cells tested. Among the NE-sensitive neurons 23 were identified as SPN's by the appearance of antidromic spikes following stimulation of the ventral rootlets that remained attached to the slices by means of concentric bipolar electrode. Lateral horn cells that exhibited antidromic spikes had mean conduction velocity of 0.7 m/s (n=12) which is close to that of rat SPN axons. The membrane depolarization elicited by NE in presumed SPN's were similar to that evoked in unidentified lateral horn cells. The sensitivity of individual lateral horn cells to NE varied considerably as NE (10 μM) applied for 15 sec evoked a depolarization ranged from 2 to over 25 mV (mean = 8.7 $\pm$ 4.5 mV, n= 16) when recorded at the resting membrane potential between -50 and -60 mV. NE depolarizations were frequently associated with cell firings. In 8 of the 12 cells sampled NE depolarizations were not affected by low Ca (0.12 mM)/high Mg (12 mM) solution or tetrodotoxin (1 μM). In the remaining cells the depolarization was partially but never completely eliminated by low Ca or TTX. These findings suggest that in addition to depolarizing lateral horn cells directly, NE may liberate a depolarizing neurotransmitter(s) from spinal interneurons. NE depolarizations were accompanied by an increase in membrane resistance in the majority (12 of 16) of cells sampled. Membrane hyperpolarization reduced the amplitude of NE depolarization and the response was nullified between -80 and -90 mV at which the spike after-hyperpolarization was also eliminated. Further hyperpolarization did not result in a reversal of polarity. The depolarizing effect of NE on lateral horn cells were effectively and reversibly antagonized by pretreating the preparations with prazosin ($\leq$ 0.1 μM) and phentolamine (1-10 μM), whereas yohimbine (1-5 μM) and propranolol (1-10 μM) were without appreciable effect. It is concluded that NE is excitatory to lateral horn cells including those identified as SPN's and that the response is mediated by $alpha_1$ subtype of adrenergic receptors. This finding in conjunction with the evidence of the presence of noradrenergic fibers in the intermediolateral cell column suggest that NE may serve as an excitatory neurotransmitter to the SPN's. (Supported by NIH Grant NS18710).

122.1 THE MECHANISM FOR THIXOTROPIC EFFECTS AT RELAXED JOINTS. Allen W. Wiegner. Clinical Neurophysiology Lab., Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Lakie et al. (J Physiol 353: 265, 1984) have described thixotropic effects at a number of human joints. That is, when the relaxed joint is subjected to a small sinusoidal torque, the amplitude of the steady-state response is increased up to several fold by a transient larger perturbation. The original, stiffer state is restored by several seconds of inactivity. We have previously found thixotropic behavior in the intact ankle joint of the rat as well as in the isolated ankle joint and isolated muscles acting at the ankle (Soc Neurosci Abstr 8: 329, 1982). In further experiments with a rat soleus muscle preparation (intact circulation, nerve cut) to explore the mechanism for thixotropy, we find the transition between stiff and loose states to be at the same magnitude of stretch (0.3% of muscle length) as the limit (range) of short range stiffness (SRS) of the relaxed muscle: when a ramp stretch or release is applied to a resting muscle, a stiff elastic response is first obtained, followed by a yield and a less stiff, often frictional response thereafter. SRS is calculated from $\Delta F/\Delta L$ in the initial stiff region. The range of SRS increases with velocity of stretch/release ($P<0.01$) and is symmetric with respect to direction (stretch or release). Elastic modulus of SRS is independent of direction and also independent of velocity over the range 0.0018-0.18 fiber lengths/sec. By stimulating the muscle and applying stretches from 0.3-7 sec thereafter, we find the time constant for redevelopment of SRS to be 2.5 sec at 34°C. Cooling the muscle from 34° to 9°C has no effect on range of SRS, but does produce a strongly viscoelastic response to stretch and, less markedly, to release. The latter asymmetry and lack of effect on range suggest that temperature effects are seen primarily in muscle structures other than those responsible for SRS. All of these properties of SRS are consistent with it being the mechanism underlying observations of thixotropy in isolated muscle preparations and contributing to thixotropy in intact joint preparations.

Both static and dynamic measurements of joint torque-angle relations in the intact and isolated joint suggest that the joint also contributes substantially (40-80%) to thixotropic effects in the rat ankle, possibly as a consequence of weeping lubrication of joints.

Thus, both the SRS of resting muscle and the lubrication properties of joints appear to contribute to thixotropic behavior seen at relaxed joints. Although these effects are small, they may be of physiological significance in that they reduce minute fluctuations in muscle length which might otherwise generate "afferent noise" from muscle spindles.

122.2 MISLEADING SPRING LIKE PROPERTIES OF MUSCLE Lloyd D. Partridge Univ. Tenn, Memphis TN 38163 and Thomas M. Hamm Dept Physiol. Univ. Arizona Health Sci Cntr., Tucson AZ 85724.

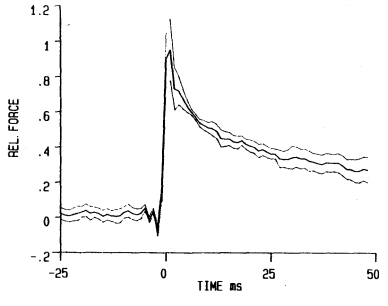
Muscle has often been considered to be spring like, with viscosity. Under restricted conditions this assumption seems to fit the data, but extension of the range of test invalidates the model. Failure is not a simple nonlinearity (Benton & Partridge '73-6 unpublished). Vibration at 200 Hz superimposed on slow movement (Hamm 1979 Dissertation) provides critical identification of changing viscous and elastic coefficients. These data, from soleus muscle of cat with the cut nerve subjected to constant stimulation, were studied in terms of power and mechanical impedance. The directly measured power output on shortening and input on stretch must balance internal elastic exchange, viscous loss, mechanical energy derived from chemical stores and mechanical potential energy loss by metabolic dissipation. For movements in physiological range, viscosity loss was small. Changes of elastic energy storage were in general smaller than the residue (net throughput from chemical process) as both varied in magnitude over the movement. As movement slowed at the low force end of the slow cycle, storage even increased while external energy was still being delivered by the muscle. Yet, at the opposite end the net stored elastic energy decreased while mechanical energy was entering the muscle. Under neural control the dominance of the power sink or source in chemical processes over the power of changes in elastic storage, makes the muscle look more like a servo-system than like a spring, though neither description is particularly good. This study shows that the modulation of chemical energy delivery and/or dissipation by mechanical factors, as well as modulation by neural activity must be included in understanding of motor control. Support by UHPHS grants NS-08608 and NS/AM 17887.

122.3 NONLINEARITIES IN ISOLATED FROG MUSCLE FIBER MECHANICAL DYNAMICS. Ian Hunter, Dept. of Physiology and Biomedical Engineering Unit, McGill University, Montreal, Canada H3G 1Y6.

When frog muscle fibers are subjected to step changes in length (input) the time course of the resulting tension (output) transient (step response) is dependent on the amplitude of the length-step perturbation. This finding has been taken as evidence that at least one of the muscle mechanical time constants (and hence the mechanical dynamics) is length-step amplitude dependent. In consequence recent models of muscle mechanical dynamics typically incorporate a length-step amplitude dependent time constant. However an alternative explanation is that the dynamics are invariant and followed by a static nonlinearity which transforms the dynamic response. Such a nonlinear system produces step responses whose "time constants" appear to be input-amplitude dependent.

In order to test whether muscle mechanics do indeed behave in this fashion single frog (Rana temporaria) tibialis anterior fibers were subjected to stochastic length perturbations of various peak-to-peak amplitudes (.05% to 3%) during tetanic stimulation at a mean sarcomere length of 2.2 μm. A nonlinear system identification technique appropriate for Wiener-type nonlinearities (i.e. a linear dynamic part followed by a static nonlinear part) was used. If muscle conforms to this type of nonlinearity then the dynamic portion of the model should be invariant and thus not require amplitude-dependent time constants. The step responses predicted by the dynamic part (before being transformed by the static nonlinearity) should therefore not be amplitude dependent. The figure shows that for the range of amplitudes tested this is indeed the case (mean and standard deviation of 35 amplitudes are shown). Thus under the conditions studied muscle mechanical dynamics is well modelled by a simple nonlinear model.

Supported by a grant from the Canadian Medical Research Council.

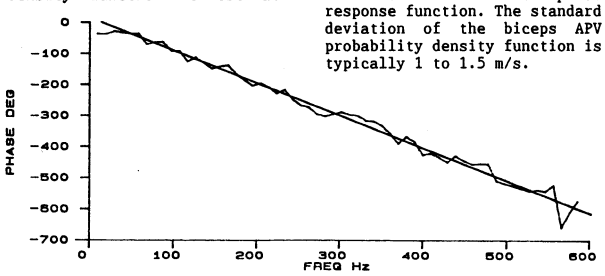


122.4 TWITCH PROPERTIES OF HUMAN MUSCLE DURING PASSIVE SHORTENING AND LENGTHENING. D. Gravel*, A.Y. Bélanger, C.L. Richards* and M. Filion. Laboratoire de Neurobiologie, Faculté de Médecine, Université Laval, Québec, Canada, G1K 7P4.

It has been shown, using tetanic contractions in animals (Joyce et al., J. Physiol., 204: 461, 1969) and maximum voluntary contractions in humans (Komi and Buskirk, Ergonomics, 15: 417, 1972), that the force generating capacity of a skeletal muscle is greater during lengthening (eccentric) than shortening (concentric) contractions. To our knowledge, there have been no studies of evoked contractions during passive muscle lengthening and shortening in human. The aim of the present study is to describe and compare the twitch properties of the plantarflexor muscle group examined at rest (isometric) and during lengthening and shortening movements of the ankle. Twitches were evoked by a supramaximal electrical shock delivered at the popliteal fossa in five healthy subjects. A KIN-COM dynamometer was used to passively move the ankle joint at a constant velocity of 30°/s. Each electrical stimulus was triggered at a predetermined angle for both lengthening and shortening movements. The peak twitch tension during muscle shortening was lower than either in lengthening or static conditions. No significant difference ($p< 0.05$) was noted between the lengthening twitch (peak tension) and the static twitch when the influence of the length was considered. There were no significant ($p< 0.05$) differences with regards to the twitch contraction time and half-relaxation time values between each testing condition. The lower peak tension of the twitch during shortening may in part be explained by a deactivation process in the contractile machinery (Edman, Acta Physiol Scand., 109: 15, 1980). Supported by l'Institut de Recherche en Santé et Sécurité du Travail du Québec (IRSST).

122.5 **COMPARISON OF BICEPS MUSCLE ACTION POTENTIAL CONDUCTION VELOCITY DETERMINED USING CROSS-CORRELATION, PHASE AND IMPULSE RESPONSE FUNCTION TECHNIQUES.** L.A. Jones, R.E. Kearney and I.W. Hunter. Dept. of Neurology and Neurosurgery, Biomedical Engineering Unit, Dept. of Physiology, McGill University, Montreal, Canada H3G 1Y6.

The usual technique for determining the action potential velocity (APV) of human muscle fibers from two surface EMG recordings involves cross-correlation. Typically the APV is calculated as the electrode spacing divided by the delay associated with the maximium absolute cross-correlation function value. However, for acceptable APV estimates the maximium of the cross-correlation function should not be used because of statistical variation in the correlation function estimates. It is preferable to use the maximum of a nonlinear function fitted to the correlation function by regression. The main problem in the cross-correlation function approach is in specifying and fitting the nonlinear function. An approach involving linear regression would clearly be preferable. We have developed a technique for estimating APV in which phase lags between the two EMG signals are determined. If there is a pure delay between the two EMGs then the phase part of the frequency response function determined between the two EMGs should be linearly decreasing with a slope equal to the delay. In practice it is found that the coherence of the frequency response function outside a 5 to 500Hz band is low. The phase slope and thus the delay is therefore conveniently estimated using coherence weighted linear regression. The figure below shows an example of the application of this technique in which the biceps APV was found to be 5.2 m/s. Neither the correlation nor the phase techniques yield estimates of the distribution of APVs. The impulse response function (inverse Fourier transform of the frequency response function) of a system consisting only of pure delays can be shown to be proportional to the delay probability density function. Using this principle we have developed another technique in which the APV probability density function is estimated from the inverse of the impulse response function. The standard deviation of the biceps APV probability density function is typically 1 to 1.5 m/s.

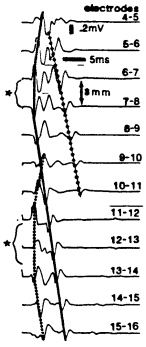


122.6 EMG MAPPING OF INDIVIDUAL MOTOR UNITS IN THE CAT TENUISSIMUS MUSCLE C.M. Chanaud, C.A. Pratt and G.E. Loeb. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205

The geometric relationships between muscle fiber architecture and whole muscle force and length indicate that long, parallel-fibered muscles [such as sartorius, biceps femoris, semitendinosus and tenuissimus(TEN)] should be well-suited for continuous force production during large muscle length changes. Although a variety of muscle fiber (and associated connective tissue) arrangements have been proposed for these muscles, few details are known about their actual architecture, which appears to incorporate anatomical features not previously considered (Rindos, et.al. Neurosci.Ab. #183.3, 1984). In this study, we used a novel electrophysiological technique to reveal details of the underlying architecture that are not easily obtained by the usual anatomical techniques.

The cat hindlimb was dissected to expose the TEN muscle, leaving the innervation and blood supply intact. A silastic rod (5X120mm) carrying 40 electrode contacts (interelectrode spacing=3mm) on the surface was inserted beneath the muscle, providing 39 possible serial pairs of bipolar EMG recording sites. The L7 and S1 ventral roots were accessed via laminectomy and proximally cut filaments were stripped down until a single TEN axon could be stimulated (1ms pulse width, 1pps). Single motor units (MU) were identified by changing the stimulus intensity near threshold and observing no change in the all-or-none potentials recorded by the EMG electrode array. The polyphasic signature was then recorded at each available site along the 120mm long array.

Sets of EMG tracings, as shown at right, demonstrated that every stimulated MU extended into both the proximal and distal portions of the muscle, which are innervated by separate nerve branches. Within a single MU, the polyphasic signature recorded at each site could be resolved into separate biphasic waveforms, defining fiber subgroups, each of which was presumably innervated by one of the numerous end-plate clusters along the muscle. End-plate regions were localized to specific regions (stars) by the polarity reversals of the shortest latency waveforms there. Each MU consisted of similar longitudinal (proximal to distal) distributions of subgroups. Subgroups belonging to a single unit were heavily interdigitated, not placed end-to-end. The subgroups ranged from 3.3-6.3 cm in length. Conduction velocity of the muscle action potentials (slopes of diagonal lines) ranged from 2.5-3.5 m/s.



122.7 STABILITY OF EMG SIGNAL PARAMETERS DURING SUBMAXIMAL PLANTARFLEXION CONTRACTIONS. A.V. Sirin* and A.E. Patla (SPON: G. Partlow) Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

This study was conducted to determine the stability of the two EMG signal parameters during isometric submaximal efforts, and analyse the behaviour of these two parameters during a 10s maximal isometric contraction. Eventual goal is to assess synergistic behaviour of the triceps surae during sustained fatiguing contractions.

A computationally fast method of estimating a fatigue index, modal frequency (MF), proposed by Bower et al (1984), was evaluated. Validation was carried out on EMG data from three muscles of the triceps surae of five subjects at 30% MVC, held for four minutes. Results showed a high average correlation (r=.901) between MF and mean power frequency which was calculated using a Fast Fourier transform algorithm.

Stability of the average EMG (AEMG) and MF was assessed by computing the coefficient of variation (CV) of five successive records of either 500ms, 1s, or 2s duration, sampled at 1024Hz. EMG signals from the three muscles of the triceps surae were analysed for six subjects while performing isometric plantarflexions at 25%, 50%, and 75% MVC. ANOVA results showed that the CV of MF was dependent on the record length at all three levels of MVC. Post-hoc tests revealed that the CV of 500ms record length was greater than that of 2s at 25% ($p<.08$), 50% ($p<.08$), and 75% ($p<.04$), and the CV of the 1s record length was greater than that of 2s at 25% MVC level ($p<.05$). The mean CV across muscles and contraction levels for record lengths of 500ms, 1s, and 2s were 12.6%, 10.5%, and 6.9% respectively. AEMG results revealed no significant differences between the CV of any of the record lengths at any contraction level, in any of the muscles. Based on these results a comparison of average Modal Frequency of 1s record length was made between five and ten consecutive record segments. T-tests revealed no significant difference between the means of five and ten record segments.

A 10s maximal isometric plantarflexion effort was evaluated. All three muscles of the triceps surae were monitored on six subjects. A moving average of 2s portions (5 segments, each 1s long) of the contractions were evaluated. ANOVA indicated that the MF of MG had a significantly ($p<.002$) greater rate of decline than either LG or SOL. The AEMG of all muscles however, did not alter significantly and neither did the plantarflexor torque.

Supported by a grant from NSERC (A#0070).

Reference:

Bower, J.S. et al. (1984). J. App. Physiol., 57, 913-916.

122.8 EFFECTS OF MOTOR UNIT DISCHARGE RATE AND FIBER DIAMETER ON FORCE- EMG RELATIONSHIPS. M. J. Blaschak* and W. Z. Rymer. (SPON: W. T. Rainey). Dept. of Physiol., Northwestern Univ., Chicago, IL 60611.

Alterations in force-emg relationships have been demonstrated in human spastic paretic muscle following supraspinal lesions (e.g. Blaschak, M., et al., Neurosci. Abst., 9:904, 1984). These alterations could arise from disruptions in the normal pattern and rate of motor unit (MU) discharge, from muscle atrophy, or from a combination of the two. The purpose of this study was to investigate the effects of motor unit discharge rate and muscle atrophy on force-emg relationships in a computer generated surface emg simulation model.

This model, developed through a modification of an earlier study (Guha, S. and Anand, S., Comp. Biol. Med., 9:213, 1979), allowed changes in initial MU discharge rate, mean maximum MU discharge rate, and fiber diameter. The amplitude and duration of the individual MU action potentials were graded according to their order of recruitment and fiber diameter.

The initial discharge rate (2, 4, 6, 8, 10/sec), the final discharge rate (13 or 24/sec), and the fiber diameter (100%, 95%, 90%, or 80%) were set, and a series of simulated emg's were produced. Following the generation of each emg series, a variety of characteristics, including the mean rectified emg value (MREC), the frequency of mean power (MPF), and the associated force, were determined. EMG characteristics were then determined for all rate and diameter conditions across matched forces.

The results indicate that changes in initial and final discharge rates are reflected as alterations in the force-MREC (FE) relationship, while changes in fiber diameter are reflected as alterations in the FE relationship and in reductions in the MPF. For example, there was an increase in the MREC per unit force as demonstrated by a 20% increase in the slope of the FE relationship as the initial rate decreased from 8/sec to 2/sec and the maximum rate was decreased from 24/sec to 13/sec. There was a 4% decrease in the MPF over the same conditions. When the discharge characteristics were held constant, a 5%, 10%, and 20% reduction in fiber diameter gave a 12%, 20%, and 38% increase in the slope of the FE relationship, and a 10%, 22%, and 38% reduction in the MPF, respectively. These findings parallel a current animal study.

The results of this simulation support that while disruption in motor unit discharge rate leads to alterations in force-emg relationships, alterations in the force-emg relationship associated with a shift of the frequency content to lower frequencies are most probably due to a combination of reductions in both the mean motor unit discharge rates and in muscle fiber diameter. NIH 5 R01 NS19331

122.9 EMG POWER SPECTRUM AND MOTOR UNIT DISCHARGE IN AN ANIMAL MODEL OF SPASTIC PARETIC WEAKNESS. R.K. Powers, M.J. Blaschak*, and W.Z. Rymer. Dept. of Physiology, Northwestern University, Chicago, IL, 60611.

There is evidence that muscle weakness following stroke is partly the result of a disorganization of motor output to the muscle, so that more EMG is required to achieve a given force level (Tang, A. and Rymer, W. J. Neurol. Neurosurg. Psych., 44:690, 1981). A similar phenomenon has been reported in an acute animal model, and has been associated with abnormally low motor unit discharge rates (Rymer, W. et al., Exp. Brain Res, 37:93, 1979). In human paretic muscle, the frequency content of the EMG power spectrum is shifted toward lower frequencies (Blaschak et al., Neurosci. Abst., 10:904,1984), and such a shift could result from lower motor unit discharge rates. However, simulation studies indicate that the frequency content of the EMG power spectrum is much less sensitive to motor unit discharge rates than to changes in muscle fiber diameter. To evaluate the effects of changes in discharge rate alone, we have measured force, EMG spectra and discharge rates in an acute animal model of weakness where muscle fiber diameter remains constant.

Force, multiunit intramuscular EMG and the activity of single motor units were recorded in the medial gastrocnemius muscle of decerebrate cats before and after a dorsal hemisection of the cord at T12. Muscle length was held constant, and force was graded by manipulation of the contralateral hindlimb.

After the hemisection, the mean rectified EMG at matched force levels was 1.2 - 4.0 times greater than that recorded before the section. Based on our present sample of motor units, minimum steady discharge rates appear to be the same before and after spinal section. However, for matched levels of motor output (EMG), average discharge rates are lower after the section, indicating that maximum firing rates are probably lower. Similar discharge characteristics have been reported for single motor units in the tibialis anterior muscle of spastic paretic humans (Rosenfalck, A. and Andreassen, S. J. Neurol. Neurosurg. Psych., 43:907-916,1980). Even in animals showing large postsection changes in motor unit discharge rates and mean rectified EMG, there were only minor shifts in the frequency content of the EMG power spectrum, reflected as a 4% decrease in the mean power frequency.

Supported by NIH Grant RO1-NS21180.

122.10 CORRELATION OF SUPRASPINALLY GENERATED MOTOR UNIT (MU) RECRUITMENT ORDER WITH MOTOR AXON CONDUCTION VELOCITY (CV). T.E. Dick, F.-J. Kong, and A.J. Berger. Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.

Phrenic motoneurons (PM) are activated rhythmically with individual MUs recruited progressively during inspiration. This rhythmic behavior is generated primarily by descending bulbospinal inputs. Recruitment order of MUs may be a function of specific connections from supraspinal neurons (Hilaire and Monteau, J. Physiol. (Paris) 75: 765-781, 1979) and/or may be determined by intrinsic factors such as motoneuron size (Berger, J. Neurophysiol. 42: 76-90, 1979). Recently Bawa et al. (J. Neurophysiol. 52: 410-420, 1984) tested the size principle by determining recruitment order of hind limb MUs activated by segmental afferents. They compared pairs of simultaneously recorded MUs and reported that in 97% of cases, MUs with lower CVs were recruited first. Their data verified the size principle of Henneman for segmentally determined recruitment order.

In this study, we correlated recruitment order and axonal CV for simultaneously recorded pairs of phrenic MUs. We recorded single MU action potentials (MUAPs) with two tungsten microelectrodes placed in the sternal region of the diaphragm of anesthetized cats. Phrenic nerve activity was recorded simultaneously on hook electrodes at two sites along the nerve. Recruitment order was obtained by comparing the onsets of MUAPs during inspiration. Axonal conduction time was determined by using the MUAP to trigger averages of the two delayed whole-nerve recordings. Repeated measures showed that conduction times varied by 20 us or less over a distance of 100 mm. This corresponds to a variability of 0.5 m/s or less in estimates of CV in axons with CVs of 50 m/s.

For the population of MUs studied, recruitment times varied from 28 to 1028 ms after onset of phrenic nerve activity, and CV varied from 29.7 to 53.8 m/s. In 92% of cases (23 of 25 MU pairs so far tested in 5 cats), the pair-wise recruitment order was directly related to axonal CV.

We conclude that in normal supraspinally generated inspiration, the recruitment order of PMs is strongly correlated with axonal CV verifying the applicability of the size principle to at least a portion of this motoneuron pool. (This research was supported by NIH grant NS 14857, the Puritan-Bennett Foundation and the Shandong Medical College.)

122.11 FORCE-FREQUENCY RELATIONS OF CAT MOTOR UNITS DURING DIFFERENT PATTERNS OF STIMULATION. H.P. Clamann and S.A. Binder-Macleod*. Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298

To match the force output of a muscle to a specific task the CNS selects the number of motor units needed (recruitment) and varies the rate of discharge of each active unit (rate coding). The relation between discharge rate and force of motor units is not well understood. A sigmoid relation between discharge rate and the force of single motor units is often described in animals, although a variety of other functions relate whole muscle force to discharge frequency of motor units in man. These may change qualitatively and quantitatively as the pattern of force output changes. To gain further insight into the force-frequency relationship in single motor units, several patterns of stimuli were applied to single motor units and the resulting force output measured.

Cats were deeply anesthetized with sodium pentobarbital. Axons of single medial gastrocnemius motor units were isolated in ventral root filaments and stimulated to determine twitch strength and contraction time, fusion frequency and maximum tetanic tension of the unit. Force output of the unit was measured at the Achilles tendon which had been detached from the calcaneus. Each unit was then stimulated with a train of stimuli varying linearly from 5 pulses per second (pps) to a maximum 20% above the unit's fusion frequency and linearly back to 5 pps. One period of such triangle-wave stimulation lasted 5 seconds. This pattern was repeated for 3 to 5 continuous periods of the triangle-wave pattern.

The force-frequency response was remarkably similar for all motor unit types and reproducible in each motor unit. It consisted of a hysteresis loop having a sigmoid ascending limb and a descending limb composed of a plateau of constant force which was maintained to a frequency of 15 to 25 pps below which force fell rapidly. With each successive trial the loop shifted to the left or lower frequencies. Force output at a given frequency was consistently greater when frequency was decreasing than when it was increasing; the difference was as great as 500%. When frequency was decreasing, force remained constant for most of the range, falling steeply below frequencies of 20 pps. When a unit was stimulated at any frequency above 30 pps which was then decreased linearly, force closely matched the descending limb of the hysteresis loop.

We conclude that motor unit force output is strongly dependent on immediate past stimulus history. The range of frequencies suitable for force modulation by rate coding is influenced by stimulus history and is poorly predicted by the unit's fusion frequency or twitch contraction time.

122.12 PATTERNS OF INNERVATION AND RECRUITMENT IN REINNERVATED HAND MUSCLES AFTER COMPLETE NERVE SEVERANCE. C. Thomas*, R. Stein T. Gordon, G. Elleker*, and R. Lee, Depts. of Physiology, Pharmacology, and Medicine, Univ.of Alberta, Edmonton, T6G 2H7 and Dept. of Clinical Neurosciences, Univ.of Calgary, T2N 4N1.

Orderly size relationships between nerve and muscle properties are redeveloped in cat triceps surae muscles, even when reinnervated by antagonistic nerves (Thomas et al., Neurosci. Abs. 10:144, 1984). Following reinnervation of a human muscle, an orderly pattern of recruitment was not observed (Milner-Brown et al., J. Neurol. Neurosurg. Psychiat. 37:670, 1974). In the human forearm, the ulnar and median nerves branch extensively to innervate muscles with different functions and separate spinal motor pools. We have studied the patterns of innervation and motor unit recruitment in reinnervated muscles of human patients who had accidentally severed and undergone fascicular resuture of the ulnar and/or median nerve in the past five years. EMG from single motor units was recorded with selective needle electrodes from ulnar reinnervated (first dorsal interosseus, abductor digiti minimi) and median reinnervated (abductor pollicus brevis, opponens pollicus) muscles. Normally innervated motor units were activated only by voluntary contraction of the muscle in which they were recorded. In patients, reinnervated motor units could also be activated by voluntary contraction of other muscles innervated by that same nerve. The original spinal motor pool of each motor unit was identified objectively as that muscle with the lowest force and EMG threshold for activation of the unit. This was confirmed by the patient as the muscle action which activated the motor unit most easily. Our data clearly show that reinnervation was non-specific as muscles were now innervated both appropriately by some of their original motor axons and inappropriately by motor axons that had previously innervated different muscles with different functions.

With spike triggered averaging, we determined the twitch tension and EMG activity associated with the slow steady firing of reinnervated motor units during isometric voluntary contractions. Following ulnar or high median nerve sections, there was no correlation between the size of the motor unit as measured by twitch tension and the voluntary force at which the motor unit fired reliably in the muscle being recorded from. This apparent absence of orderly recruitment in any one muscle may be due to recruitment of motor units from spinal pools with widely different functions. Following median nerve section at wrist level, where the reinnervated muscles have more synergistic actions, orderly recruitment appeared to be re-established. Thus, it would seem that the size principle of motor unit recruitment could be re-established after nerve section in humans, if motor axons innervate their original muscles or ones with closely synergistic functions.

122.13 THE INFLUENCE OF SPINAL CORD TRANSECTION ON POTENTIAL FOR INCREASING THE NUMBER OF SERIAL SARCOMERES IN SKELETAL MUSCLE FIBERS. P.V.Loubert* (SPON: P. Lacy). Dept. of Anatomy and Cell Biol., Univ. of Michigan, Ann Arbor, Michigan 48109.

Muscle fibers have been shown to have a remarkable capacity for increasing and decreasing their optimal length to accommodate changes in their functional environment. This occurs by addition and subtraction of serial sarcomeres within the muscle fibers (Goldspink, 1972; Williams, 1976). It has also been shown that length adaptations can occur to a normal degree in denervated muscles (Goldspink, 1974). McLachlan (1983) showed that denervation slows the rate of sarcomere adjustment, and Tabary (1971) theorized that these adjustments are inhibited in the presence of spastic cerebral palsy.

The present study utilizes a ratio of sarcomere numbers per soleus muscle fiber/unit length of tibia for assessing the relative changes in muscle fiber length. The use of this measurement tool is based upon a positive linear correlation between tibia length and the number of sarcomeres per soleus muscle fiber ($R=0.74$, $p<0.001$), and the consistent ratio of number of sarcomeres/mm tibia ($x=147.5\pm4.8$) over a wide range of sizes and ages in rats. The use of this tool normalizes for differences in sarcomere number due to size differences and changes due to normal growth during the course of the experiments.

The effects of spinal cord transection on the ability of soleus muscle fibers to increase the number of sarcomeres within the fibers was studied. Female rats received surgical transections of their spinal cords, followed by casting of their right ankles in complete dorsiflexion. Spinal transection was used to isolate the muscle from descending CNS influences, and casting was used to increase the functional length of the soleus muscles. Casting produced a significant increase in the number of sarcomeres in the soleus muscle fibers of the casted limbs ($p<0.001$). This indicates that muscle fibers retain the ability to increase the number of sarcomeres in series when upper motor neuron influence is removed.

This study was supported in part by the MPTA Institute for Education and Research, the Program in Physical Therapy at the Univ. of Michigan - Flint, and NIH grant NS-17017.

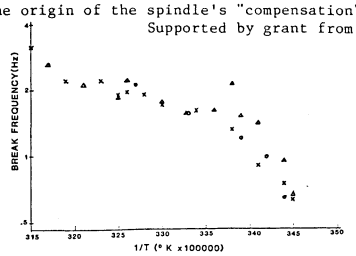
122.14 TEMPERATURE DEPENDENCE OF THE DYNAMIC BEHAVIOR OF MUSCLE SPINDLES M.N. Kruse* and R.E. Poppele Lab. of Neurophysiol., Univ. of Minnesota, Minneapolis, MN 55455

The dynamic behavior of mammalian muscle spindles, which is characterized by a rate or velocity dependence, can be observed in the frequency domain as an increasing response amplitude and phase lead to stretch frequencies greater than 1 Hz. At lower stretch frequencies, the response amplitude is nearly constant and there is a smaller phase lead. The transition or break between low and high frequency behavior occurs at a characteristic frequency corresponding to a single rate constant. The frequency at which the amplitude of spindle responses begins to increase is near the frequency at which the amplitude of changes in muscle tension begins to decrease when a tetanic tension is modulated sinusoidally. Thus, a rate constant associated with spindle dynamics acts to "compensate" for muscle dynamics in a simple stretch reflex.

We have determined the temperature dependence of the frequency characteristics of isolated cat muscle spindles taken from tenuissimus muscle using small amplitude sinusoidal stretches. Data from 2 primary endings and 1 secondary show the same change in break frequency with temperature on an Arrhenius plot which can be fitted well with either one or two lines; see Figure below. The equivalent activation energy (Ea) for the single line is about 9 Kcal/M and thus too low to be explained by a single active process such as channel gating. It is more consistent with a passive mechanism like one involving the mechanical properties of the intrafusal muscle.

The equivalent Ea's for the two line fit are 6 Kcal/M (25-44°C), and 23 Kcal/M (16-25°C). This interpretation of the data implies that there is more than a single process involved in determining the break frequency. The apparent increase of Ea associated with temperatures below 25°C could indicate the involvement of a membrane bound process, in which the increase accompanies a change of state in the membrane. However, the temperature data alone are insufficient to resolve the question about the origin of the spindle's "compensation" for muscle dynamics.

Supported by grant from NSF (81-19871).



122.15 EFFECT OF Ca^{++} CHANNEL BLOCKERS AND Ni^{++} ON K^+ CONTRACTURES IN FROG TWITCH FIBERS. M. Huerta, R. Gamboa-Aldeco* and E. Stefani. Department of Physiology and Biophysics. CINVESTAV-IPN. Apdo. Post. 14-740. 07000 México, D.F.

Twitch fibers have an inward Ca^{++} current (I_{Ca}), which is located in T-System and a Ca^{++} influx during excitation. The role of I_{Ca} during K^+ contractures remains unclear. A reduction in external Ca^{++} causes a decrease on the amplitude and duration of K^+ contractures (Cota & Stefani, 1981. J.Physiol., 317:303). However, Ni^{++} substitutes Ca^{++} on the time course of K^+ contractures (Caputo. The Regulation of Muscle Contraction. Academic Press. 1981. pp 81-95). In order to obtain further information on the role of I_{Ca} on K^+ contractures, we studied K^+ contractures after decreasing or eliminating I_{Ca} with Ca^{++} channel blocker agents (Nifedipine and Diltiazem) or after replacing Ca^{++} by Ni^{++}.

Tension was isometrically recorded from isolated twitch muscle fibers of tibialis from Rana pipiens. The control saline was (mM): CH_3SO_3Na 117.5, CH_3SO_3K 2.5, $(CH_3SO_3)_2Ca$ 1.8 (pH 7.4) and contained 10^{-6} g/ml of TTX. The experiments were performed at room temperature (20-22 °C). The exposure of the fibers to control saline with Diltiazem (1 μM) added for 3 min. prior to the contracture (60-190 mM K^+) decreased the peak tension (66 ± 10%,n=9; $\bar{X}$ ± S.E.) and shortened the half amplitude duration (15 ± 7%,n=8). This effect was evident after the second contracture, probably due to the blocking mechanism of Diltiazem (Gonzalez-Serratos & Valle-Aguilera, 1983. Biophys. J., 41:395a). This action was reversible. Similar results were obtained when the fibers were preincubated for longer periods (22-30 min). Nifedipine (0.1-10 μM) produced similar effects (peak tension reduction: 57 ± 8%,n=16 and half amplitude duration decrease: 47 ± 4%,n=18). This blocking effect was evident from the first contracture. When external Ca^{++} was omitted from the contracture fluid (low Ca^{++} saline: 0 mM Ca^{++} + 3 mM Mg^{++}) a decrease on the amplitude and duration of K^+ contracture was found and when Ca^{++} was equimolarly substituted for Ni^{++} (1.8 mM Ni^{++} + 3 mM Mg^{++}) a further decrease of the tension was observed (peak tension decreased about 70%). This indicate that Ni^{++} was not able to restore the normal temporal course and amplitude of K^+ contracture. This effect was reversible. The present results can be explained by a modulation of I_{Ca} on K^+ contractures or by specific interactions between external Ca^{++} and Ca^{++} binding sites in the membrane.

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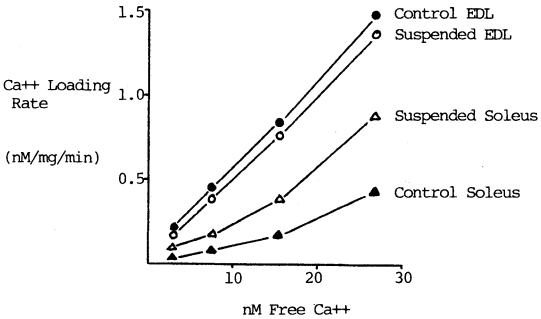
*Fellowship by CONACyT. MEXICO.

122.16 CALCIUM TRANSPORT IN SARCOPLASMIC RETICULUM FROM FAST AND SLOW RAT MUSCLES AFTER HYPOKINETIC SUSPENSION. G.T. Patterson, R.C. Gupta*, C. Petrone*, K.E. Misulis* and W-D. Dettbarn. Pharmacology Dept., Vanderbilt Univ. Med. Ctr., Nashville, TN 37232.

This laboratory and others have shown that hindlimb suspension causes acceleration of contractile speed in soleus muscle. We report here the effects of this non-neurogenic disuse on the sarcoplasmic reticulum (SR) calcium (Ca++) transport in the soleus and extensor digitorum longus (EDL) muscles from rats.

SR of functional integrity and high purity was isolated from EDL and soleus muscles of rats suspended 2 weeks and control rats by the procedure of Mrak and Fleischer (Muscle & Nerve 5:439, 1982). Ca++ loading rates were determined by a Millipore method. ATPase activity was determined by the measurement of inorganic phosphate production.

The Ca++ loading rates of SR from EDL and soleus of control and suspended rats are shown in the following figure:



Control EDL Ca++ loading rates are more than twice that of control soleus. The SR from EDL of suspended rats had a 10% reduction in Ca++ loading rates, while SR from suspended soleus had about a 45% increase. Preliminary results on Ca++-stimulated ATPase activity of SR from EDL and soleus of suspended rats indicated changes have occurred corresponding to the changes found for Ca++ loading rates.

These results on Ca++ loading rates in soleus correlate with the observed increased contractile speed and supplies more evidence that fiber type conversion in soleus occurs during suspension disuse. Whether a change in neural activity or the loss of weightbearing activity is responsible for these changes in the soleus are currently being studied. Supported by NASA Grant #NAG 2-301.

122.17 ACETYLCHOLINESTERASE FROM LAMPREY SKELETAL MUSCLE IS FOUND IN GLOBULAR AND ASYMMETRIC FORMS. Leo Pezzementi, Ellen Reinheimer*, and Maureen Pezzementi*. Oberlin College, Oberlin, OH 44074 and Birmingham-Southern College, Birmingham, AL 35254.

Last year, we reported preliminary work on the acetylcholinesterase activity of frozen lamprey skeletal muscle (Soc. Neurosci. Abstr. 10, 140.7). Here we present data from experiments performed on fresh muscle in the presence of protease inhibitors. Lamprey skeletal muscle acetylcholinesterase activity appears to be due to acetylcholinesterase, not pseudocholinesterase. Activity is inhibited by BW 284c51, but not by Iso-OMPA or ethopropazine. The enzyme has a high affinity for acetylthiocholine and is inhibited by high concentrations of substrate. A large fraction of the enzyme activity is a glycoprotein, since it is precipitated by concanavalin A-agarose. The precipitation is inhibited by glucose. Optimal extraction of acetylcholinesterase is obtained in a high-salt detergent-containing buffer. Fractional amounts of esterase are extracted in buffers lacking salt and/or detergent. The data suggest that globular and asymmetric forms of enzyme are present. Velocity sedimentation on sucrose gradients shows that the enzyme sediments predominantly as a broad peak of activity that could correspond to A_4 and/or G_4; additionally, there is usually a small peak of activity that could represent A_{12}. Sequential extraction of esterase in conjunction with velocity sedimentation reveals that the enzyme is found in G_2, G_4, A_4, A_8, and A_{12} forms. Some of these may be the products of proteolysis. This work was supported by a Cottrel College Science Grant from the Research Corporation and a grant from the Committee on Research and Development of Oberlin College.

122.18 ACETYLCHOLINESTERASE IN SERUM AND MUSCLES OF DIABETIC RATS. K.A. Skau. Coll. of Pharmacy, U. Cincinnati, Cincinnati, OH 45267.

Experimental diabetes in laboratory animals produces changes in the somatic nervous system such as reductions in conduction velocity and axonal transport. There have been few studies on changes in the end-organ (skeletal muscle) in response to these neuronal alterations. Acetylcholinesterase (AChE) is a sensitive indicator of muscle and nerve function as the molecular forms of this enzyme are regulated by such factors as axonally transported trophic factors, muscle contractile activity and neuronal acetylcholine release. In addition, AChE accumulates in the serum of patients with various neurological diseases. This study examined the potential usefulness of AChE in serum and muscle as indicators of neuronal or myopathic changes in diabetic rats.

Sprague-Dawley rats (180gm) were injected i.v. with streptozotocin (50mg/Kg) to produce a moderate diabetes (serum glucose of 37.6mM vs. 9.1mM for age-matched controls). Blood was withdrawn from a tail vein at 10 and 16 weeks, allowed to clot, centrifuged to sediment formed elements, and the serum was assayed for AChE activity using a radiometric assay. Assay tubes contained 45μM iso-OMPA to inhibit non-specific cholinesterases, and replicate samples were assayed in the presence of iso-OMPA and BW284C51 (10μM) to serve as blanks. At 16 weeks after diabetes induction, the animals were sacrificed and skeletal muscles were removed, homogenized in buffer (50mM Tris HCl, pH 7.4; 0.2mM EDTA) containing high salt (1.0M NaCl) and detergent (1% Triton X-100) to solubilize all forms of the enzyme. The molecular forms were separated on linear 5-20% sucrose density gradients.

Diabetic rats showed typical signs and symptoms of moderate hyperglycemia such as reduced growth rate, glycosuria, cataracts (after 4 mo.), gastric distention, diarrhea and bladder hypertrophy. At 10 wks and 16 wks after induction the diabetic animals exhibited 49% and 103% increases in serum AChE activity. Although the source of this increased activity is not clear, it should be noted that patients with amyotrophic lateral sclerosis, Alzheimer's dementia and progressive muscular atrophy have been shown by others to exhibit elevated serum AChE.

Hemidiaphragms (HD) and extensor digitorum longus (EDL) muscle have three major peaks of AChE activity corresponding to the G_1, G_4 and A_{12} forms. In diabetic rats the total AChE as well as the density of AChE (activity/gm of tissue) was reduced when compared to age-matched controls. In both HD and EDL the A_{12} form was relatively unaffected while the G_4 form was significantly reduced. The G_1 form was variable, being reduced in some muscles and unchanged in others. It is generally accepted that the A_{12} form is regulated by neurotrophic factor while the other forms may be regulated by muscle activity as well. Thus the disruption of AChE in diabetic muscle does not seem to be a simple denervation phenomenon.

PAIN: CENTRAL PATHWAYS II

123.1 SELECTIVE PRESYNAPTIC DEPOLARIZATION OF C FIBRE TERMINALS IN THE FELINE SPINAL CORD. O. Calvillo, M. Ghignone, L. Quintin. Department of Anesthesiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430

Clonidine, an α_2 adrenoreceptor agonist is a powerful antinociceptive agent: behavioral observations rated it 60 times more potent than morphine. There are α_2 adrenoceptors in various regions of the the mammalian central nervous system including Laminae II-III of the spinal cord, which receives a large number of nocicepive afferent fibres. In this region C fibre input is amenable to presynaptic modulation.(1) We studied the effects of clonidine on the excitability of primary afferent terminals of cutaneous low and high threshold fibres. Changes in the excitability of these terminals can be an important determinant of synaptic efficacy. Experiments were done on cats, anesthetized with nembutal paralyzed with pancuronium bromide and ventilated mechanically. The spinal cord was exposed in the lumbosacral region and transected at T13-L1. The sural and posterior tibial nerves were dissected and mounted on electrodes for stimulation or recording. Primary afferent terminal excitability was tested by delivering constant current pulses (up to 375 A 0.5 ms duration) through metal microelectrodes. Antidromic action potentials were recorded from the sural nerve. Two responses were observed. The first had a short latency (2-3 ms) with a conduction velocity of 47-63 m/s identified as A fibre action potential. The second had a much longer latency (250-300 ms) and conduction velocity of 0.9-1.1m/s, its threshold was about 15 times greater than the short latency component belonging to C fibre action potential.(1) Excitability was calculated from the current ratio required to evoke a reponse that served as control to that current that would have been required to obtain the same response after drug administration. Clonidine in doses 1-5 μg Kg^{-1} did not effect the excitability of A fibre primary afferent excitability (n=5). Clonidine increased the excitability of the C fibre primary afferent terminals by about 85% (n=12). This effect was reversed by yohimbine (n=4) and phentolamine (n=8), thus suggesting mediation by α_2 addrenoceptors. Clonidine selectively increased the excitability of the C fibre primary afferent terminals signaling primary afferent depolarization (PAD). During PAD there is a depression of synaptic efficacy so that the responses of second order neurones can be selectively suppressed. Clondine did not affect the excitability of the A fibre terminals, suggesting that the noradrenergic sympathetic mechanism which mediates the antinociceptive action of clonidine is specific for nociceptive spinal pathways. In summary clonidine in spinalized cats selectively produces PAD of C fibre primary afferent terminals without affecting the A fibre terminals.

1. Calvillo, O., et al. 1982 Neurosci 7, 1389-1400.

123.2 EXTRACELLULAR RECORDING OF DORSAL HORN SENSORY NEURON RESPONSES TO NOXIOUS THERMAL STIMULI: CRITICAL DEPENDENCE ON CHOICE OF ANESTHETIC. J.M.Peets* and B.H.Pomeranz. Dept. of Zoology, U. of Toronto, Toronto, Canada M5S 1A1.

In previous reports (Peets and Pomeranz, Pain, Suppl. 2;S370), we have studied the spinal pharmacology of high and low frequency transcutaneous electrical nerve stimulation (TENS) induced analgesia. Rats were intrathecally catheterized, given TENS treatment (either high (100 Hz) or low (4 Hz) frequency) and intrathecal injection of a variety of endorphinergic and serotonergic enhancers and blockers. These studies indicated that, on a spinal level, low frequency TENS analgesia , as determined by tail flick latency, appears to be mediated by opiate receptors. High frequency TENS analgesia does not appear to be endorphin mediated. Furthermore, we could not implicate spinal serotonin receptors in either high or low frequency TENS analgesia.

To further study this analgesia, we sought to record from dorsal horn sensory neurons in the anesthetized rat. Using extracellular multiunit recording techniques, we attempted to record responses to noxious thermal stimuli from these neurons. Rats were monitored for EKG and blood pressur in all experiments, and circulation in superficial cord vasculature was carefully observed. A variety of anesthetics was assayed, and in our experience, clear and reasonably consistent responses to noxious thermal stimuli to the skin were found only in pentobarbital-infused rats, but not in urethane, urethane-chloralose or halothane/nitrous oxide anesthetized rats at a variety of anesthetic depths.

Preliminary results indicate that the responses of dorsal horn sensory neurons to noxious thermal stimuli could be inhibited by low frequency TENS (4 Hz, 20 min.) administered bilaterally to the heels of the rat. We feel that the choice and depth of anesthetic are critical to the success of such recordings, and hence to the study of subtle modulating influences on thermal nociception in the rat.

123.3 SPINAL CORD LAMINAE I AND II NEURONS WHICH PROJECT TO THE CONTRALATERAL PONTINE RETICULAR FORMATION IN THE CAT.
A.R. Light, E.J. Casale*, and D. Menetrey*. Department of Physiology, UNC-Chapel Hill, Chapel Hill, N.C. 27514

Relatively few reports have been made on the physiological properties of lamina I neurons which project to rostral brain structures in the cat. In the present experiments we identified by antidromic activation 13 superficial lumbar dorsal horn neurons which projected to the contralateral parabrachial region of the pons.

Adult cats were anesthetized with sodium pentobarbital throughout the experiments. Monopolar tungsten microelectrodes were used for antidromic stimulation, and horseradish peroxidase (HRP)-filled micropipettes were used for recording both extra- and intracellularly from the somadendritic region of the neurons in the lumbosacral spinal cord.

All 13 rostrally-projecting neurons were nociceptive specific. Eight responded only to noxious mechanical stimulation and 5 responded both to noxious mechanical stimulation and to noxious thermal stimulation.

The conduction velocities of the axons projecting to the contralateral parabrachial region ranged from 0.8 to 10.0 meters/sec (mean 3.5 meters/sec). In addition, in two tested neurons, antidromic activation from the thalamus was also observed. In these two cases the axons' conduction velocity slowed down considerably between the parabrachial region and the thalamus.

Five of the neurons were labeled by iontophoresis of HRP from the recording micropipettes and subsequent diaminobenzidine-H_2O_2 histochemistry. Two of the neurons were small cells located in lamina I while 3 were small neurons located in outer lamina II.

These data suggest that some small, nociceptive specific neurons located in laminae I and II have slowly conducting axons that project contralaterally at least as far rostrally as the pontine parabrachial region. At least some of these also send axon collaterals to the contralateral thalamus.

Supported by NINCDS grants NS-16433 and NS-00534.

123.4 CONVERGENCE OF JOINT AND CUTANEOUS OR INTRAORAL AFFERENT INPUTS TO NOCICEPTIVE NEURONES IN TRIGEMINAL (V) SUBNUCLEUS CAUDALIS (MEDULLARY DORSAL HORN). J.G. Broton, J.W. Hu* and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto M5G 1G6, Canada.

Recent studies in our laboratories have shown that there is extensive convergence of cutaneous, tooth pulp, visceral and muscle afferent inputs to single neurones in subnucleus caudalis of the V brainstem sensory nuclear complex. Most of the neurones showing this extensive convergence were functionally classified as cutaneous nociceptive neurones, and it was suggested that such convergent patterns may contribute to mechanisms of referred as well as deep pain. In the present study we wished to determine if neurones in caudalis also receive convergent afferent input from the temporomandibular joint (TMJ). The extracellular activity of single caudalis neurones was recorded in chloralose-anaesthetized cats. Each neurone encountered was tested to see if it could be activated by electrical stimulation of the TMJ and if it could be functionally classified on the basis of its responses to tactile and noxious orofacial stimuli as a low-tnreshold mechanoreceptive (LTM), wide dynamic range (WDR) or nociceptive-specific (NS) neurone. Electrical stimuli were also applied to facial skin and oral mucosa. For some neurones, the exposed and isolated TMJ capsule was also probed with a mechanical stimulus and algesic chemicals (7% NaCl, KCl, bradykinin, histamine; isotonic saline as control) were injected directly into the TMJ to determine if neurone activity could be evoked.

A total of 45 LTM, 7 WDR and 11 NS neurones in caudalis was tested with electrical stimulation of the TMJ; each of these neurones could be activated by electrical stimulation and tactile (LTM; WDR) or noxious (WDR; NS) stimulation of its facial or intraoral receptive field. Whereas only 8 of the 45 LTM neurones were activated by TMJ stimulation, 4 of the 7 WDR neurones and 8 of 11 NS neurones could be excited by electrical stimulation of the TMJ at latencies ranging between 4-12 msec. A comparable latency range was noted for their electrically evoked responses from cutaneous or mucosa afferent inputs. Most of the nociceptive neurones tested could also be excited by mechanical stimulation of the TMJ and by algesic chemicals injected into the TMJ.

These data indicate that many neurones in V subnucleus caudalis that receive nociceptive afferent inputs from superficial orofacial sites are also involved with transmission of joint afferent information that may also be of a nociceptive character. The findings are therefore consistent with our recent findings of convergence of other deep afferent inputs to caudalis and may explain the poor localization, spread and referral of TMJ pain. (Supported by NIH grant DE04786 to B.J.S. and DE05392 to J.G.B.).

123.5 AN ELECTROPHYSIOLOGICAL STUDY OF FELINE TRIGEMINAL SUBNUCLEUS CAUDALIS NEURONS EXCITED BY MIDDLE MENINGEAL (DURAL) ARTERY STIMULATION. Karen D. Davis* and Jonathan O. Dostrovsky. Department of Physiology, University of Toronto, Toronto, Canada M5S 1A8.

Vascular headaches, such as migraine, are thought to occur when intracranial and/or extracranial arteries dilate. The dural vasculature is known to be extremely pain sensitive and is one of the structures from which headaches are presumed to originate. Although in both anatomical and clinical studies the trigeminal nerve has been implicated in mediating these sensations no electrophysiological evidence has been presented to support this assumption. Thus a search was conducted for neurons in the trigeminal subnucleus caudalis (SNC) which could be excited by stimulation of the middle meningeal artery (MMA) which supplies the dura mater.

Experiments were performed on 7 chloralose anesthetized cats. A small unilateral craniotomy was performed to expose the major branches of the middle meningeal artery (MMA) and the ipsilateral caudal medulla was exposed by retraction of the neck muscles. Mean arterial blood pressure, expired CO_2 and rectal temperature were maintained within their physiological range throughout the experiment. Extracellular single unit recordings in SNC were made during bipolar stimulation (0.1-1.0ms single or double pulses, <15mA) of the MMA. Neurons were classified as either nociceptive specific (NS), wide dynamic range (WDR) or low threshold mechanoreceptive (LTM) according to their responses to nociceptive mechanical, thermal or nonnociceptive mechanical stimulation delivered to the facial skin and/or cornea.

Recordings were obtained for 33 SNC neurons excited by MMA stimulation of which 20 were examined in detail. Most of these cells (85%) were found to have cutaneous receptive fields in the ophthalmic skin and occasionally included the cornea. The few remaining cells (15%) had receptive fields located in the maxillary skin. With the exception of one LTM neuron, all neurons were classified as NS or WDR. The mean latency to neuronal activation from MMA stimulation was 13.3ms which suggests that small, slowly conducting myelinated fibers may be involved in mediating these responses. These results have provided evidence for the existence of neurons in SNC which can be excited by MMA stimulation and suggest that they are probably involved in vascular headache originating in the dural vasculature. These results may also provide an explanation for the phenomenon of vascular head pain which is referred to the periorbital region of the face.
This project was supported by USNIH 1R01 DE05404.

123.6 ANTIDROMIC ACTIVATION OF NEURONS IN THE MEDULLARY DORSAL HORN FROM STIMULATION IN NUCLEUS SUBMEDIUS. J.O. Dostrovsky and J.G. Broton, (SPON: M. Charlton). Dept. of Physiology, Univ. of Toronto, Toronto, Canada M5S 1A8.

Craig and Burton, in a recent anatomical study (J. Neurophysiol., 45,443-466,1981), have described the existence of a specific projection from lamina I of the spinal and medullary dorsal horn (MDH) to nucleus submedius in the medial thalamus and have suggested that nucleus submedius may be a "pain center".

We have investigated this pathway using antidromic stimulation techniques in 5 chloralose anesthetized cats. A small-tipped tungsten microelectrode was stereotaxically placed in or near nucleus submedius and used to stimulate the axons of ascending neurons. A second microelectrode was placed in the MDH (trigeminal subnucleus caudalis) at or just caudal to the level of the obex to obtain extracellular recordings from single neurons. Antidromic invasion was based on constant latency, all or none responses at threshold, and ability of the action potentials to follow two stimuli at intervals of less than 3 ms. In most cases collision of antidromic and orthodromic spikes was also demonstrated.

Twenty-four neurons were recorded in the superficial region of the MDH in or close to lamina I which could be antidromically activated from the thalamic electrode by low stimulation currents (mean 37μA, range of 8 to 90μA). The mean antidromic latency was 5.1ms with a range of 2.0 to 14.5ms. All stimulation sites and some recording sites were verified histologically. For 17 of these neurons it was possible to determine the response characteristics to mechanical and thermal stimulation of the skin. Seven of them were excited by noxious pinch of the nose or whisker pad, and were similar to the lamina I nociceptive specific neurons described by others. Two neurons were excited by a vigorous tap or flick, and a third occasionally by extreme pressure to the nose and these responses although not typical were considered to be due to activation of nociceptors. One neuron was excited by both pinch and nonnoxious pressure and was classified as a wide dynamic range neuron. In addition to these nociceptive neurons, four neurons were excited by cooling and were classified as 'cold' cells, and for two neurons no orofacial mechanical or thermal receptive field could be found.

This study has confirmed using electrophysiological techniques the existence of a direct pathway from the superficial MDH to the region of nucleus submedius and has shown that most of the information transmitted in this pathway is related to noxious stimuli. However, it is not clear from the present study whether innocuous thermal information is transmitted to nucleus submedius or to a nearby region of medial thalamus. Supported by USNIDR grant 1R01 DE05404 and NIH postdoctoral fellowship to JGB.

123.7 TOOTH PULP DRIVEN NEURONS IN THE LATERAL BANK OF THE PRESYLVIAN SULCUS OF THE CAT. M.A. Biedenbach, Dept. of Physiology, Univ. of Texas Health Science Center, San Antonio, Texas 78284.

Electrical stimulation of tooth pulp is generally employed to activate neural mechanisms of nociception. In the cerebral cortex, tooth pulp driven neurons are known to occur in the primary somatosensory region and their functional properties are interpreted to indicate a role in sensory-discriminative aspects of nociception, e.g. stimulus localization, discrimination of stimulus intensity levels. Other cortical areas, although not yet identified, are thought to participate in additional aspects of nociception, e.g. affective-emotional, or pain modulating. The lateral bank of the presylvian sulcus is not part of the primary somatosensoy cortex but has a descending projection to the trigeminal nucleus.

In chloralose anesthetized cats unit potentials were recorded in the lateral bank of the presylvian sulcus while stimulating contralateral and ipsilateral canine tooth pulps. Only a small fraction of neurons in this cortex responded to the stimuli. The typical response to a tooth pulp stimulus consisted of one or a few spikes up to a short burst of spikes. On the basis of spike latency neurons divided into two main groups: an early group discharging between 12 and 30 msec. and coinciding with an early gross potential - and a later group discharging between 40-80 msec., coinciding with a second more variable gross potential. The late discharging group of neurons was the most numerous. In a given neuron, increasing the stimulus intensity from threshold to maximum resulted in a few additional spikes per response and a small decrease in latency. In some early discharging units in addition a second burst occurred during the late gross potential. However, these stimulus intensity produced response changes could be obtained alternatively by keeping stimulus intensity constant but going from a single shock to a pair of shocks. Some neurons responded only to ipsilateral, some only to contralateral and some to both tooth pulp stimuli.

Of course, more extensive studies are required to establish whether these tooth pulp driven neurons play a role in sensory-discriminative or affective aspects of nociception, or in a modulating system through the descending projection to the trigeminal nucleus. Supported by NIH grant NS 16934 and Biomed. Res. Support grant RR 07187.

123.8 RESPONSE CHARACTERISTICS OF NOCICEPTIVE NEURONS IN THE HAMSTER SUPERIOR COLLICULUS. M.A. Larson* and B.E. Stein. Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298.

Most discussions regarding the superior colliculus (SC) center about its involvement with innocuous (usually visual) stimuli. However, investigators in the 1940's and 1950's believed it had a functional role in pain because electrical stimulation of the SC evoked pain in man and behavior indicative of pain in cats. It was not until recently that Stein and Dixon (1978) showed that some SC cells required frankly noxious stimuli for activation or for evoking the greatest number of discharges. These findings were confirmed by Rhoades, et al., (1983). The aim of the present study was to determine how these nociceptive SC cells code the intensity of graded mechanical and thermal stimuli.

Fifty-five nociceptive SC cells were studied in anesthetized hamsters. Two populations of cells were found: (a) wide dynamic range (WDR) cells (n=25) that responded to gentle mechanical, as well as, noxious mechanical stimuli (<100 mg/mm^2-noxious pinch); and, (b) nociceptive specific (NS) cells (n=30) that required higher intensity (1 g/mm^2 - 30 g/mm^2) mechanical and noxious thermal stimuli.

The stimulus-response profiles of WDR and NS neurons to graded skin temperatures closely approximated a linear function (r^2=.977) and the slopes for WDR and NS cells were not significantly different. Thermal thresholds for both groups were 42-46°C and responses increased monotonically from 44-50°C. Thermal responses of the WDR neurons were best described by a power function with an exponent of 2.3 ($f=T^{2.3}$, r^2=.988) and this is consistent with data from dorsal horn WDR neurons and the results from previous psychophysical studies using these same stimuli. NS responses were described by a power function with an exponent of 2.5 ($f=T^{2.5}$, r^2=.988) similar to, but, slightly steeper than the function describing NS neurons in the dorsal horn and with data from psychopysical studies.

These data show that the two populations of SC nociceptive neurons are not only capable of detecting and coding the graded intensities of noxious stimuli, but are remarkably similar to neurons in traditional pain related regions (e.g. dorsal horn, trigeminal n. caudalis, thalamus). It is reasonable to postulate that there is significant survival value in potentially harmful stimuli having ready access to a structure involved in alerting and orienting an animal to peripheral stimuli.

Supported by a grant from the Jeffress Foundation.

123.9 SOMESTHETIC THALAMIC UNIT RECORDINGS AFTER FORELIMB DEAFFERENTATION BY C5-T2 GANGLIONECTOMIES IN RATS. J. Ovelmen Levitt, T. Makachinas*, J. N. Young*, E. Rossitch, Jr.* and B. S. Nashold, Jr. Division of Neurosurgery, Duke University Medical Center. Durham, NC 27710

After unilateral forelimb deafferentation (C5-T2), rats exhibit a behavioral syndrome consisting of licking and biting to the point of amputation of the completely denervated portions of the limb, and, scratching in the adjacent ipsilateral partially denervated area. This phenomenon may be related to dysesthetic sensations referred to the denervated limb which in turn could be generated by abnormal central neuronal activity. Recently, Emmers (1981) has described 2-5 repetitive discharges for 500 msecs. from certain rhythm-emitting neurons in the posterolateral ventrobasal thalamus(SII) of rats after strong, noxious stimulation. This activity rhythm was found to be dependent on excitatory synaptic interconnections with neurons in the centrum medianum-parafascicular nuclear complex, and has been interpreted by Emmers as signalling a painful stimulus. We undertook to study under what conditions neuronal activity of this character was present in SI and SII thalamus after forelimb deafferentation.

Sixty-five thalamic units were recorded in 13 rats: 51 units in 10 control preparations and 14 cells in 3 animals that had 35 day C5-T2 unilateral ganglionectomies. Cells were identified in the face and forelimb area of SI and SII somesthetic thalamus. Responses to subcutaneous electrical stimulation were examined as was the character of spontaneous activity. After initial anesthesia of 90 mg/kg Ketamine, the recordings were done under light (10mg/kg/hr Ketamine) anesthesia and paralysis with Flaxedil.

In the forelimb area of the contralateral SI there was little or no spontaneous activity in either the control preparations or those that had 35 day chronic C5-T2 ganglionectomies. In SII forelimb area there was low level spontaneous activity (1-10 Hz irregular rhythm) of both controls and denervated animals.

Cells in SII were found to repond to wide and often bilateral fields. Rhythm emitting neurons were located in SII in control preparations which responded to strong contralateral face and/or forelimb stimulation. After denervation strong stimulation to the face produced a prolonged after-discharge which continued for seconds. Ipsilateral face and forelimb stimulation also produced an after-discharge.

Although we have not observed spontaneous repetitive discharges of rhythm-emitting neurons located in the forelimb area of SII, contralateral to 1 month C5-T2 ganglionectomies, there was an increased reponse in SII to strong stimulation of innervated receptive fields.

123.10 SPINOTHALAMIC TRACT CELLS IN THE DORSAL HORN OF THE CAT CERVICAL SPINAL CORD. D.G. Ferrington, L.S. Sorkin and W.D. Willis. Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, TX 77550.

Spinothalamic tract (STT) cells in the spinal cord of sub-human primates, carnivores and rodents respond to innocuous and noxious mechanical and thermal stimuli. In the cat there is little quantitative information about the coding of stimuli by STT cells in the dorsal horn. This is in contrast to the wealth of information available for STT cells in the monkey dorsal horn. In the cat, STT cells were found mostly in laminae I, V and IV of the cervical cord, while in the lumbar cord STT cells are predominantly in the ventral horn. We recorded extracellularly the activity of 56 STT cells in the cervical dorsal horn of chloralose anesthetized cats. Identification of STT cells by antidromic activation, mainly from the medial part of the posterior nucleus of the thalamus, was based on three criteria; constant latency of response; an ability to follow high frequency stimulation of the thalamus; and collision of anti- and orthodromic action potentials. Recovery of lesions or dye marks from histological sections indicated that most cells were located in the lateral two-thirds of laminae V and IV.

The cells were divided into 4 groups by their responses to innocuous and noxious levels of mechanical stimuli and manipulation of subcutaneous tissue. The groups were: low threshold (LT) - responded only to innocuous mechanical stimuli; high threshold (HT) - responded only to noxious mechanical stimuli; wide dynamic range (WDR) - responded to innocuous stimuli but with a greater response to noxious stimuli; and deep (D) - responded to manipulation of subcutaneous tissue. For 31 cells classified, the proportions in each class were 10% LT, 13% HT, 61% WDR and 16% D. Fourteen cells were tested for their response to 30 sec duration thermal stimuli in the range 43-55°C. Nine cells had thresholds $\leq$ 55°C (range 47-55; mean = 51 ± 8°C; SD) and showed a graded response to increasing stimulus intensity.

It is concluded that cat STT cells in the cervical dorsal horn respond to noxious thermal and mechanical stimuli and are capable of signalling to higher centers the intensity of a noxious thermal stimulus applied to the skin. In addition, the small receptive field sizes of these cells and their conduction velocities (range 9-76 m/s; mean = 38 ± 17 m/s; SD) suggest that the cells might play an important role in signalling the location of painful stimuli.

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123.11 SOMATOSENSORY PSYCHOPHYSICAL RESPONSES OF PATIENTS WITH BURNING MOUTH SYNDROME (BMS). M. Grushka*, B.J. Sessle and T.P. Howley*. Fac. of Dentistry, University of Toronto, Toronto, Canada M5G 1G6.

BMS is a disorder primarily of post-menopausal women who experience a constant intra-oral burning sensation. Although little is known of the aetiology of this disorder, a strong neurological or psychogenic component is often inferred. We have initiated studies to determine whether there is any objective evidence of sensory dysfunction in the oral and perioral tissues of patients suffering from BMS. The study was carried out in 72 BMS patients (mean age ± S.D.: 57.5±10.6 years) in whom the most frequent site of pain was the tongue tip. We administered psychophysical tests of tactile and 2-point discrimination thresholds (by means of Semmes-Weinstein Pressure and Weinstein anaesthesiometers, respectively) on the tongue (4 sites), lower lip, anterior palate and right and left cheeks; oral stereognostic ability was also determined with the use of 20 geometric forms. In addition, a thermal stimulator (Thermal Devices Inc., Minnesota) was used to determine, in °C, thermal detection and pain thresholds, pain tolerance, and warm magnitude estimation at the tongue tip and lower lip. A group of 43 volunteers (mean age 56.2±9.6 years) served as age and sex-matched controls and the data were compared by t-tests or ANOVA.

No statistically significant ($p>0.05$) differences in mean values between BMS and control subjects were found, for any of the 8 sites tested, in tactile thresholds (range of $\log_{10}$ values, in mg: BMS, 1.04±.39 to 1.95±.46; controls, 1.03±.39 to 2.23±.49) or 2-point discrimination thresholds (range of values, in mm: BMS, 1.54±.75 to 14.00±5.62; controls, 1.45±.72 to 14.81±3.78). Mean oral stereognosis scores (BMS, 13.76±3.56; controls, 14.39±2.46), thermal detection thresholds (tongue tip: BMS, 0.73±.25; controls, 0.73±.23; lower lip: BMS, 0.76±.26; controls, 0.88±.27) and heat pain thresholds (tongue tip: BMS, 44.45±3.27; controls, 44.88±2.58; lower lip: BMS, 44.79±3.31; controls, 45.29±2.45), as well as warm magnitude estimation also were not significantly different. However, for heat pain tolerance (defined as the maximum temperature which subjects could withstand for 30 seconds) the mean value was significantly lower for BMS patients than for control subjects at the tongue tip (BMS, 46.43±2.32; controls, 48.12±1.12, $t=3.24$, $p<0.01$) but not at the lower lip (BMS, 47.32±2.44; controls, 48.27±1.60, $t=1.64$, $p>0.05$). In addition, no significant differences in heat pain tolerance were found between these two sites for each group.

These data suggest that changes do not appear to occur in tactile, stereognostic, and thermal detection and estimation capabilities of BMS patients. However, alterations may occur in peripheral or central pathways subserving some thermal pain functions, and this dysfunction may be localized to the tongue.

PEPTIDES: RECEPTORS II

124.1 MODULATION OF CENTRAL CHOLECYSTOKININ RECEPTORS BY GUANYL NUCLEOTIDES. L.P. Wennogle, H. Wysowskyj*, and B. Petrack. Neuroscience/Cardiovascular Research, CIBA-GEIGY Corp. Summit, New Jersey 07901.

The concept of endogenous modulators of neuronal activity has led to a tremendous interest in neuropeptides. A classic example of this principle is cholecystokinin (CCK) modulation over mesolimbic dopaminergic transmission in the mammalian CNS. In spite of intense interest, the mechanism by which CCK exerts an influence over neurotransmission is unknown; the connection between central CCK receptors and nucleotide regulatory proteins is poorly understood. We report here findings which clarify the link between central CCK receptors and guanyl nucleotides and indicate a possible mechanism of CCK action. Guanylyl-imidodiphosphate (GppNp) inhibited CCK binding to mouse cortical membranes in a complex fashion. CCK binding (Wennogle et al. Life Sciences 36, 1485, 1985), using a Bolton-Hunter iodinated CCK8 (^{125}I-CCK8), was reduced by micromolar GppNp, but binding plateaued at a value of 65%. Dissociation of radioiodinated ^{125}I-CCK8 was markedly increased when GppNp was included at the moment of initiation of ^{125}I-CCK8 dissociation by unlabeled CCK. Competitive interaction between GppNp and CCK could not account for the effect on dissociation and we concluded that GppNp is acting at a distinct site on the CCK-receptor complex. Scatchard analysis of ^{125}I-CCK binding in the presence and absence of GppNp revealed no change in receptor number at saturation, but a small decrease in affinity was caused by GppNp. The results are consistent with a conformational change in CCK receptors induced by guanyl nucleotides. A number of displacing ligands were tested on ^{125}I-CCK8 binding. The ratio of IC_{50} without/with GppNp was taken as a measure of the relative affinity of the displacer for the CCK receptor. By this analysis, some displacers had a GppNp ratio less than one, indicating that GppNp decreased their affinity for the receptor population. This group of compounds included CCK8, t-boc-CCK4, and caerulein; these agents are considered receptor agonists. Another group of agents, including benzotript and CBZ-CCK26-32, had GppNp ratios greater than one, indicating greater affinity in the presence of GppNp. These agents are considered receptor antagonists. The shift ratios ranged from 0.6 for agonists to 1.5 for antagonists. These observations suggest that central CCK receptors are regulated by guanyl nucleotides, perhaps indicating a dopamine modulatory mechanism.

124.2 NEUROTENSIN RECEPTORS IN THE FOREBRAIN AND MIDBRAIN OF THE PIGEON. S.E. Brauth, C.A. Kitt, A. Reiner and R. Quirion. Dept. of Psychology, Univ. of MD, College Park, MD, Dept. of Pathology, Neuropathology Lab., Johns Hopkins Univ., Balto., MD, Dept. of Anatomy & Cell Biology, Univ. of MI, Ann Arbor, MI and Douglas Hosp. Res. Centre, Verdun, Quebec, Canada.

Autoradiographic methods were used to assess the distribution of binding sites for 3-H Neurotensin (NT) in the forebrain and midbrain of the pigeon. Within the telencephalon the highest levels of NT-receptors were observed within the hyperstriatum ventrale (HV). Moderately high levels of NT-receptors were observed within the archistriatum, neostriatum intermedium and hyperstriatum accessorium. These telencephalic regions and HV are thought to be comparable to portions of mammalian neocortex. Low levels of binding sites were observed within the striatal complex including the laterally situated paleostriatum augmentatum and medially situated lobus parolfactorius. The lowest levels of NT-receptor sites in the telencephalon were observed within the paleostriatum primitivum (PP, believed comparable to mammalian globus pallidus), ectostriatum (comparable to layer IV of mammalian extrastriate visual cortex), Field "L" (comparable to layer IV of mammalian auditory cortex), hippocampus, septum and preoptic area.

Within the brainstem high levels of NT-receptors were observed in the lateral habenular nuclei, the ventral tegmental area of Tsai, nucleus tegmentipedunculopontinus, pars compacta (comparable to the mammalian substantia nigra, pars compacta), locus ceruleus and the nucleus subceruleus dorsalis. The latter four cell groups contain numerous catecholaminergic neurons. Corresponding catecholaminergic cell groups in mammalian forms also contain high levels of NT-receptors. Lower levels of NT-binding sites were observed within the following nuclei of the avian thalamus and midbrain: nucleus rotundus, nucleus triangularis, nucleus spiriformis lateralis, nucleus spiriformis medialis and nucleus ectomamillaris. Low levels of NT-receptors were also observed within the retinal terminal layers of the tectum (i.e. layers 1-7). This latter finding is consistent with immunohistochemical studies demonstrating the presence of a NT-related hexapeptide (i.e. LANT-6) within avian retinal efferents (W.D. Eldred and A. Reiner, unpub. obs.).

NT-receptors in the lateral spiriform nucleus may be a consequence of the input this cell group receives from PP. Immunohistochemical studies have shown that neurons of PP are rich in LANT-6. The tritiated NT used as ligand in the present study may have some affinity for the putative LANT-6 receptor of the avian brain. The basis for the high levels of NT-receptors in HV is uncertain. Although numerous telencephalic and thalamic cell groups which may project to HV contain abundant NT-positive neurons, the precise source of NT-containing inputs to HV is currently unknown. Supported by Grants No.BNS 8312571 (SEB) and NS 19620 (AR).

124.3 CHARACTERIZATION OF AVP EFFECTS ON B-50 PHOSPHORYLATION IN RAT HIPPOCAMPAL SYNAPTIC MEMBRANES. A. Hinko* and A.F. Pearlmutter* (SPON: T.H. Chiu). Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699.

Our laboratory has reported previously the characteristics of specific AVP binding in the presence of Ni^{2+} to rat hippocampal synaptic plasma membranes (SPM) (Costantini, M.G. and Pearlmutter, A.F., J. Biol. Chem. 259: 11739, 1984). We have extended our investigation to determine if Ni^{2+}, AVP, and AVP analogs affect membrane protein phosphorylation in hippocampal SPM. Hippocampi were excised from anesthetized male Sprague-Dawley rats (100-150 g) and homogenized in 10 vol 0.32 M sucrose containing protease inhibitors. SPM preparation was done at 0-4°C. A crude mitochrondrial/synaptosomal pellet was prepared and resuspended in H_2O to cause synaptosomal lysis. The lysate was centrifuged and the supernatant layered onto a discontinuous gradient of 0.4 M and 1.0 M sucrose. After ultracentrifugation, the SPM material floating on the interface between the sucrose layers was collected and used for phosphorylation studies. Phosphorylation conditions were: 15-25 µg SPM, 10 mM Mg^{2+}, 0.1 mM Ca^{2+}, ± 5 mM Ni^{2+}, 20 mM tris, pH 7.4. After 5 min preincubation at 30°C, 5 µM ATP with 2 µCi [γ-^{32}P] ATP was added. Reactions were stopped 20 sec later with a solubilization solution. Peptides were added 30 sec prior to ATP. Proteins were separated by SDS-PAGE and analyzed by autoradiography or liquid scintillation counting of the bands. Ni^{2+} caused a reduction in overall SPM protein phosphorylation, but Ni^{2+} did not alter the changes in phosphorylation caused by the peptides. The peptides (10^{-4} - 10^{-3} M) reduced phosphorylation of a presynaptic phosphoprotein (B-50) in the following order of potency: AVP = oxytocin > desglycinamide AVP > deamino-8-D-arginine-AVP > $d(CH_2)_5$Tyr(Me)AVP = [pGlu^4,Cyt^6]AVP-(4-9). This generally corresponds to their relative efficacy in displacing [^{3}H]AVP from high affinity SPM binding sites. However, when 10^{-6} - 10^{-4} M AVP was added after maximum ^{32}P incorporation (30 sec after ATP addition), AVP markedly inhibited B-50 dephosphorylation over a 30 min period. In conclusion, AVP can affect both phophorylation and dephosphorylation of B-50 in hippocampal SPM; these effects depend upon incubation conditions. The physiological importance of these in vitro effects of AVP on SPM phosphorylation remains to be examined. This research was sponsored by NIH grant, NS 17848.

124.4 **CHARACTERIZATION OF BRADYKININ ANTAGONISTS IN A CULTURED NEURONAL CELL LINE.** **K. M. Braas[1], D. C. Manning[1], V. S. Wilson[*2], D. C. Perry[3], J. M. Stewart[3], R. J. Vavrek[*3], and S. H. Snyder[1]. [1]Dept. of Neuroscience, Johns Hopkins Univ. Medical School Baltimore, MD 21205, [2]Dept. of Pharmacology, George Washington Univ. Medical School Washington D.C. 20037, [3]Dept. of Biochemistry, Univ. of Colorado Medical School Denver CO 80262.**

The nonapeptide bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is present in a variety of tissues and appears to be involved in inflammation, pain and cardiovascular regulation. Bradykinin nonapeptide (Perry and Snyder, J. Neurochem. 43:1072, 1984) and immunohistochemically visualized bradykinin (Correa et al, Proc. Natl. Acad. Sci. 76:1489, 1979) occurs in neurons in brain. Until recently, specific sequence-related antagonists of bradykinin activities have not been available. Substitution of the proline in position 7 with D-phenylalanine (D-Phe) has been shown to convert bradykinin agonists to specific antagonists (Stewart and Vavrek, In: Kinins 1984, Greenbaum, L.M. ed., Plenum Press, NY, 1985). The antagonism is specific for bradykinin agonists in the rat uterus and guinea pig ileum smooth muscle assays and in the rat blood pressure kinin assay. Potency is enhanced when the phenylalanines at positions 5 and 8 are replaced with β-(2-thienyl)-L-alanine (Thi). To ascertain if these antagonists act at bradykinin receptors in neuronal cells, we have employed the cultured neuronal cell line NG108-15 which has many properties of normal differentiated neurons. NG108-15 cells plated at an initial density of 4000 cells/cm² were cultured with Dulbecco's modified Eagle medium supplemented with 10% newborn calf serum and HAT for 7 days. Binding of [³H]-bradykinin to NG108-15 cell membranes was assayed at 25°C in 25 mM Tes, pH 6.8, containing 1 mM 1,10-phenanthroline, 0.1 mM bacitracin, 1 mM dithiothreitol and 0.1% BSA for 90 min. Bound [³H]-bradykinin was separated from free by filtration over GF/B filters pretreated with 0.1% polyethyleneimine. Binding of [³H]-bradykinin was saturable, specific and of high affinity (Kd, ~50 pM), which is similar to that observed in previous studies for the N1E-115 cell line and guinea pig ileum. Competition experiments were performed using [³H]-bradykinin (50 pM) and unlabeled agonist or antagonist peptides. [³H]-bradykinin binding sites in NG108-15 cells displayed Ki values of 90 pM for bradykinin, 300 pM for [Thi⁵,⁸]-bradykinin and 10-15 nM for the antagonists [D-Phe⁷]-bradykinin and [Thi⁵,⁸,D-Phe⁷]-bradykinin. Substitution of hydroxyproline in position 3 or addition of Lys-Lys to the N-terminal does not decrease the potency of the [Thi⁵,⁸,D-Phe⁷] antagonist. Potencies of other antagonists with additional amino acid modifications and substitutions are currently being evaluated. The ability of these antagonists to inhibit bradykinin receptor-mediated cGMP formation and accumulation of [³H]-inositol phosphates in NG108-15 cells under investigation.

124.5 EVIDENCE FOR SPECIFIC NEUROPEPTIDE Y BINDING SITES IN RAT BRAIN MEMBRANES USING [^{3}H]NEUROPEPTIDE Y. J.C. Martel*, R. Quirion and S. St-Pierre* (SPON: E. Ramon-Moliner). Douglas Hospital Research Centre, Verdun, Quebec H4H 1R3 Canada and F,culté de Médecine, Université de Sherbrooke (Quebec) J1H 5N4 Canada.

Neuropeptide Y (NPY), a 36 amino acid member of the pancreatic polypeptide family, has been found to be widely distributed in mammalian brain with particularly high concentrations in hypothalamus and olfactory tubercle (P.C. Emson and M.E. DeQuidt, TINS, 7:31-34, 1984). Although NPY is thought to act as a neurotransmitter, little is known on the distribution of its receptors in brain. Earlier report on NPY binding in rat brain membranes (with radioiodinated NPY) have demonstrated the presence of specific binding sites for that peptide in the central nervous system (A. Saria et al., Eur. J. Pharm., 107:105-106, 1985). Here we report on the existence of NPY binding sites in rat brain using a tritiated ligand.

Rats were killed by a blow on the neck and decapitated. Brains were rapidly removed and put in ice cold Krebs-Ringer buffer. Brains (minus cerebellum) were homogenized using a Brinkmann polytron at setting 5 for 15 seconds and centrifuged for 15 minutes at 39,000 g. Pellets were rinsed with ice cold Krebs-Ringer, resuspended and centrifuged for another 15 minutes at 39,000 g. Final pellets were rinsed and resuspended in ice cold Krebs-Ringer (4.5 ml per brain). 100 µl of this membrane preparation were incubated for 60 minutes at room temperature in 400 µl of Krebs-Ringer buffer plus 0.1% BSA, 0.05% bacitracin and 2.0 nM [^{3}H]NPY (70 Ci/mmol, Amersham). Incubations were terminated by rapid filtration under reduced pressure through GF/C filters pretreated with 1.0% polyethyleneimine (PEI). Specific binding was defined as the radioactivity bound in presence or absence of 1.0 µM NPY. Under these conditions, specific binding ranged between 70-80%. This high specific binding is probably due to the pretreatment of filters with PEI and the extensive washing of filters with ice cold Krebs-Ringer. Non radioactive NPY displaced [^{3}H]NPY binding with a IC_{50} of 4.3 nM while human pancreatic polypeptide displaced [^{3}H]NPY with an IC_{50} of 900 nM. These data indicate that [^{3}H]NPY is a useful, newly available, ligand to study NPY receptor binding sites in the central nervous system.

124.6 VIP(10-28) IS AN ANTAGONIST OF VIP ADENYLATE CYCLASE STIMULATION* IN HT29 CELLS. J.T. Turner, C.J. Ray-Prenger and D.B. Bylund. Dept of Pharmacology, Univ. of Missouri Sch. of Med., Columbia, MO 65212.

One major limitation in studying many of the neuropeptides has been the lack of specific antagonists. In the HT29 human colonic adenocarcinoma cell line, the neurotransmitter vasoactive intestinal peptide (VIP) binds to specific receptors, increases adenylate cyclase activity and stimulates glycogenolysis (Rousset, et al., FEBS Letts 126:38, 1981). During our characterization of the HT29 VIP receptor-cyclase system, we observed that the 19 residue carboxy terminal fragment of VIP, VIP(10-28), inhibited ^{125}I-VIP binding in both intact cells and in membranes and also inhibited VIP-stimulated, but not basal or forskolin-stimulated, adenylate cyclase activity. In a representative experiment, the addition of 3 µM VIP(10-28) produced a 5 fold increase (2 nM-10 nM) of the ED_{50} for VIP stimulation of the enzyme without changing the efficacy of VIP, consistent with competitive antagonism. Two peptides structurally related to VIP, PHI-27 and PHM-27, also stimulated HT29 adenylate cyclase and the antagonism of the effects of these peptides by VIP(10-28) was also studied. A dissociation constant for VIP(10-28) from Schild plots with each agonist was determined. Although the potency of VIP(10-28) is low, the proteolytic cleavage of an endogenous agonist to yield a specific antagonist suggests an intriguing possible in vivo regulatory mechanism. This work was supported in part by a grant from the American Heart Association, Missouri Affiliate.

124.7 TACHYKININ RECEPTORS IN MAMMALS. J. E. Maggio[1], C. R. Mantyh and P. W. Mantyh[2], [1]Harvard Med. Sch. Dept. of Pharmacology, Boston, MA 02115 and [2]Center for Ulcer Res. and Educ., VA Wadsworth Center, Los Angeles, CA 90073.

Substance P (SP) is the best known member of the tachykinin family of bioactive peptides, which share the conserved carboxyl-terminal sequence -Phe-X-Gly-Leu-Met-NH_2. SP was once thought to be the only tachykinin in mammals, but in 1983 four groups independently reported that mammalian tissues also contain two additional tachykinins, substance K (SK) and neuromedin K (NMK), so named to reflect their homology with the amphibian tachykinin kassinin. The mammalian techykinins differ in their central and peripheral pharmacologies, suggesting that they may mediate different physiological functions through different receptors. Using ^{125}I-labelled Bolton-Hunter conjugates of tachykinins as radioligands, we have studied tachykinin receptors in the rodent CNS and periphery using homogenate binding and autoradiography.

The radioligands (BHSP, BHSK, BHNMK, etc.) were synthesized by conventional methods and purified to >2000 Ci/mmol by reverse-phase HPLC. The same ligands were used for both homogenate binding and autoradiography at 0.1 nM concentration in 0.05 M TrisHCl pH=7.5 containing 0.02% BSA, 40 mg/l bacitracin, 4 mg/l leupeptin, 5 mg/l chymostatin and 3 mM $MnCl_2$. Both binding and autoradiography experiments were worked up by extensive washings after 1-2 hr incubations with ligand. In test-tube binding experiments BHSP, BHSK, BHNMK were most effectively displaced ($IC_{50} < 10^{-8}$ M) by SP, SK, NMK, respectively, suggesting that each mammalian tachykinin has a distinct set of binding sites. Autoradiographic studies supported this conclusion and further revealed that each of the tachykinin binding sites has a distinct regional distribution. Striking geographical differences were noted in the cerebral cortex, amygdala, hippocampus, hypothalamus and olfactory bulb.

In addition, studies with Bolton-Hunter conjugates of the nonmammalian tachykinins kassinin (BHK) and eledoisin (BHE) suggested further subclassification of mammalian tachykinin receptors. Thus BHK appears to label a particular subset of SK receptors while BHE labels a particular subset of NMK receptors in rat brain. The mammalian tachykinin system is apparently more complex than previously supposed.

In summary, these studies demonstrate that each of the three known mammalian tachykinins has a distinct set of binding sites with a distinct regional distribution in the CNS and periphery. These presumptive receptors can be further subclassified on the basis of their ligand binding properties.

124.8 ELECTRON MICROSCOPIC VISUALIZATION OF NEUROTENSIN BINDING SITES IN RAT SUBSTANTIA NIGRA. E. Moyse*, M. Vial*, K. Leonard*, P. Kitabgi*, J.P. Vincent*, W. Rostène and A. Beaudet. (SPON: B.E. Jones). Montreal Neurol. Inst., 3801 University St., Montreal, Canada H3A 2B4; INSERM U-55, 84 rue du Fbg. St. Antoine, 75012 Paris, France; C.N.R.S., Fac. Sci., Parc Valrose, 06034 Nice, France.

Specific binding sites for neurotensin (NT) were localized in rat brain by high resolution radioautography using Tyr^3-monoiodinated NT (spec. act: 2000 Ci/mmole). The effects of histological processing on the binding and retention of ^{125}I-NT were first assessed on 20 µm-thick cryostat sections of the midbrain incubated for 1 h at 4°C. Binding of ^{125}I-NT to unfixed tissue sections was highly specific (> 90% of total) with a K_D of 7.7 nM and a B_{max} of 76 fmol/mg prot. These binding kinetics remained unchanged following pre-fixation of the brain with .75% paraformaldehyde, .1% glutaraldehyde and 1% tannic acid. The radioautographic distribution of ^{125}I-NT binding sites was similar in unfixed and pre-fixed sections, and conformed with that previously observed using ^{3}H-NT, with high labeling densities in the substantia nigra and ventral tegmental area. Post-fixation of the labeled sections with 4% glutaraldehyde followed or not by 2% OsO_4 ensured regionally proportional retention of more than 70% of the specifically bound ligand during subsequent dehydration, suggesting that the majority of bound ^{125}I-NT molecules had been cross-linked by glutaraldehyde onto or in the vicinity of their specific binding sites. For high resolution radioautographic visualization of these sites, 75 µm-thick pre-fixed vibratome slices from the ventral midbrain tegmentum were incubated with .3 nM ^{125}I-NT, rinsed in ice cold buffer, post-fixed as above, dehydrated in ethanols and flat embedded in epon. Semi-thin and thin sections were cut from each slice and respectively processed for light and electron microscopic radioautography according to standard dipping techniques. Light microscopic radioautographs exhibited an overall labeling pattern similar to that observed in cryostat sections. Within the substantia nigra, the label was diffusely distributed over the neuropil and selectively accumulated along the plasma membrane of the perikaryon and proximal dendrites of a sub-population of pars compacta neurons. There was also a conspicuous labeling of the capillary endothelium but this labeling, unlike the rest, was displaced neither by native NT nor by one of its natural metabolites (NT 1-8), even at concentrations of 100 µm. In electron microscopic radioautographs, bound ^{125}I-NT molecules were mostly detected as isolated silver grains overlying more than one cellular profile. A significant number of these grains appeared to be associated with neuronal, particularly axodendritic, membrane interfaces. Only a few, however, could be ascribed to synaptic junctions by resolution circle analysis. The present results demonstrate the applicability of ^{125}I-Tyr^3-NT to high resolution radioautographic detection of NT binding sites in rat brain and suggests that in the substantia nigra: (1) part of these binding sites is localized on the perikaryon and dendrites of a sub-population of pars compacta neurons and (2) only a small proportion is actually associated with synaptic junctions.

124.9 AUTORADIOGRAPHIC LOCALIZATION OF CALCITONIN GENE RELATED PEPTIDE BINDING SITES IN THE RAT BRAIN, GUINEA PIG PERIPHERY AND HUMAN SPINAL CORD. P.W. Mantyh, C.R. Mantyh*, N.C. Brecha, L. Kruger, and C. Sternini. Center for Ulcer Research and Education, Bldg. 115, Room 219, LA, CA, 90073 and Brain Research Institute, UCLA, LA, CA, 90024

Calcitonin Gene Related Peptide (CGRP) is widely distributed throughout the CNS and periphery and has been shown to possess potent in vivo actions when administered either centrally or peripherally.

Here we have used autoradiographic techniques to localize and quantify specific high affinity binding sites for human ^{125}I-CGRP (Amersham) in the rat brain, guinea pig periphery and human spinal cord. Rat and guinea pig tissue was removed immediately after death and the human tissue was obtained less than 10 hours postmortem. Serial tissue sections (30um) were obtained and preincubated in a solution of 50 mM Tris HCl (pH 7.4), 2% BSA, .05% bacitracin, and 5 mM $MgCl_2$ (10 min, 20°C). Sections were transferred to the same solution with 100pM ^{125}I-CGRP added (60 min, 20°C) and subsequently washed in 50mM Tris-HCl (4 times, 1 min each, 4°C). The slides were dried and exposed for 5 days against LKB Ultrofilm to generate autoradiograms. Autoradiograms were analyzed using densitometry. Specific binding was defined as that binding that was displaced by 1 uM human CGRP but not by unrelated peptides. Initial experiments determined that these incubation conditions resulted in the highest specific/non-specific binding ratios.

Autoradiograms revealed that in the rat CNS high concentrations of CGRP binding sites are present in the lateral septum, entorhinal cortex, lateral nucleus of the amygdala, solitary nucleus, dorsal motor nucleus of the vagus and the pars caudalis of the spinal trigeminal nucleus. In the guinea pig periphery high concentrations of CGRP binding sites are present in the epithelium of the lung, the collecting tubules of the kidney, the longitudinal smooth muscle of the duodenum, ileum, and colon and the smooth muscle of the vas deferens. In the human spinal cord high concentrations of CGRP binding sites are present in lamina 1,2,and 5-9. Our results indicate that CGRP binding sites in the rat, guinea pig and human can be labeled with high specific/non-specific binding ratios using human ^{125}I-CGRP as the radioligand and that if these specific binding sites do correspond to functional CGRP receptors it appears that CGRP modulates a diverse set of cells in both the CNS and the periphery. Supported by NS-5685, AM-17328, a Smith Kline and Beckman Award to to P.W.M., and Sloan Fellowships to P.W.M. and N.C.B.

124.10 CHARACTERIZATION OF INSULIN RECEPTORS IN THE CHOROID PLEXUS OF THE RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY AND COMPUTER DENSITOMETRY D.A. Davidson*, E.S. Corp*, D. Figlewicz*, S.C. Woods, D. Porte Jr.*, D.M. Dorsa, D.G. Baskin, Depts. Medicine, Pharmacology, Psychology, and Biological Structure, Univ. of Washington, and VA Medical Center, Seattle, WA 98108.

We recently reported the presence of specific binding for insulin in the choroid plexus (CP) of the rat brain with in vitro autoradiography. We sought to determine if these binding sites exhibited pharmacological properties similar to those of peripheral insulin receptors. Therefore, we exposed lateral ventricle choroid plexus to [125-I]-human insulin (0.05 nM; SA = 300 µCi/µg for 18 hours at 4°C) using frozen sections of rat brain. Specific binding was defined as radioactivity displaced by 10 µM unlabeled porcine insulin. Techniques of in vitro receptor autoradiography and computerized densitometry on the resulting LKB film images were used to obtain measurements of insulin binding properties. Iodoinsulin binding was determined by a standard curve relating optical density to CPM/sq. mm. radioactivity in the tissue slice. Insulin binding to the choroid plexus showed time and temperature dependency, saturability and reversibility. Competition with [125-I] insulin for insulin specific binding sites was observed with the addition of increasing concentrations of unlabeled porcine insulin. No competition for insulin binding sites was observed with the addition of 1µM ribonuclease or glucagon. To establish specificity, competitions with insulin analogs were performed. The equimolar ratios of the IC-50 values for competition with human iodoinsulin to binding sites on the CP were: chicken insulin = 0.5; porcine insulin = 1; proinsulin = 3.75; tuna insulin = 11; desoctapeptide insulin = 95; IFG-1 = 667. The relative order of binding affinities of the various insulin analogs was similar to that of insulin receptors in peripheral tissues. Scatchard analysis revealed that the data could be fit using a two binding site model (N = 3 rats). The Kd (mean ± SD) of the high and low affinity binding sites were 2.0 nM ± 1.0 nM and 170.0 nM ± 60.0 nM, respectively. To determine the microanatomical localization of these receptors, intact choroid plexus was removed from the lateral and fourth ventricles and incubated with [125-I] insulin as described above. After embedding in methacrylate, sections of choroid plexus were prepared for autoradiography with NTB2 emulsion. Results showed most autoradiographic grains were on the CSF surface of the choroidal epithelial cells. There was a marked reduction of grains on both surfaces with the addition of 1 µM unlabeled porcine insulin. These findings support the conclusion that binding sites with characteristics of insulin receptors are present in the choroid plexus. This raises the new hypothesis that CP insulin receptors are involved in choroid plexus function and regulation of CSF composition.

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124.11 CHARACTERIZATION OF ^{3}H-VASOPRESSIN BINDING SITES IN THE DEVELOPING RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY. F. Petracca*, D. Baskin, J. Diaz, and D. Dorsa (SPON: D. Dorsa). GRECC, VA Medical Center, and Depts. of Pharmacology, Psychology, Biological Structure and Medicine, Univ. of Washington, Seattle, WA 98195

We have recently reported binding of ^{3}H-Arginine^{8}-vasopressin (AVP) in several regions of the developing rat brain (Soc. Neurosci. Abstr., Vol 10, Part 2, p. 1042, 1984), including the caudate nucleus, septum, dorsal hippocampus, cingulate gyrus, and amygdala. We observed that the pattern of distribution of the sites changes dramatically over the course of the early postnatal period, suggesting that vasopressin may play a role in brain development. In the present study, we examined the specificity and saturability of the ^{3}H-AVP binding sites which we have observed in the developing rat brain, to determine whether they are specific vasopressin receptors.

To determine saturability, Day 10 Long-Evans rat pups were sacrificed, and 20-micron, slide-mounted brain sections were incubated for 30 minutes in Tris buffer solution containing ^{3}H-AVP (concentrations ranging from .25 nM to 10 nM) in the presence or absence of 1 μM unlabeled AVP. For specificity studies, Day 6 brains were incubated in buffer solution containing 2.5 nM ^{3}H-AVP in the absence or presence of a 1 μM concentration of one of the following unlabeled peptides: AVP, oxytocin, or luteinizing hormone releasing hormone (LHRH). Slides were placed in contact with LKB Ultrofilm for 30-50 days. Concentrations of bound AVP were determined by computer densitometry, using a standard curve relating optical density to fmol ^{3}H-AVP/mm^{2} established using tissue containing known quantities of tritium.

In the cingulate gyrus, specific binding of ^{3}H-AVP increased to 45% of total at 2.5 nM and did not further increase over the range of ligand concentrations tested. In the septum, when exposed to 2.5 nM ^{3}H-AVP, we observed total binding (TB) of 0.15 fmol/mm^{2}, 53% of which (0.07 fmol/mm^{2}) was specific binding as defined using 1 μM unlabeled AVP. In the cingulate gyrus, TB was 0.18 fmol/mm^{2}, and specific binding was 0.10 fmol/mm^{2}, (55%). Specific binding of ^{3}H-AVP was also equally displaced in these areas when the tissues were incubated in the presence of 1 μM oxytocin but was not reduced by LHRH, a peptide of similar size but which is structurally unrelated to AVP. Results of the present study indicate that the ^{3}H-AVP binding which we have observed in the neonatal Long-Evans rat is saturable and demonstrates specificity for neurohypophyseal peptides. Whether this site is a specific receptor for AVP will require further specificity studies using similar methodologies.

This work was supported by NIH Grant NS 20311.

124.12 PHARMACOLOGIC CHARACTERIZATION OF VASOPRESSIN RECEPTORS IN THE BRAIN, LIVER AND KIDNEY OF THE BRATTLEBORO RAT. L. Shewey*, L.E. Cornett*, and D.M. Dorsa (SPON: C. Chavkin) GRECC, VA Medical Center and Depts. of Pharmacology and Medicine, Univ of Washington, Seattle, WA 98195 and UAMS, Little Rock, AR 72205

Recently, specific binding sites for ^{3}H-Arginine^{8}-vasopressin (AVP) have been localized and characterized in several tissues of the rat. In the homozygous Brattleboro rat (HO-BB), a recessive trait leads to a near complete inability to synthesize AVP in the brain, which results in diabetes insipidus (DI). HO-BB rats show several other behavioral and physiological deficiencies, which can be reversed by treatment with AVP suggesting that they do have functional receptors for this peptide. The present studies were performed to determine the effect of AVP absence on the pharmacological characteristics of the AVP receptors of the CNS and periphery in the Brattleboro rat.

For this study, age matched adult male heterozygous (HE-BB) and homozygous (HO-BB) rats were sacrificed, and liver, brain and kidney tissue removed and dissected. Membranes were prepared from pooled tissue, stored at -70°C and thawed for use. HE and HO-BB rat membranes were incubated in the same assay for 45 minutes at 22°C with ^{3}H-AVP (S.A. = 40 Ci/mmol) with or without 1 μM cold AVP.

The results of saturation experiments (using ^{3}H-AVP in concentrations from 0.25 nM-10 nM) yielded a single-site best fit as determined by a computerized Scatchard analysis, and are summarized in the following table: K_d (nM), B_{max} (fmol/mg protein).

		Liver	Renal Medulla	Hind-brain	Hippo-campus	Amygdala	Septum
		n=3	n=4	n=4	n=4	n=3	n=3
K_D	HE	4.8±.1	4.8±0.09	2.3±0.7	1.9±0.4	1.9±0.4	1.9±0.7
	HO	3.9±.32*	3.0±0.2*	3.3±0.7*	3.8±0.4**	2.8±0.2	3.2±0.7
B_{max}	HE	123±72	83±22	10±2.1	10±0.3	10.3±2.8	8.4±4
	HO	151±67*	145±48*	22±10*	16±3.0*	17.8±10.6	18.2±7*

Results shown are means ± standard deviation
* = p<.05 ** = p<.01

These data indicate that vasopressin receptors are present in greater numbers in the tissues studied of the HO-BB rat. This may be due to "up-regulation" in the absence of endogenous AVP. In addition, AVP receptor affinity may be altered in the Brattleboro rat. These changes in pharmacologic properties of AVP receptors could dramatically influence the physiologic and behavioral sensitivity of these animals to exogenously administered AVP.

124.13 SOMATOSTATIN BINDING SITES ARE DECREASED IN RAT FRONTAL CORTEX AFTER LESION OF THE ASCENDING CHOLINERGIC FIBERS FROM THE NUCLEUS BASALIS MAGNOCELLULARIS. J. Epelbaum, A. Enjalbert, M. Hamon, C. Kordon and Y. Lamour. INSERM U. 159, U. 161 and U. 114, Paris, France.

Evidence has accumulated that Alzheimer's disease, senile dementia of the Alzheimer's type and Parkinson's associated dementia are linked to relatively selective reductions in cholinergic and somatostatinergic parameters in the cerebral cortex and hippocampus. In this work we attempted to define more precisely the possible relationship between ascending cholinergic neurons from the basal forebrain and the intrinsic somatostatinergic neurons in cerebral cortex.

In a first set of experiments we compared the regional distributions of cholineacetyl transferase (ChAT) activity, somatostatin (SRIF) levels and 125I CGP23996 (a non reductible somatostatin analog) binding sites in 10 different cortical regions and hippocampus of the rat brain. There was a significant correlation (r = 0.72) between cortical ChAT activity and SRIF levels. However, no significant correlation was observed between SRIF levels or ChAT activity and 125I CGP23996 binding.

In a second set of experiments, the nucleus basalis magnocellularis (NBM) was lesioned by local ibotenic acid injections (10 ug in 0.8 ul). This resulted in a 40 % decrease in ChAT activity in the frontal and parietal cortices. SRIF levels were unchanged in the lesioned animals while 125I CGP23996 binding was also diminished in the frontal cortex (12.6 + 3.0 fmol/mg protein vs 89.4 + 33.2 fmol/mg protein in the control group means + sem n = 7). Examination of individual animals revealed a positive correlation (r = 0.76) between the decrement of ChAT activity and that of 125I CGP23996 binding in the frontal cortex.

These results suggest that 1) the regional distribution of cholinergic and somatostatinergic neurons (and/or terminals) are closely related in the rat cerebral cortex. 2) SRIF binding sites in the frontal cortex may be located on cholinergic terminals.

124.14 GUANINE NUCLEOTIDE INHIBITION OF BOMBESIN RECEPTOR BINDING. J.B. Fischer and A. Schonbrunn*. Laboratory of Toxicology, Harvard School of Public Health, Boston, MA 02115.

Amphibian bombesin (BBS) and its mammalian analog gastrin-releasing peptide (GRP) are part of a family of "brain-gut" peptides showing structural homology at their carboxy termini. Specific, saturable binding sites for this family of peptides have been defined in neural, exocrine and other tissues. Peripheral injection of BBS or GRP causes increased release of several hormones, including insulin, prolactin and growth hormone. This laboratory has characterized functional BBS receptors which mediate BBS stimulation of hormone secretion in two clonal cell lines, the HIT insulin-secreting pancreatic islet cell line (P.N.A.S. 81:1822, 1984), and the GH_4C_1 pituitary tumor cell strain, which secretes prolactin and growth hormone (ENDO. 110:352, 1982; J. BIOL. CHEM. 258:7527, 1983). To further study the biochemical mechanisms involved in the action of BBS we have studied the binding of [^{125}I-Tyr^{4}]BBS and [^{125}I]GRP_{14-27} in membranes prepared from these cells. The binding of both ligands was measured as described for [^{125}I-Tyr^{1}]somatostatin (ENDO. 114:1784, 1984). Membranes (10-30 μg of protein) were incubated at 37°C to equilibrium (60 min), then collected by centrifugation. BBS displaced both radiolabeled ligands with an IC_{50} of about 0.06 nM. Guanine nucleotides were found to inhibit the equilibrium binding of both ligands to membranes whereas App(NH)p had no effect. In GH_4C_1 membranes maximal inhibition was 70-90% whereas in HIT membranes maximal inhibition was only 30-50%. However the IC_{50} values for various GTP analogs were essentially the same in the two membranes types: $GTP\gamma S$, 0.1 μM; Gpp(NH)p, 0.2 μM; GDPβS, 3 μM; GTP, 0.1 μM; GDP, 2 μM; GMP, >100 μM. To learn more about the guanine nucleotide regulatory site which was coupled to the BBS receptor we prepared membranes from cells treated with maximal doses of cholera toxin or Bordatella pertussis toxin (islet-activating-protein). These toxins are known to ADP-ribosylate nucleotide regulatory proteins associated with adenylate cyclase-coupled receptors, and to affect guanine nucleotide regulation of agonist binding to such receptors. Toxin treatment did not affect the affinity or number of binding sites for [^{125}I]BBS or [^{125}I]GRP, or alter the Gpp(NH)p inhibiton of peptide binding. Furthermore, BBS had no effect on adenylate cyclase activity in these cells. Therefore the nucleotide regulatory site associated with the BBS receptor in both pituitary and pancreatic cells is different from those linked to adenylate cyclase-coupled receptors.

124.15 AGE-RELATED CHANGES OF VASOPRESSIN RECEPTORS IN THE RAT. M.A. Miller* and D.M. Dorsa (SPON: R. Steiner) GRECC, VA Medical Center and Depts. of Medicine and Pharmacology, Univ. of Washington, Seattle, WA 98195.

Recent reports have indicated that cellular responsiveness to exogenous arginine^8-vasopressin (AVP) declines with age in the rat. This decline has been attributed by some to a desensitization phenomenon induced by chronic AVP hypersecretion by the hypothalamo-neurohypophyseal system in aged animals. Others, however, have failed to confirm an elevation of basal AVP levels with aging in the rat and have suggested instead a diminished capacity to secrete AVP. We reasoned that changes of vasopressin responsiveness of tissues may result from age-related changes in vasopressin receptors themselves. We have, therefore, compared AVP receptor affinity and number in membranes prepared from the liver (V_1 type receptors) and kidney (V_2 type receptors) of young (3 mo) and old (24 mo) male rats and assessed plasma AVP levels in the two groups. Young (n=10) and old (n=7) Fischer 344 rats (supplied by the NIA Aging animal program) were killed by decapitation and trunk blood was collected for RIA. Liver and kidney tissue was pooled for each group and crude membrane fractions were prepared, frozen, and stored at -70°C. Saturation analysis was performed using ^{3}H-AVP (S.A. = 40 Ci/mm) over a concentration range of 0.25nM-10nM. Specific binding was defined by addition of a thousand-fold excess of unlabeled AVP. Binding site parameters were obtained by Scatchard-Rosenthal analysis.

Results of the RIA showed that the post-decapitation levels of AVP in old rats ($\bar{x}$ ± SE: 3.9±1.1 pg/ml, n=7) did not differ significantly from young animals (3.0±0.8 pg/ml, n=9). In addition, we found no significant difference in the K_d values measured for AVP receptors in either the liver (young: 0.74±0.08nM, n=3; old: 0.75±0.05 nM, n=4) or kidney (young: 1.92±0.19 nM, n=5; old: 1.99±0.23 nM, n=5) with aging. However, the number of AVP receptors in the liver of old rats (151±9.5 fm/mg protein, n=4) was significantly reduced ($p<0.01$) below that observed in young rats (271±22 fm/mg protein, n=3). While there was a tendency for AVP receptor numbers to be reduced in kidney tissue of old rats as well (old: 96±17.4 fm/mg protein, n=5; young: 138±36 fm/mg protein, n=4), this reduction was not significant. In summary, we found reduced numbers of vasopressin receptors in peripheral tissues of aged rats even though circulating vasopressin levels were similar to young animals. This suggests that diminished binding of vasopressin in aging may explain reduced vasopressin responsiveness. In additon, the lower receptor number may be due to factors other than receptor "down-regulation" in aged animals.

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124.16 THYROTROPIN RELEASING HORMONE BINDING SITES IN ADULT HUMAN SPINAL CORD. M. Gudesblatt and K.H. Sonnenfeld (SPON: Gerald Cohen). Department of Neurology, Mt. Sinai Sch. of Med., New York, NY 10029.

Thyrotropin releasing hormone (TRH) has been found to be widely distributed, but discretely localized throughout the central nervous system (CNS). TRH has an excitatory action on spinal motor neurons and has multiple and sometimes conflicting electrophysiological actions on cortical neurons. Specific high affinity (K_d=2 nM) and low capacity binding sites have been demonstrated in the adult human brain (Parker, C.R., and A. Capdevila, Peptides 5:701-706, 1984). In addition, high affinity TRH binding sites have been localized by radioautography to Laminae II and IX of adult human spinal cord (Manaker, S., et al., Neurology 35:328-332, 1985). This study was undertaken to investigate the characteristics of TRH receptors in adult human spinal cord.

Post-mortem segments of adult human spinal cord were rapidly removed, frozen, and stored at -70°C. Tissue in ice cold sodium phosphate buffer (20 mM, pH 7.4) containing Bacitracin (50 µg/ml) was homogenized using a Brinkman Polytron PT 10 (one, 15 sec pulse). Following centrifugation (30,000g, 15 min) and washing, twice, the pellet was resuspended in fresh buffer (100 mg wet weight/400 ml buffer) using a Dounce tissue homogenizer. Binding was measured by incubating the homogenate at 0°C for 4 hours with ^{3}H-(3-Me-His^2)TRH. The ^{3}H-(3-Me-His^2)TRH bound to tissue was separated from free ligand by rapid filtration through Whatman GF/B filters followed by three washes with 3 ml of cold buffer. Nonspecific binding was determined by measuring in parallel incubations the amount of ^{3}H-(3-Me-His^2)TRH bound in the presence of 1 µM unlabeled ligand. Samples were counted on a Beckman liquid scintillation counter.

Measureable specific binding was present in adult human lumbar and sacral spinal cord. Binding obtained equilibrium within 4 hours. Specific binding to increasing amounts of tissue was linear over the range of tissue concentrations used in these experiments. ^{3}H-(3-Me-His^2)TRH binding was displaced by increasing concentrations of TRH. Unlabeled (3-Me-His^2)TRH was 10 times more effective in displacing tritiated ligand than was TRH. No significant displacement occured using the constituent amino acids of TRH, or agonist and antagonists for other receptors. Scatchard plot analysis of ^{3}H-(3-Me-His^2)TRH binding to adult human spinal cord indicates that the affinity constant (K_d) is approximately 4.5±2.0. These results are consistant with highly specific binding sites in adult human spinal cord with similar characteristics to the adult human brain TRH binding sites, previously reported. The specfic role that this receptor plays in spinal cord function requires further investigation.

124.17 DISTRIBUTION OF ^{3}H-SOMATOSTATIN BINDING SITES IN RAT AND HUMAN BRAIN.
CA Whitford[1*], DR Weightman[2*], CR Snell[3*], JM Candy[1*], CA Bloxham[4*], JA Edwardson[1*] (SPON: R.M. Gould) [1]MRC Neuroendocrinology Unit, and [4]Department of Pathology, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE; [2]Department of Medicine, University of Newcastle upon Tyne; [3]Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN.

Somatostatin binding sites in the brain have previously only been demonstrated using iodinated analogues of somatostatin. We report here the use of a tritiated ligand which is chemically and biologically identical to the natural ligand ([4-^{3}H (Phe^6)]-somatostatin-14) to demonstrate the distribution of high affinity binding sites which have pharmacological properties consistent with somatostatin receptors, in the rat and human brain.

In the rat brain, the density of ^{3}H-somatostatin binding sites on membrane fragments at 0.7nM ligand concentration was highest in the cerebral cortex and hippocampus (75-76 fmol/mg protein) and lowest in the medulla/pons and cerebellum (5 fmol/mg protein). Intermediate levels of binding were found in the striatum, midbrain and hypothalamus (14-23 fmol/mg protein). Preliminary studies using tritium film autoradiography show that areas with the highest density of binding sites include the deep layers of cerebral cortex, lateral septal nucleus, hippocampus, amygdala, claustrum, and habenula.

A more detailed study of the regional distribution of ^{3}H-somatostatin binding sites on membrane fragments in the human brain at 0.7nM ligand concentration showed that the cerebral cortex had a very high density of binding sites with the highest levels in the frontal and temporal cortex (62-70 fmol/mg protein), lower levels in the parietal and cingulate cortex (51-53 fmol/mg protein) and lowest in the occipital cortex (33 fmol/mg protein). The density of binding sites in the nucleus accumbens, caudate nucleus, hippocampus, hippocampal gyrus and claustrum was from 40-50 fmol/mg protein, while in the septum, putamen and amygdala, it was between 30-33 fmol/mg protein. Regions with a low level of binding included the globus pallidus (2 fmol/mg protein), hypothalamus (14 fmol/mg protein), substantia nigra (7 fmol/mg protein) and thalamus (4 fmol/mg protein). In contrast to the rat, the highest density of binding was in the cerebellar cortex (74 fmol/mg protein). Tritium film autoradiography revealed that the highest density of binding sites in the cerebellar cortex was present in the granule cell layer.

We conclude that the distribution of high affinity somatostatin binding sites is similar in rat and human brain, with the exception of the cerebellum.

124.18 QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF SOMATOSTATIN BINDING SITES IN DISCRETE AREAS OF RAT BRAIN. R. McCarty* L. Plunkett* K. Shigematsu*and J. M. Saavedra (SPON: I. Hanbauer). Section on Biochemical Pharmacology, NHLBI-NIH and Laboratory of Clinical Science, NIMH, Bethesda, MD. 20205

There is now excellent evidence from pharmacological and electrophysiological studies that somatostatin (SS) serves as a neurotransmitter or modulator of neural activity in many areas of the central nervous system. In the present study, we have studied the distribution of SS binding sites in discrete areas of rat brain by incubation of tissue sections with ^{125}I-SS, autoradiography, computerized densitometry and comparison with ^{125}I-standards. With this approach, we sought to examine the regulation of SS binding sites in various brain regions.

Adult male Sprague-Dawley rats were sacrificed and the brains were rapidly removed and frozen at -30°C. Sixteen micron coronal sections were cut in a cryostat at -14°C, thaw mounted onto subbed slides and kept overnight at 4°C. Sections were then incubated in 150 mM Tris (pH 7.4) which contained 5 mM $MgCl_2$, 0.1 mM bacitracin and 0.1% BSA and then incubated for 60 minutes in fresh buffer containing ^{125}I-SS, diluted 1:10 with unlabelled SS to yield concentrations of 0.13-2.0 nM. Non-displacable binding was studied in adjacent sections incubated in parallel with 10^{-5}M unlabelled SS. Sections were washed 3 times for 5 minutes each in Tris buffer at 4°C, dried under a stream of cool air and apposed to tritium-sensitive Ultrofilm. Displacable binding ranged from 50-85%.

Highest concentrations of SS binding sites in rat brain (fmol/mg protein ± SEM) were found in the claustrum (151 ± 29), central nucleus of the amygdala (90 ± 11), deep layers of the cerebral cortex (61 ± 9), lateral olfactory nucleus (58 ± 13), CA1 and CA2 regions of hippocampus (57 ± 11), medial and lateral septal nuclei (54 ± 9) and medial habenula (44 ± 5). Significant concentrations of SS binding sites were not found in the striatum or midbrain and brainstem areas. Scatchard analysis of 4 brain areas with high dendities of SS binding sites revealed an inverse relationship between maximum binding capacity (B_{max}) and binding affinity (K_a).

Our findings indicate that regulation of brain SS binding sites may be studied as one approach to examining the involvement of central SS pathways in various physiological and behavioral states.

124.19 WHOLEMOUNT LOCALIZATION OF FMRFAMIDE-IMMUNOREACTIVE NEURONS AND CHARACTERIZATION OF RECEPTORS TO FMRFAMIDE IN THE APLYSIA CNS. R. E. McCaman and J. K. Ono, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA, 91010.

Previous studies have revealed a wealth of synaptic connections within the various ganglia of the Aplysia nervous system in which the chemical mediators are not conventional monoaminergic transmitters nor amino acids. Thus, we have begun to explore the possibility that some peptides may be involved in mediating conventional synaptic transmission, i.e., short latency, and relatively rapid conductance changes. The recent isolation of authentic FMRFamide (FMRFa) from the Aplysia CNS and the characterization of the nucleotide sequence encoding the amino acid sequence clearly establish the presence of this tetrapeptide in Aplysia. Furthermore, we have demonstrated selective and reproducible immunoreactive staining of an abundance of neurons in wholemounts of all the Aplysia ganglia using an antiserum obtained from Price & Greenburg. Previous reports indicate that FMRFa can induce excitatory and inhibitory responses in some Aplysia neurons (Stone et al., Neurosci. Absts. 7:636, 1982; Rubin et al., Neurosci. Abst. 10:1116, 1984). We have further characterized the various responses to FMRFa on different Aplysia neurons and find that these responses are due to two basic types of receptors, found either alone or in combination in various neurons. The depolarizing response appears to be due to an GNa^+ which reverses at approximately +10 mv and the hyperpolarizing response, as previously characterized by Rubin et al., is due to an GK^+. Both of these responses appear to have about the same threshold (10^{-7}M) to perfusion of FMRFa and their latencies, rise times and durations are demonstrably similar to those mediated by ACh, 5HT, and/or Hm applied under similar conditions and involving similar conductance mechanisms. We have tested various FMRFa analogues and find that some evoke a response to both the GNa^+ and the GK^+ (YFMRFa), others activate either the GNa^+ (d-amino acid substituted analogues, FMRdFa and FMdRFa) or the GK^+ (YGGFMRFa). Analogues lacking the C-terminal amide (FMRF) or other substitutions (LPLRFa) appear inactive.

The known presence of FMRFa in the Aplysia CNS along with our observations on the specificity of the receptors, the nature of the conductance changes mediated by FMRFa and the selective cellular localization of this peptide suggest that it may be a likely candidate for mediating chemical transmission within the ganglia. Discovery of monosynaptic connections between specific FMRFa-containing neurons and re-identifiable follower cells should provide an excellent opportunity for testing this possibility. (Supported by N.I.H. grant NS 18862)

124.20 **INTERACTIONS BETWEEN TYR-MIF-1 AND THE MU OPIATE RECEPTOR-SELECTIVE PEPTIDE MORPHICEPTIN (B-CASOMORPHIN 1-4 AMIDE) AT THEIR RESPECTIVE BINDING SITES.** J.E. Zadina and A.J. Kastin. Veterans Admin. Med. Cntr. and Tulane Univ. Sch. of Med. New Orleans, LA 70146.

The presence of a peptide very similar to TYR-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) in rat brain has been supported by RIA (Kastin et al, Pharmac. Biochem. Behav. 13:901,1980; Br. Res. Bull., 7:697, 1981), HPLC (Kastin et al., unpub. observ.), and by the presence of specific, high affinity binding sites (Zadina et al., Pharmac. Biochem. Behav. 17:1193, 1982). Prominent among the actions demonstrated for Tyr-MIF-1 is antagonism of morphine-induced analgesia in the tail-flick test (Kastin et al., Pharmac. Biochem. Behav. 21:937, 1984).

Morphiceptin (Tyr-Pro-Phe-Pro-NH_2), is an amidated tetrapeptide fragment of the bovine milk protein, beta-casein. Its potent opiate agonist activity is indicated by the analgesia elicited after icv injection (Brantl et al., Life Sci. 28:1903,1981; Chang et al., Life Sci. 30,1547,1982) and it is selective for mu opiate receptors (Chang et al., PNAS 78:75, 1981).

Using in vitro assays, we have found that specific binding of ^{125}I-Tyr-MIF-1 to rat brain membranes is displaced with high potency by Morphiceptin and some of its analogs. In addition, Tyr-MIF-1 can displace ^{125}I-Morphiceptin from its binding sites.

Rat brain membranes were prepared as described (Zadina et al,1982), extensively washed, and preincubated at 37°C. The membranes were incubated for 18 (Tyr-MIF-1 assay) or 24 hr (Morphiceptin assay) at 4°C with 1nM iodinated peptide, an enzyme inhibitor (bestatin), and varying concentrations (1-1024 nM) of unlabeled peptide or analog. Nonpecific binding was determined as binding in the presence of a 10uM concentration of the corresponding unlabeled peptide. The biphasic displacement curves were analyzed by nonlinear curve-fitting programs (Allfit and Ligand). In the ^{125}I-Tyr-MIF-1 assay, morphiceptin as well as its active analog, NmePhe^3-D-Pro^4 morphiceptin (PL017), and the parent heptapeptide, beta casomorphin, all showed potent displacement activity. D-Pro^2 substitution however, which eliminates opiate activity, also eliminated its binding activity, and indicated a stereospecific reaction. Enkephalin analogs, dynorphin, kyotorphin, dermorphin, EKC, cyclazocine, DAGO and DHM were all inactive. Morphiceptin, PL017, and Tyr-MIF-1 all effectively displaced ^{125}I-Morphiceptin, but the D-Pro^2 morphiceptin analog was inactive. These results indicate a possible mechanism for the interaction of Tyr-MIF-1 and opiate substances.

REGENERATION: NEURONAL AND GLIAL RESPONSES

125.1 ACTINOMYCIN D DISINHIBITS POSTTRANSLATIONAL PROTEIN MODIFICATION BY AMINO ACID ADDITION IN REGENERATING RAT SCIATIC NERVES IN VITRO. R.V. Riccio, G. Chakraborty*, and N.A. Ingoglia. Department of Physiology, New Jersey Medical School, Newark, NJ 07103.

Posttranslational modification of proteins (PTPM) by amino acid addition has been shown previously in preparations of a 150k xg supernatant of rat sciatic nerves. The demonstration of this reaction is dependent on removing low molecular weight "regulatory" molecules by fractionating the supernatant by Sephacryl S-200 chromatography (excluding molecules < 125 kD), or by removing molecules <30 kD by ultrafiltration prior to the in vitro assay. We now report that addition of 2-20 μM Actinomycin D (Act D) to the 150 k xg supernatant of sciatic nerves 2 hrs post-crush allows for the expression of PTPM activity to levels similar to those obtained by either of the above molecular weight exclusion methods.

Rat sciatic nerves were crushed and 2 hrs later a 150k xg soluble supernatant was prepared from a homogenate of segments taken 1 cm proximal to the crush site (P1). The supernatant was divided and either fractionated by S-200 chromatography, filtered through a Centricon C-30 filter, or incubated with 2-20 μM Act D (20', 37°C). The void volume of the S-200 column, the C-30 retentate, and the samples containing Act D were then incubated with ^{3}H-lysine and a reaction mixture at 37°C for 30' and analyzed for incorporation of radiolabel into protein. Untreated supernatant, and supernatant incubated with Act D at 0°C or 4% mannitol (vehicle) at 37°C served as controls. Act D significantly increased ^{3}H-lysine incorporation above all controls and approached that seen after S-200 or C-30 treatment. Addition of Act D to the S-200 or C-30 fractions further increased ^{3}H-lysine incorporation above the untreated fractions.

These data indicate that the expression of the PTPM reaction can be released by the addition of Act D to the 150k xg supernatant in addition to removing putative inhibitory agents by molecular weight exclusion. (Supported by NINCDS grant NS19148 from NIH).

125.2 INCREASES IN POSTRANSLATIONAL PROTEIN MODIFICATION AND PROTEIN SYNTHESIS IN RAT SCIATIC BUT NOT OPTIC NERVES FOLLOWING INJURY. S.S. Athwal, R.V. Riccio and N.A. Ingoglia, Dept. of Physiology, New Jersey Medical School, Newark, NJ 07103

Posttranslational protein modification by amino acid addition (PTPM) occurs in both rat optic and sciatic nerves, but 18 hrs. after a crush injury there is a 2-fold increase in activity in the sciatic nerve and a 25% decrease in optic nerve activity (Athwal, S.S. et al. Soc. Neurosci. Abstract, V.10; 1031, 1984). The present experiments investigate these reactions at earlier time points after a nerve crush, and compare them with glial and Schwann cell protein synthetic activity over the same time period.

The right optic or sciatic nerve was crushed, and 10 minutes to 3 days later nerve segments just proximal to the crush were removed and assayed for PTPM or protein synthesis. In protein synthesis experiments, rats were injected intravenously with a mixture of ^{3}H-amino acids one hour prior to sacrifice. PTPM activity was determined by incubation of a fraction of the 150K xg supernatant with ^{3}H-Lys as described elsewhere (Zanakis, M.F. et al., J. Neurochem., 43:1286, 1984). Incorporation of ^{3}H-amino acids into proteins for both PTPM and protein synthesis was determined by extraction in hot and cold TCA. In each experiment, contralateral sham operated nerves were used as internal controls. PTPM in sciatic nerves increased 1.5 times control levels 10 minutes after crush and continued to increase to a maximum of 9.5 times control by 2 hrs. post-crush. In optic nerves, activity was decreased by approximately 50% of controls throughout the time course studied. Protein synthesis was also depressed by approximately 50% in crushed optic nerves, but in sciatic nerves an initial depression (up to 1 hr. post crush) was followed by a doubling of protein synthetic activity at 2 hrs. In sciatic nerves, protein synthesis remained elevated up to 3 days later.

These results indicate that in a nerve which is capable of regeneration (sciatic), PTPM increases immediately (10 minutes post crush) following injury, but a nerve incapable of regeneration (optic) is incapable of activating the modification reaction. The finding that protein synthesis is not activated in injured optic nerves suggests that nerve crush stimulates gene activation in Schwann cells of the sciatic nerve but not in glia of the optic nerve. These findings may be significant for understanding the reasons for the lack of a regenerative response in central mammalian nerves. (Supported by NINCDS grant NS19148 from NIH).

125.3 IMMUNOHISTOCHEMICAL LOCALIZATION OF A NEURONAL GROWTH-ASSOCIATED PROTEIN (GAP 43) IN NEONATAL RAT BRAIN. C. B. McGuire*, J. J. Norden, S. S. Bock, and J. A. Freeman. Dept. of Anatomy, Vanderbilt University Medical School, Nashville, TN 37232.

It is likely that axon growth, terminal formation, and synaptogenesis involve the synthesis and transport of specific growth-associated proteins (GAPs) to the developing axonal endings. A search for candidate proteins involved in rat neurogenesis has yielded a 43 kDa membrane protein (GAP 43) which is synthesized and axonally transported by rat retinal ganglion cells during periods of axon growth and synaptogenesis in the developing visual system. In order to further study the role of GAP 43 in neuronal development, we have produced a specific rabbit antiserum against the 43 kDa protein purified by preparative two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). We have used this antiserum to immunohistochemically localize GAP 43 in frozen sections of neonatal rat brains.

Brains from rats 0-30 days old were sectioned and processed for immunohistochemistry, using a standard peroxidase anti-peroxidase procedure. In order to control for the specificity of anti-GAP 43 immunostaining, we partially purified GAP 43 from a membrane fraction (1000,000 x g) of 7 day old rat brains by DEAE ion-exchange chromatography followed by one-dimensional preparative PAGE and used it to pre-absorb GAP 43 immunoreactivity on adjacent brain sections. We found that GAP 43 is localized to very fine, irregular profiles in the retinal recipient layers of the developing superior colliculus throughout the time-course of retinal ingrowth and synaptogenesis. This result indicates that GAP 43 is localized to axon terminals during e periods of axon growth and maturation. Several other brain areas showed similar immunoreactivity, including the molecular layer of the dentate gyrus, where staining is most pronounced during the period of rapid synaptogenesis, postnatal days 7-21. We propose that GAP 43 is involved in the formation and maintenance of presynaptic membrane specializations. Two biochemical studies are consistent with this hypothesis. First, a role for GAP 43 in neurogenesis is supported by our finding that GAP 43 is enriched in growth cones isolated from fetal rat brains. Second, we have observed small but significant quantities of GAP 43 in adult rat brains indicating a role for GAP 43 in normal nerve function. We are presently trying to elucidate, by immunoelectron microscopy, the subcellular localization of GAP 43 and the precise developmental stages at which GAP 43 is being incorporated into individual axon endings in an effort to discover the function of this neuronal growth-associated protein. (Supported by NIH Grant NS18103 and NEI Grant EY01117 to J.A.F.).

125.4 LOCALIZATION OF A SPECIFIC 37kDa PROTEIN-LIKE IMMUNOREACTIVITY IN THE MAMMALIAN PNS AND CNS DURING WALLERIAN DEGENERATION. G.Stoll* and H.W.Müller (SPON:K.H.Mauritz). Dept. of Neurology, Univ. of Düsseldorf, F.R.Germany.

As a specific response to denervation the synthesis of a 37kDa protein was significantly stimulated in the nerve sheaths of mature PNS and CNS of rat. The synthesis of the 37kDa protein was inhibited in the peripheral nerves when proper reinnervation was established whereas 37kDa synthesis continued at maximal rate in non-regenerating CNS for at least 3 months. However, the cellular localization and function of the 37kDa protein in Wallerian degeneration in unknown.

Specific antibodies raised in rabbits to purified 37kDa protein isolated from regenerating sciatic nerve were used to identify the cell types expressing this protein in PNS and CNS. Paraffin and plastic thin sections were prepared from sciatic nerve and optic nerve and stained by avidine-biotinylated peroxidase-complex technique. In sham-operated control sciatic nerve the antigen was recognized at the outer surface of the myelin sheaths and the Schwann cell bodies suggesting a distribution similar to basal lamina. Two weeks after crushing the sciatic nerve the amount of antigen was significantly increased in the distal nerve segment. At this time the antigen was localized predominantly within a cell type that morphologically resembled macrophage. A small fraction of the antigen remained associated with the myelin and Schwann cell surface similar to control nerves.

In sham-operated control optic nerve the antibody to peripheral 37kDa protein recognized only granular structures distributed within the cell body and processes of astrocytes. In contrast to sciatic nerve the total amount of detectable 37kDa-like immunoreactivity did not increase in the optic nerve within 2 weeks after a crush lesion. However, the immunoreactivity disappeared from most of the astrocyte cell bodies and reappeared in vesicular or granular structures distributed evenly throughout the cross-section of the nerve. Recognition of epitope(s) in CNS by an antibody raised to 37kDa protein from peripheral nerve suggests homology of the central and peripheral 37kDa proteins. Since myelin resorption is one of the important functions of macrophages in Wallerian degeneration the role of the 37kDa protein in PNS and CNS will be discussed in this regard.

125.5 POST-TRANSLATIONAL MODIFICATION OF THE 48 kD GROWTH-ASSOCIATED PROTEIN IN THE REGENERATING GOLDFISH RETINA. N.I. Perrone-Bizzozero and L.I. Benowitz. Dept. of Psychiatry and Program in Neuroscience, Harvard Med. Sch.; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178.

Regeneration of the optic nerve in goldfish involves a shift in the retinal ganglion cells' program of protein synthesis and axonal transport (Grafstein & Murray, Exp. Neurol. 25:494, 1969; Benowitz et al, J. Neurosci. 1:300, 1981; Heacock & Agranoff, Neurochem. Res. 7:771, 1982). Among the membrane-bound proteins that are conveyed to the developing nerve terminals by rapid axonal transport, presumably to participate in phenomena such as axon elongation, growth cone motility, and target recognition, the most striking change associated with regeneration is a 50-100 fold increase in a group of proteins, the principal component of which has a molecular weight of 48,000 daltons (48 kD) and a pI of 4.7 (Benowitz & Lewis, J. Neurosci. 3:2153, 1983). Proteins presumed to be similar to this are found in a number of other developing and regenerating systems, both in lower vertebrates and in mammals, and it has been proposed that the expression of these and several other growth-associated proteins (GAPs) may be a basic feature of axonal outgrowth (Skene, Cell 37:697, 1985). In the present studies we examined certain aspects of the biosynthesis of the 48 kD protein in order to understand better the cellular level at which changes in its expression come about during growth, and to gain further insight into its biochemical properties. Intact or regenerating goldfish retinas were pulse-labeled in vitro with ^{35}S-methionine or ^{3}H-proline for 20-30 minutes, and then either frozen or incubated for another 30-60 minutes with an excess of non-radioactive amino acids. Results of these pulse-chase experiments show that the 48 kD protein is post-translationally modified over a period of about 30 minutes, and is synthesized only when the retina is undergoing axonal regeneration. Tunicamycin, an inhibitor of N-glycosylation through the dolichol phosphate pathway, prevents the 48 kD and several other proteins from appearing when regenerating retinas are pre-incubated with the inhibitor for 1 hour. Tunicamycin prevents outgrowth of axons from the goldfish retina in culture (Heacock, Brain Res. 241:307, 1982). The observation that the 48 kD protein is one of only 2-3 regeneration-specific proteins whose synthesis is blocked by this inhibitor therefore supports the idea that this protein may be essential for axonal outgrowth. Research in progress is attempting to identify the unglycosylated precursor of the 48 kD protein to ascertain whether it is a growth-specific m-RNA translation product. Supported by NIH EY05690 and American Paralysis Association Contract 84-04.

125.6 QUANTIFICATION OF THE SYNAPTIC VESICLE PROTEINS, SYNAPSIN I AND p38, AND THE ASTROCYTE-SPECIFIC PROTEIN, GLIAL FIBRILLARY ACIDIC PROTEIN, FOLLOWING TRIMETHYLTIN-INDUCED INJURY TO THE RAT CENTRAL NERVOUS SYSTEM. M.E. Viana*, T.O. Brock and J.P. O'Callaghan. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 and Northrop Services, Inc., Research Triangle Park, NC 27709.

Lesion- or chemical-induced injury to the central nervous system (CNS) is associated with complex biochemical and morphological responses. In order to characterize and quantify these responses, we are examining changes in nervous-system proteins (NSP) and brain morphology produced by exposure to known neurotoxicants (O'Callaghan and Miller, TIPS 4: 388, 1983). In the present investigation, we characterized the effects of trimethyltin (TMT), a neurotoxic organometal that produces cytopathological changes in rat CNS that are largely restricted to the limbic system. NSP measurements consisted of radioimmunoassays of synapsin I and p38 (formerly p36; see Jahn, et al. PNAS 81: 1684, 1984), two synaptic vesicle-associated proteins, and glial fibrillary acidic protein (GFAP), an astrocyte-specific protein. Computer-assisted densitometry also was employed to quantify NSP resolved by two-dimensional gel electrophoresis (2D-PAGE). Morphological indices consisted of 1) morphometry of hippocampal pyramidal cells and 2) immunocytochemistry of GFAP.

Acute administration of TMT (0.0-9.0 mg/kg, i.v.) to adult LE rats caused both dose- and time-dependent decreases in synapsin I and p38 in the hippocampus. Large reductions in synapsin I (73%) and p38 (60%) were observed following 9.0 mg/kg. Both of these proteins were decreased maximally at 5 weeks after TMT administration but approached control values 12 weeks after administration. Resolution of hippocampal proteins by 2D-PAGE revealed a TMT-induced loss of a protein tentatively identified as neurofilament 68K; this same protein also was reduced in samples of frontal cortex from TMT-treated rats whereas synapsin I and p38 values were not affected. TMT (0.0-9.0 mg/kg) caused large dose- and time-dependent increases in GFAP in both hippocampus and frontal cortex. Maximal effects were observed 5 weeks after TMT administration and were of similar magnitude (500-600% of control) in both regions; by 12 weeks GFAP values had decreased to near control values. Microdissection of slices of dorsal hippocampus demonstrated regional differences in the extent to which TMT affected GFAP with area CA3 showing the greatest increase (6000% of control).

Morphological examination of the hippocampal formation revealed a loss of pyramidal cells and predominant GFAP immunoreactivity in astrocytes throughout Ammon's horn and fascia dentata. Collectively, these data suggest that NSP may be used to characterize cell-type-specific responses to CNS injury.

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125.7 METABOLIC ACTIVITY IN GOLDFISH OPTIC TECTUM: EFFECTS OF OPTIC NERVE CRUSH AND REGENERATION. M. Murray and N. Berman. Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

The staining method for cytochrome oxidase is sensitive to long term changes in neuronal activity resulting from denervation. The goldfish optic system provides a favorable system to examine the effects of denervation and reinnervation on metabolic activity of tectal neurons and the surrounding neuropil using this method. Synaptic number in the stratum fibrosum et griseum superficialis (SFGS), the main recipient lamina for optic axons, fails by 40% after optic nerve crush. Regenerating optic axons enter the SFGS by 3 weeks postoperatively but the normal number of contacts does not return until after 3 months postoperatively.

We crushed the right optic nerve in large goldfish and then sacrificed them at intervals from 2 days to 2 years postoperatively. Fish were perfused with paraformaldehyde: glutaraldehyde, the brains were cut and the sections were incubated with cytochrome and diaminobenzidine, mounted and coverslipped. Sections were examined in the light microscope and the density of the brown reaction product was quantitated using a Quantimat 920 image analyzer. Differences in the intensity of staining between the control (right) and experimental (left) SFGS were determined. The density of staining in the stratum marginale, the most superficial lamina which receives no optic input, was equivalent between left and right sides in all animals. Little difference in staining of the SFGS between two sides was apparent at 2 days postoperatively. By 4 days, the density of staining of SFGS was substantially reduced on the experimental side. Recovery of control levels of staining occurred by 3 months postoperatively. We conclude that the metabolic activity of the SFGS parallels the synaptic density and may act as a sensitive indicator of reinnervation.

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125.8 **EARLY APPEARANCE OF A25 (A MODIFIED RAPIDLY TRANSPORTED POLYPEPTIDE) IN FROG SCIATIC NERVE FOLLOWING DAMAGE, AND THE EFFECTS OF A CONDITIONING LESION.** G.W. Perry, Bruce Tedeschi* and David L. Wilson. Department of Physiology and Biophysics, Univ. of Miami School of Medicine, MIAMI, FL 33101.

Previously, a protein designated A25 was seen only at regenerating nerve tips, and only several days after nerve damage. A25 (apparent size of 70,000 daltons) appears to be the product of post-translational modification of a rapidly transported protein, and is retrogradely transported along intact axons (Tedeschi and Wilson, 1983 J. Neuroscience 3,1728). Using a new solubilization procedure we now detect A25 much earlier after nerve damage, but still only at or distal to a site of damage. We have also detected another protein, designated A30, with a greater molecular weight (about 100,000) in normal sciatic nerve that was previously obscurred by background labelling of fluorographs in the less acidic regions of our 2D-gels. Intriguingly, A30 disappears, or is absent, in damaged nerve segments whenever A25 appears. Both polypeptides exhibit microheterogeneity, and we are exploring the possibility that A30 is the precursor of A25. As the nerve elongates distally from the site of damage, A25 is detectable, but not A30, along the entire length of the regenerated portion of nerve.

The appearance of A25 was enhanced in damaged nerves that had received a prior "conditioning lesion". However, when rapidly transported proteins were collected prior to arrival at regenerating nerve regions, a prior conditioning lesion appeared to have relatively little effect on the majority of transported proteins, including A30, beyond those changes seen previously following a single lesion (Perry and Wilson, 1981 J.Neurochemistry 35,1203). In addition, with our new solubilization procedure, we have detected changes following nerve damage in a few other rapidly transported proteins, including A30.

We conclude that A25 appears at damaged nerve sites much earlier than was previously seen, and that another rapidly transported protein, A30, may be its progenitor. It is also tempting to speculate that among the roles possible for A25 may be a role in signalling the cell body about axon damage.

125.9 CHANGES IN THE LEVELS OF IN VITRO SYNTHESIZED INTERMEDIATE FILAMENT PROTEINS ACCOMPANYING GOLDFISH OPTIC NERVE REGENERATION. P. Tesser* and N. Schechter. Departments of Psychiatry and Behavioral Science, and Biochemistry SUNY at Stony Brook, New York 11794

Changes in protein and mRNA synthesis accompanying goldfish optic nerve regeneration have been investigated by in vitro translation of RNA derived from retinas at various time points after nerve crush. The resulting translation products were examined by 2-dimensional gel electrophoresis and immunoprecipitation.

We have concluded that the level of total protein synthesis remains virtually constant during regeneration as judged by the level of in vitro protein synthesis which is directed by equivalent amounts of total RNA at each time point. Furthermore, the amount of total RNA which can be isolated from retina also remains relatively constant during regeneration. However, the synthesis levels of certain structural proteins show large increases.

Previously, the proteins designated as ON_{1-4} had been identified as intermediate filament proteins of the goldfish visual pathway (Quitschke and Schechter, J. Neurochem., 42:569, 1984). ON_1 and ON_2 are of neuronal origin and their levels of synthesis are linked to nerve regeneration. Conversely, ON_3 and ON_4 are non-neuronal and change very little in abundance during regeneration. We have used polyclonal antibodies specific for either ON_1 and ON_2 or ON_3 and ON_4 and a general monoclonal anti-intermediate filament antibody for further characterization. Antibodies raised against the optic nerve ON_1 and ON_2 proteins react specifically with the in vitro synthesized ON_1 and ON_2 proteins thus confirming their retinal origin. Increased synthesis of ON_1 and ON_2 is observed ten days after crush and reaches a maximum at about twenty days; by 85 days they are translated at levels equal to those before crush. Antibodies to ON_3 and ON_4 do not react with any proteins translated from retinal RNA. The monoclonal antibody reacts with both ON_1 and ON_2 as well as with several putative intermediate filament proteins. We also observe on 2-D gels a previously unidentified protein which is not detectable before crush and is synthesized at high levels twenty days after crush.

Because ON_1 and ON_2 are synthesized as two distinct proteins in vitro and because no post-translational modifications occur during the in vitro reaction, it appears that ON_1 and ON_2 differ slightly in amino acid composition and are encoded by two distinct mRNAs.

It is possible to conclude that regeneration of the goldfish optic nerve involves very specific changes in gene expression rather than a general increase in mRNA and protein synthesis. (This research was supported by grant EY 05212 from the NIH.)

125.10 CONSEQUENCES OF PARTIAL AXOTOMY FOR PRODUCTION OF NEUROTRANSMITTER VESICLES AND ROUTING OF MATERIAL MOVING BY FAST TRANSPORT IN THE AXONAL TREE. D.J. Goldberg and R.T. Ambron. Depts. of Pharmacol. and Anat. and Cell Biol., Columbia U. CPS, NY, NY 10032.

It is known that axotomy reduces the export from the cell body of materials associated with synaptic transmission. We previously showed, using a giant invertebrate neuron with a simple axonal tree, that the reduction in export of transmitter storage vesicles seems to be an expression of a feedback regulation that closely couples vesicle export to the extent of the synaptic field (Science, 213: 913, 1982; J. Neurosci., 4:1800, 1984). We have now sought to determine whether export is regulated by decreasing the rate of synthesis of transmitter vesicles or by slowing their exit from the cell body after synthesis. Two lines of evidence indicate that synthesis decreases. First, we counted serotonergic vesicles in electron micrographs of several areas of the cell body of the Aplysia neuron GCN 3 days after proximal transection of one branch of the bifurcate axon. Export of vesicles is stably decreased by half at this time, and were this due to an increase in the time required for formed vesicles to exit the cell body, we would expect to see them accumulate in the cell body. We observed no such increase in their numbers. Second, we measured the extent of incorporation of ^{3}H-fucose into a 75kD putative vesicle glycoprotein. Reduced incorporation was evident 1 day after transection of one branch, and was dramatic when both branches were transected. Fucosylation of other glycoproteins was relatively unaffected.

We also found previously that distal axotomy of one branch of the axon causes, within hours, transmitter vesicles to be preferentially routed into the intact branch, despite the fact that the transected branch remains capable of carrying its full normal complement of vesicles. We wondered whether this routing was selective for vesicles, especially since considerable sprouting occurs at the proximal stump of the transected branch. We thus analyzed, using SDS PAGE, the partitioning between the two axonal branches of 6 fucosyl glycoproteins 2 weeks after distal transection of one branch. Included among these glycoproteins was at least one whose transport moderately increases after transection, as well as the 75kD putative vesicle protein. We found that there was considerable variability in the partitioning ratios of the glycoproteins within an individual axon during our relatively brief assay period. No difference was consistent from one axon to the next, however. We conclude that there is stochastic variation in the composition of material received by axon endings but, at least insofar as the glycoproteins we have studied in detail are concerned, there is no selectivity in the partitioning of material.

125.11 REGENERATION ASSOCIATED CHANGES IN AXONAL MICROTUBULE PROTEIN TRANSPORT IN DORSAL ROOT GANGLION CELLS OF RAT. M.M. Oblinger. Dept. of Biological Chemistry & Structure, The Chicago Medical School, North Chicago, IL 60064.

Axotomy of peripheral sensory axons induces a series of metabolic changes in dorsal root ganglion (DRG) neurons that involve the axonal cytoskeleton. For example, a decrease in the synthesis and subsequent axonal transport of neurofilament proteins in slow component a (SCa) occurs during regeneration of peripheral DRG axons (Oblinger & Lasek, 1983, Soc. Neurosci. Abstr., 9:148; 1985, in press). The faster moving slow component of transport, SCb, had not been previously studied during regeneration of DRG axons. SCb moves at a rate similar to observed axonal outgrowth rates in the DRG cell and conveys a significant portion of the axonal cytoskeleton, including some of the tubulin and most of the actin. Recent evidence indicates that the tubulin conveyed in SCb differs in its subunit composition and solubility properties from that conveyed with neurofilaments in SCa (Tashiro et al., 1984, J. Neurochem., 43:1220). The present study examined the effects of axotomy on the SCb associated microtubule proteins in mature peripheral sensory axons of albino rats.

Two peripheral axotomy conditions were examined: axotomy induced by either a forceps crush of the sciatic nerve or by removal of a 2mm segment of nerve. The peripheral axotomy site was 48-52 mm from the L5 DRG. For comparison, nerves from normal or sham-operated animals and animals that sustained a central crush lesion of the L5 dorsal root were used. Injections of ^{35}S-methionine into the L5 DRG were made 1-2 weeks after axonal injury and animals were sacrificed 3,5 and 7 days after labeling. Aliquots of successive 2 mm segments of the sciatic nerve, proximal and distal to the injury, were subjected to gel electrophoresis and fluorography. The tubulins, actin and several additional SCb proteins were excised from gels and the radioactivity measured.

In response to axotomy induced by either a cut or crush of the sciatic, the amount of SCb tubulin was significantly increased in peripheral DRG axons compared to the amount in control conditions. Additionally, a marked increase in the rate of transport of SCb tubulin was apparent in regenerating systems compared to control axons. This rate increase was evident by analysis of the movement of the labeled tubulin peak or front with time. Less dramatic changes in the transport profiles of other SCb proteins were found. The alterations in SCb tubulin transport may contribute to the mechanism of the priming lesion effect documented in the DRG system (Oblinger and Lasek, 1984, J. Neurosci., 4:1737). That is, the increased elongation rates that result when peripheral axons are lesioned 1-2 weeks after a first lesion may be related to the availability of increased amounts and rates of microtubule protein needed to support faster and more extensive neurite elongation.

125.12 CHANGES IN CELL PROLIFERATION IN THE GOLDFISH RETINA FOLLOWING OPTIC NERVE CRUSH. D.B. Henken and M.G. Yoon. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1

In goldfish new cells are continuously added to the retina throughout life. In this study we examined how optic nerve crush (ONC) affects the rate of cell birth in the retina. Unilateral ONC's were performed on 25 fish. Either 3, 6, 9, 12, 15, 20, 25, 30, or 40 days later both eyes were injected with tritiated thymidine (^{3}H-TdR, 6.7 Ci/mmole, ICN). The fish were sacrificed one day after ^{3}H-TdR administration and embedded in paraffin for standard light microscopic autoradiographic examination of labelled cells.

Differential changes in the labelling pattern of the experimental eye, relative to the control eye, were seen at various post-operative periods. ^{3}H-TdR incorporation in the deep layers of the retina (ganglion cell and optic fiber layers) was enhanced as early as three days following ONC. The increased rate of labelling in the experimental retina (as compared to the control) reached a peak at around 12 days and returned to control levels by day 30.

Differential labelling was also found in the photoreceptor layer. Enhanced labelling was seen in the experimental retina 10-12 days following ONC. At 15 days and onward, however, labelling in the experimental retina fell below that of the control.

The differential labelling described here in response to ONC occurred through all topographic regions of the retina, although labelling was always clearly highest in the peripheral germinal zones. These results suggest that axotomy and regeneration of ganglion cell axons affect the rates of cell birth in the photoreceptor layer as well as in the deep layers of the retina in adult goldfish.

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125.13 MORPHOLOGY OF REGENERATED RETINAL AXONAL TERMINAL ARBORS IN THE GOLDFISH TECTUM. C.A.O.Stuermer, M.Beckmann* and E.Kalko*. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, FRG.

Regenerated goldfish retinal axons and terminal arbors (TAs) were labeled by applying crystals of HRP either to the stumps of the resectioned optic nerve (early regenerates) or to cut axon bundles in retina at defined retinal positions (late regenerates). Two to four days later the tectal lobes were removed, reacted with DAB, fixed, dehydrated, cleared, whole mounted under Permount and coverslipped for light microscopic examination.

Most regenerated axons and TAs resemble the normal in that the axon enlarges to form the stem segment of the arbor which first bifurcates into two major branches and then into branchlets. The TA's stem and major branches were either oriented in the same direction as their parent axons - or particularly in peripheral tectum - the stem and/or one or both major branches were perpendicular to their axon. Axons and TAs extend horizontally and contribute to superficial and deep layers of the synaptic layer SFGS (Stuermer, J.Comp.Neurol. 229:214, 1984).

At early regeneration stages (15 days) the front of axons and TAs were confined to the rostrocentral and dorsal and ventral peripheral tectum. Along their length, the axons had regularly spaced elongated thickenings which were about 20 times longer than the spindle-like enlargement of the normal. TAs ranged between 50x90µm up to 180x350µm. These TAs showed several peculiarities: the stem and the major branches appeared broader and longer than normal. They were lobular and contained irregularly shaped bulges and protrusions. The major branches divided into numerous processes some of which exhibited growth cone-like globular or pear-shaped swellings with tender twigs. Occasionally, a third branch arose from the proximal part of the stem. Close to the entrance of the optic tract we noted axons ending in growth cone-like enlargements which possessed filopodia-like extensions.

Axons and TAs at 2,4,6 and 12 months had normal morphologies and sizes (Stuermer, 1984). Most TAs were located at retinotopic sites (Neurosci. Abstr.10, 1984).

Thus we conclude that regenerating TAs in goldfish are basically of the same extent and shape as normal TAs. They do not exceed excessively the normal dimensions.

125.14 SEROTONIN-LIKE IMMUNOREACTIVITY IN NORMAL AND REGENERATING CEREBRAL GANGLIA OF THE SNAIL MELAMPUS. R.L. Ridgway and S.B. Moffett, Dept. of Zoology, Washington State University, Pullman, WA 99164-4220.

In the pulmonate snail Melampus the capacity to regenerate central nervous system tissues following injury extends into adulthood and may include the replacement of lost neurons. For example, after unilateral cerebral ganglionectomy the cerebral connectives, commissure, and distal nerves regrow, converge, and often form a new ganglion bud. Ultrastructural studies have established the presence of morphologically differentiated neurons in long term (>6 months) regenerating ganglia (Moffett and Austin, J. Comp. Neurol. 207:177-182, 1982). Serotonergic cells are among the strong candidates for replacement in the event of loss because of their importance to such behaviors as feeding and locomotion. We have used immunohistochemistry to identify serotonergic cells in normal and regenerating cerebral ganglia.

Normal cerebral ganglia of adult Melampus contained 30-35 cells having serotonin-like immunoreactivity, most of which were found in 4 spatially distinct clusters. The size, number, and axonal distributions of neurons were characteristic for each cluster. Two large (35-50 µm diam.), single neurons were also among the immunoreactive cells of each ganglion. One of these, C1, appears homologous to the "metacerebral giant cells" of other gastropod species. It sends axons to the ipsilateral buccal ganglion and median lip nerve, and to the contralateral cerebral ganglion.

All of the regenerating snails had undergone left cerebral ganglionectomy 1-5 years prior to sacrifice. The degree of ganglion bud development varied from a simple swelling to an enlargement one-half the size of control ganglia. This variability cannot be directly correlated to post-operative time since poorly developed buds were found among the longest term regenerates. Serotonin-like immunoreactivity within poorly developed buds consisted of profusely distributed neurites arising from cells of neighboring (non-excised) ganglia. Buds of intermediate development were characterized by neuropil regions and distinct axon tracts, and sometimes by the presence of small, scattered immunoreactive cells. Some of these cells were located in adjacent nerve trunks. We are still in the process of examining ganglion buds of more advanced development.

Our findings to date confirm the reestablishment of axonal pathways following injury in Melampus and show that the regenerative capacity of this nervous system is sufficient to generate new serotonergic cells.

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125.15 RELEASE OF A DIFFUSABLE SIGNAL FROM INJURED PERIPHERAL NERVE STIMULATES MACROPHAGE INFILTRATION DURING WALLERIAN DEGENERATION.
P.Minweger* and H.W.Müller (SPON: H.J.Freund). Dept. of Neurology, Univ. of Düsseldorf, F.R.Germany.

Sciatic nerves of young adult rats were injured by cryo-lesions at upper thigh level. One group of animals received repetitive i.p. injections of silica quartz dust. This substance is cytotoxic for cells of the monocyte-macrophage series. Removal of myelin in the distal nerve stump undergoing Wallerian degeneration was significantly retarded in animals receiving silica as compared to the control group.

When segments of autologous sciatic nerve were transplanted into Millipore diffusion chambers of 0.22 um pore size which were then implanted in the peritoneal cavity the myelin sheaths persisted for at least 3 weeks. This method eliminated the access of non-resident cells to the transplant but allowed excellent survival of denervated sheath cells within the nerve segment (Beuche and Friede, J.Neurocytol.,13:767,1984). In contrast, in diffusion chambers of 5.0 um pore size which allowed penetration of non-resident cells myelin was removed at a time course similar to Wallerian degeneration in situ. The cells migrating through the latter membrane could be identified as macrophages. To exclude the possibility that (a) macrophages infiltrate the nerve tissue by random migration, and (b) other hematogenous cells (pre-) infiltrating the nerve release the signal which attracts macrophages we carried out experiments using a novel diffusion chamber cell trap. To a diffusion chamber of 0.22 um pore size which contained the nerve an additional chamber (trap) was attached consisting of an outer membrane of 5.0 um pore size and a common 0.22 um membrane shared by the two chambers. Under these conditiones removal of the myelin sheaths in the nerve was inhibited as described for the 0.22 um chamber. However, a large population of macrophages accumulated in the trap chamber within one week after transplantation. Our data suggest the release of a diffusable signal from injured peripheral nerve which specifically attracts non-resident macrophages to infiltrate the nerve tissue during an early period of Wallerian degeneration.

125.16 THE EFFECT OF IMMUNOSUPPRESSIVE DRUGS ON REMYELINATION.
R.M. Herndon and L.C. Triarhou, Ctr. for Brain Research, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642, and Dept. of Pathology, Div. of Neuropathology, Indiana Univ. Sch. of Med., Indianapolis, IN 46223

Anti-inflammatory and immunosuppressive drugs are being used in the treatment of multiple sclerosis (MS) with increasing frequency. Since many of these drugs are antimitotic and there is evidence that division of oligodendrocytes is essential to remyelination, we investigated the effect of dexamethasone, azathioprine, cyclophosphamide, and silica quartz dust (which suppresses macrophage activity) on remyelination following lysolecithin-induced demyelination. Four week old rats were anesthetized and subjected to midthoracic laminectomy. Two µl of 1% lysolecithin were then injected into the ventrolateral white matter using a glass micropipette attached to a Hamilton syringe held in a micromanipulator. Animals were anesthetized and killed by perfusion at 8, 12, 28 and 60 days post surgery. Dexamethasone was given by continuous infusion using Alzet minipumps in a dose of 0.56 mg/rat/day for 14 days beginning on the day of surgery. Azathioprine and cyclophosphamide were given by intraperitoneal injection in a dose of 15 mg/kg/day beginning either 5 days before surgery, the day of surgery, or beginning 5 days after surgery. The silica quartz dust was injected intraperitoneally in a dose of 200 mg the day before surgery. Dexamethasone appeared to decrease the extent of the demyelination but produced a clear and substantial delay in remyelination which was most evident 4 weeks after the lesion. Both azathioprine and cyclophosphamide unexpectedly enhanced remyelination (by 50% at 28 days) when given over a 5 day period before surgery. When given beginning immediately after or 5 days after lysolecithin injection, the effect was still significant, although less pronounced. Silica quartz dust decreased the macrophage response and delayed the clearance of myelin debris but had little apparent effect on remyelination. These findings raise the possibility that some of the improvement seen following immunosuppressive therapy in MS patients could be due to enhanced myelin regeneration.

125.17 EVIDENCE FOR REMYELINATIVE POTENTIAL OF ADULT OLIGODENDROCYTES: COMPUTER RECONSTRUCTIVE ANALYSIS OF TISSUE CULTURE EXPERIMENTS.
M.K. Wolf, M. Brandenberg*, and S. Billings-Gagliardi. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605; and the Image Graphics Laboratory, Department of Neuroscience, Children's Hospital Medical Center and Department of Neuropathology, Harvard Medical School, Boston, MA 02115.

Previous work has established that when a fragment of mouse optic nerve is added to an organotypic culture of mouse cerebellum, the optic nerve oligodendrocytes are capable of migrating into the cerebellar tissue and forming myelin around cerebellar axons. This system was developed to study mutant mice with CNS hypomyelination, and previous work has utilized immature (0 to 14 postnatal day) optic nerve. We now find that adult optic nerve oligodendrocytes have similar capabilities. In six consecutive trials, adding fragments of 50 to 93 postnatal day mouse optic nerve to cultures of newborn mouse cerebellum which lacked resident myelinating oligodendrocytes, either because of a genetic mutation or previous drug treatment, produced myelin in some cultures each time. A few cultures produced abundant myelin, as previously obtained with immature optic nerve. Small size of the fragments, and dissection in Ca- and Mg-free saline seemed critical for success. This is direct experimental evidence that adult mammalian oligodendrocytes can potentially regenerate myelin following CNS injury. The experimental system can now be used to look for factors which promote regenerative behavior and to characterize this behavior in quantitative terms. For computer analysis, serial sections of a culture are mapped directly onto computer datafiles, via a digitally controlled microscope, a vector display system, and a digitizing tablet. Each such section map consists of contours delineating the position of the optic nerve and myelin. Contours are "tagged" so as to identify the corresponding structure in the section. The maps are then registered, stacked, and displayed as 3-dimensional reconstructions, which can be rotated and pseudocolored with perceived enhancement or suppression of any feature. Displays are recorded either by an X-Y pen plotter or by photography of the display screen. Additional software permits extraction of numerical data, such as interstructure distances, from the same section database. Numerical analysis completed to date shows that oligodendrocytes are capable of migrating at least 1.6 mm. Graphic analysis suggests that the true migration trajectories may be much longer.
Supported by NIH Grant NS-11425 (Javits Award), and contract #RC84-05 from the American Paralysis Association to SB-G and MKW, and by NIH Grant NS-20820 (Javits Award) to Richard L. Sidman.

125.18 INCREASED NUMBERS OF MICROGLIAL CELLS IN THE HYPOGLOSSAL NUCLEUS AND DORSAL MOTOR NUCLEUS OF THE VAGUS NERVE IN THE CAT FOLLOWING PERIPHERAL NERVE TRANSECTION: J.L. Cova* and H. Aldskogius. Depts. of Anat. and Neurosci., Med.Coll. Ohio, OH. 43699 and Dept. of Anatomy, Karolinska Institutet, Stockholm, Sweden.

Numerous previous studies have described an extensive proliferation of glial cells, particularly microglial cells, in the vicinity of axotomized motorneurons of rodents and lagomorphs. In a study on the adult and adolescent cat using paraffin sections (4 µm thick) no significant changes were observed in total number and density of perineuronal glial cells in the spinal cord ventral horn after brachial plexotomy (Cova, J.L., Aldskogius, H., Anat.Embryol. 169:303, 1984). However, microglial cells normally constitute only a small fraction of the entire glial cell population. Therefore, a significant increase of this type of cell could occur without altering the total number of glial cells significantly. In a recent study using plastic-embedded, 0.5 µm thick sections to permit differential counts of various glial cell types, no changes in their number or density were observed following crush injury of the hypoglossal nerve in the cat (Cova, J.L., Aldskogius, H., J.Comp.Neurol., 233:421, 1985). The present study extends these previous studies by examining the effect on the glial cell population in the hypoglossal nucleus and dorsal motor nucleus of the vagus nerve after nerve section. The data have been related to the longterm effects of nerve section on neuronal numbers in these two brainstem nuclei.

The left hypoglossal and right vagus nerves were transected in adult cats under barbiturate anesthesia. Ten, 20, 35 and 80 days later the animals were reanesthetized and perfused with aldehyde solutions. Blocks containing the hypoglossal nucleus and dorsal motor nucleus of the vagus nerve from both sides of animals surviving 10-35 days were embedded in Vestopal W, sectioned at 0.5 µm, stained with toluidin blue and analyzed quantitatively with regard to the number of various glial cell types as described before (Cova, J.L., Aldskogius, H., J. Comp.Neurol., 233:421, 1985). The brain stem from the 80-days survivals were embedded in paraffin, serially sectioned at 10 µm and stained with cresyl violet. Neuronal numbers were estimated from nucleolar counts in every tenth section. Intact animals were used as controls.

There was about a two-fold increase in the number of microglial cells in both examined nuclei ipsilateral to nerve transection. No changes were observed with regard to oligodendrocytes and astroglial cells. Neuronal counts indicated a slight cell loss in the hypoglossal nucleus and a somewhat more marked cell loss in the dorsal motor nucleus of the vagus nerve. These findings show that a perineuronal glial cell reaction occurs in the cat following peripheral nerve section. However, it appears to be considerably less prominent than in rodents and lagomorphs and may be related to a moderate degree of axotomy-induced neuronal degeneration.

125.19 NERVE INJURY STIMULATES APOLIPOPROTEIN SECRETION BY NON-NEURONAL CELLS. G. Jack Snipes*, and John A. Freeman. (SPON: D. Buxbaum). Dept. of Anatomy, VAnderbilt Univ, Nashville TN 37232.

Nerve trauma initiates significant changes in the overall composition of proteins secreted by non-neuronal cells in the rat. The expression of one such protein, as 37 kDa protein, correlates with the time-course of nerve growth both during neurogenesis and during nerve regeneration. In addition, the secretion of a 26 kDa protein by non-neuronal cells is associated specifically with central nervous system development and its response to injury. We now report that we have identified the 37 kDa protein as apolipoprotein E (apo E), and we present evidence that the 26 kDa protein is apolipoprotein Al (apoAl).

We have produced a highly specific rabbit antibody against the 37 kDa protein isolated from regenerating sciatic nerves. This antiserum recognizes a 36 Kda protein in rat serum which we have purified and identified as apolipoprotein E on the basis of its molecular weight, isoelectric point, and amino acid composition. In addition, the anti 37K antiserum recognizes apo E on western blots of authentic samples of high density lipoproteins (HDLs) and very low density lipoproteins (VLDLs) isolated from rat serum. The nerve 37 kDa protein essentially comigrates with apo E by two-dimensional electrophoresis, and shares a similar amino acid composition with apo E. The purified apo E specifically blocks the immunoprecipitation of [35-S] methionine labeled 37 kDa protein synthesized by non-neuronal cells. Thus, on the basis of its molecular weight, isoelectric point, amino acid composition, and immunological properties, we conclude that the 37 kDa protein associated with nerve development and regeneration is apo E.

Apolipoproteins and lipids together form lipoprotein complexes. In order to identify additional apolipoproteins synthesized in the nervous system, we labeled non-neuronal cell proteins with [35-S] methionine and immunoprecipitated apo E containing lipoprotein complexes with the anti-apo E antibody. The immunoprecipitated [35-S] labeled apolipoproteins, synthesized by non-neuronal cells, were identified by 1D- and 2D-PAGE. We found that optic, but not sciatic nerves secrete a 26 kDa apolipoprotein which we have identified as apo Al on the basis of its association with apo E, its molecular weight, and its isoelectric point.

We propose that these apolipoproteins are synthesized by phagocytic cells during development and following nerve injury for the purpose of mobilizing lipids produced as a consequence of axon and myelin degeneration. Additionally, it is interesting to consider the possibility that these lipoproteins are utilized by developing and regenerating neurons to deliver lipids necessary for axon growth.

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125.20 METHOTREXATE DID NOT INHIBIT REACTIVE GLIOSIS AFTER FIMBRIA TRANSECTION IN ADULT RAT BRAIN. K.A. Ayyad* and J.S. Wendt* (SPON: Robert S. Sloviter). Dept. of Neurology, VA Medical Center and Univ of Tex Health Science Center, Dallas, TX 75216.

Reactive gliosis may be an impediment to CNS regeneration in mammals. Pre-treatment of adult rats with methotrexate (MTX) (2.5mg/kg I.P.) for 5 days followed by cortical lesions has been reported to inhibit glial proliferation (Immunopharmacol 5:91,1982). Also, intraventricular injection of MTX (10ul of a 200nM solution) just prior to perforant pathway transection has been reported to inhibit glial proliferation in denervated dentate gyrus in the rat (Brain Res 265:160,1983). The present pilot study was conducted to determine whether administration of MTX according to either of these protocols would inhibit reactive gliosis after fimbria transection in the rat.

In the first experiment 9 adult Sprague-Dawley rats received MTX 2.5mg/kg x 5 days; 7 control animals received corresponding saline injections. On day 5 hippocampal fimbria transections were performed stereotaxically with a fine surgical blade. By post-operative day 5, 8 of 9 MTX animals had died of intracerebral and/or gastrointestinal hemorrhage. One control died. The last MTX animal and 2 controls were sacrificed on post-op day 11, and frozen horizontal brain sections were stained for astrocytes by Cajal's gold chloride method. Extensive reactive gliosis was noted in all animals, and the MTX animal also had an intracranial hematoma.

In the second experiment fimbria transection was accompanied by intralesional injection of 10ul phosphate buffer with MTX (200nM, 20mM, 2mM or 0.2M) into the lesion site. In one animal the 0.2M dose was repeated 48 hr post-surgery. Two control animals received fimbria transection surgery and phosphate buffer injections. After sacrifice at 13 days post-op and tissue processing as above, all animals showed extensive gliosis around the lesion site.

These results suggest that MTX does not substantially inhibit reactive gliosis around a traumatic brain lesion.

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PSYCHOTHERAPEUTIC DRUGS: ANXIOLYTICS

126.1 MIDAZOLAM DISCRIMINATION IN RATS: GENERALIZATION TESTS AND PRETREATMENT STUDIES. I.P. Stolerman, H.S. Garcha* and I.C. Rose*. Departments of Pharmacology and Psychiatry, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, England.

The discriminative stimulus effects of drugs can provide highly specific methods for quantifying their central effects and for studying their mode of action. For studies on benzodiazepines, there may be advantages associated with training on a short-acting, water-soluble compound which appears not to have active metabolites. Rats were, therefore, trained to discriminate midazolam from saline with a two-bar operant conditioning procedure and food reinforcement (tandem VI-1 FR-10 schedule). Midazolam (0.4 mg/kg s.c.) or saline was injected 5 minutes before 10-minute training sessions. After 35-40 training sessions, 5-min extinction tests took place twice weekly, with training continuing on intervening days. Results were calculated with both quantitative and quantal indices of response, which yielded similar findings.

Midazolam (0.025 - 0.4 mg/kg) yielded a clear dose-response relationship: drug-appropriate responding, expressed as a percentage of the overall numbers of responses, averaged 3% after saline as compared with 97% after midazolam (0.4 mg/kg). Dose-related generalization occurred with several other benzodiazepines (flunitrazepam, 0.025 - 0.4 mg/kg s.c.: clonazepam, 0.1 - 0.8 mg/kg i.p.: diazepam, 0.5 - 8 mg/kg i.p.: chlordiazepoxide, 0.5 - 8 mg/kg i.p.: all 30 min before tests). Generalization to pentobarbitone (1.25 - 10 mg/kg s.c.) took place only at doses which severely suppressed overall rates of responding. Several drugs without anxiolytic or sedative-hypnotic effects were not generalized, even in doses which reduced response rates markedly (amphetamine, morphine, nicotine, oxotremorine, quipazine, and picrotoxin). The midazolam stimulus was detected as early as 2.5 min after injection, it was maximal from 5 - 15 min. and it disappeared after 30 - 90 min.

The benzodiazepine antagonist Ro 15-1788 (1 - 32 mg/kg i.p. 20 min before tests) completely blocked the discriminative effect of midazolam in a dose-related manner, and was not itself generalized. The GABA antagonist picrotoxin (0.2 - 1.6 mg/kg s.c. 20 min before tests) blocked generalization to pentobarbitone (7.5 mg/kg). However, picrotoxin did not block midazolam and Ro 15-1788 did not block generalization to pentobarbitone. Under the conditions used, the midazolam discriminative stimulus seemed to provide a highly specific behavioural assay for benzodiazepine-like central effects. The discriminative effects of midazolam and pentobarbitone in rats may be mediated through different, independent receptor sites.

126.2 INTERACTION OF PLATELET ACTIVATING FACTOR (PAF) WITH NEUROACTIVE DRUGS AND WITH NEURAL CELLS. E. Kornecki, R. Lenox, and Y. Ehrlich. Neuroscience Research Unit, Dept. of Psychiatry, Univ. of Vermont, Burlington, VT 05405.

Platelet activating factor (PAF) is a naturally occurring phospholipid (1-O-alkyl, 2-acetyl, glyceryl-3 phosphorylcholine) with potent biological effects. PAF has antihypertensive activity, induces bronchoconstriction and contraction of smooth muscle, and is one of the most powerful activators of platelets. We have reported previously that the triazolobenzodiazepine drugs, alprazolam and triazolam, act as specific and potent inhibitors of PAF-induced activation of human platelets (Science: 226, 1454, 1984). These findings suggest that the effectiveness of triazolobenzodiazepines in the treatment of certain neuropsychiatric disorders (e.g. agoraphobia and panic attacks) may involve interactions of the drugs with processes mediated by PAF or PAF-like phospholipids in the central nervous system. In the present study we have extended our investigation on the interaction of various neurotropic drugs with PAF-induced platelet functions, and examined the effects of PAF on neural cells grown in culture. Human platelets obtained from drug-free, 10 hr fasted, normal volunteers were collected and washed by passing platelet-rich-plasma through a Sepharose 2B column equilibrated in Tyrode solution (pH 7.4) containing 0.5mM $CaCl_2$. Platelet aggregation, secretion and shape change were measured with a Chronolog Lumi-aggregometer. The most potent inhibitor of PAF-induced platelet aggregation was the triazolobenzodiazepine triazolam (IC_{50}= 300 nanomolar). Inhibition by alprazolam gave an IC_{50} value of 500 nanomolar. Diazepam, a benzodiazepine without a triazole ring, had an IC_{50} value of 130 micromolar. Other benzodiazepines (chlordiazepoxide, RO5-4864, RO11-3624, oxazepam) had low potencies (IC_{50} values in the range of 45 to 90uM). Similarly, when using psychotropic drugs of other classes (imipramine, trazodone, pargyline, phenylzine) we found that higher drug concentrations (IC_{50} values from 20 to 500um) were required to inhibit PAF-induced platelet aggregation.

In parallel studies, cells of neuronal origin (clone NG108-15) were grown in a chemically defined (serum-free) medium supplemented with insulin, transferrin and linoleic acid, in the presence or absence of PAF for 1-6 days. Low PAF concentrations (0.1-1uM) enhanced the morphological differentiation of these cells and increased the proportion of cells bearing long neurites. In contrast, 5uM PAF induced loss of neurites and subsequently cell death.

These results suggest that low concentrations of PAF may play a regulatory role in neuronal function, whereas higher concentrations of PAF may contribute to neuronal damage induced by trauma. The triazolobenzodiazepine drugs, alprazolam and triazolam, may exert some of their therapeutic effects by inhibiting PAF-mediated cellular responses in the nervous system.

126.3 FURTHER INVESTIGATION OF THE ANTI-ANXIETY ACTIVITY OF SR 41378. A. Perio* R. Calassi*, J.P. Terranova*, R. Roncucci*, K. Biziere (SPON: L. Tapia-Arancibia). Sanofi Recherche, CRCM, Rue du Pr. J. Blayac, F-34082 Montpellier, France.

SR 41378 is the 3-(4'-hydroxy-piperidinyl)-6-(2,4 dichlorophenyl)-pyridazine. It is a potent hypnotic drug in animals (Biziere K. et al, Brit. J. Pharmacol., 84 : 168 P, 1985). As most of the sleep-inducing agents, it exhibits sedative myorelaxant and anticonvulsant activities in mice. In rats, it proved to relieve behavioral suppression induced by electric shocks in an approach-avoidance conflict test. This effect is not directly mediated by benzodiazepine receptors. Moreover, the compound genreralizes to the clonazepam cue in a drug discrimination paradigm (Biziere K. et al ., Brit. J. Pharmacol., 84 : 116P, 1985). The present experiment further investigates the anxiolytic properties of SR 41378.

Methods : i) Antagonism of the pentetrazole cue : male rats were trained to discriminate pentetrazole (20 mg/kg ip) from saline in a two lever operant chamber (method from Colpaert F.C. et al., Arch. Int. Pharmacodyn. Ther., 218 : 268, 1975). SR 41378 was injected ip 10 min prior to pentetrazole (i.e. 25 min before testing). ii) Male Sprague Dawley rats were trained in the conflict paradigm (procedure by Davidson A.B. and Cook L., Psychopharmacologia, 15 : 159, 1969). On test days animals were placed in Skinner boxes (Campden) 1h, 2h and 4h after dosing with SR 41378 (10 and 20 mg/kg ip), each session lasted 30 min. iii) Antagonism by CGS 8216 : the antagonist was injected ip 5 min before 10 and 20 mg/kg of SR 41378, the conflict test was carried out 30 min later.

Results : SR 41378 counteracted the pentetrazole cue. At 10 mg/kg it alleviated the behavioral suppression induced by electric shocks at the 1h session, and at 20 mg/kg it released punished responding for at least 2h. CGS 8216 completely reversed the desinhibitory action induced by 10 mg/kg of SR 41378 but not the reduction of responding elicited by 20 mg/kg.

Conclusions : The antagonism of the pentetrazole cue further confirms the anxiolytic property of SR 41378. In the conflict test, SR 41378 at low dose level is active for 1 hour. CGS 8216 reverses the desinhibition induced by SR 41378 but not the sedation. In a previous experiment RO 15-1788 did not antagonize SR 41378 in the same procedure. However it must be remembered that CGS 8216 unlike RO 15-1788 antagonizes also the desinhibitory activity of barbiturates and carbamates (Petrack B. et al., 14th CINP congress, FLorence, june 1985). Therefore the mechanism of action of SR 41378 could be similar to that of barbiturates or carbamates.

126.4 SR 41378, A HYPNOTIC AMINOPYRIDAZINE : ELECTROENCEPHALOGRAPHIC ASSESSMENT IN THE RAT. V. Santucci*, M. Fournier*, J.P. Chambon and K. Bizière. Dept. of Neurobiology, Sanofi Recherche, Rue du Pr. J. Blayac, 34082 Montpellier Cedex, France.

SR 41378, 3-(hydroxy-piperidinyl)-6-(2,4 dichlorophenyl)-pyridazine, is a chemically original psychotropic compound with both anticonvulsant and hypnotic properties (Santucci et al., Neurosci. Lett., supp. 18:204, 1984 ; Bizière et al., Brit. J. Pharmacol., 84:198, 1985). The purpose of the present study was to evaluate the electroencephalographic (EEG) as well as hypnotic effects of SR 41378 in comparison to those of diazepam (DZP), pentobarbitone (PB) and secobarbital (SB). The compounds were studied per se, against the insomnia induced by the i.p. injection of caffeine (10 mg/kg) and in interaction with the benzodiazepine antagonist CGS 8216 (10 mg/kg). Male Sprague-Dawley rats (200-400 g) were chronically implanted with cortical electrodes in order to record cable-transmitted EEG. The duration of slow-wave sleep (SWS) was measured by visual inspection. The EEG was continuously recorded for 6 h, in sessions separated by 7-day intervals. The sessions were distributed according to a modified latin square matrix paradigm. When administered alone, SR 41378 (10, 30, 100 mg/kg p.o.) induced the appearance of fast-frequency activities in the EEG which were also present under PB (10, 30, 60 mg/kg p.o.), SB (30, 60 mg/kg p.o.) and, to a lesser extent, DZP (1, 3, 10 mg/kg p.o.). Moreover, like PB and SB, SR 41378 induced spindle-like activities, mainly visible during wakefulness. SR 41378 increased SWS duration in a dose-dependent manner, which was also the case for DZP, PB and SB. SR 41378 was equipotent to PB and exhibited a similar time course of activity. SR 41378 (30, 100 mg/kg p.o.), as well as PB (30, 60 mg/kg p.o.) antagonized the wakefulness-inducing effects of caffeine. It was found that CGS 8216 induced a diphasic profile of activity, with facilitation of wakefulness followed by increase of SWS, as opposed to symmetrical profile for DZP. CGS 8216 reduced the hypnogenic activity of SR 41378 (10 mg/kg p.o.), PB (30 mg/kg p.o.) and DZP (10 mg/kg p.o.). As SR 41378, like the barbiturates, does not bind to the benzodiazepine receptor, the mechanism of their interaction with CGS 8216, at the EEG level, remains to be elucidated. It is concluded that SR 41378 has a potent hypnogenic activity in the rat, which resembles more that of barbiturates, than that of DZP.

126.5 SEX DIFFERENCES IN BEHAVIORAL RESPONSES TO THE PUTATIVE ANXIOLYTIC TVX Q 7821. J. E. Johnston,* J. P. Ryan, and R. L. Isaacson. Department of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, New York 13901.

The putative anxiolytic, TVX Q 7821, has been compared to diazepam in regard to its effects on aggressive and fear-related behaviors, sensorimotor skills, and pentylene-tetrazol-induced convulsions. This drug has been shown to bind with high affinity and regiospecificity to 5-HT_1, rather than benzodiazepine, receptors in calf and rat hippocampus (Glaser et al., Neurosci. Abst. 10: 259, 1984; Traber et al., Brain Res. Bull. 12: 741, 1984). The present study compared TVX Q 7821 to the behavioral effects of the serotonergic agents methysergide and quipazine in the open field in both male and female rats. The animals were pretreated with either saline, quipazine, or methysergide and subsequently received either saline or TVX Q 7821 (all drugs 10 mg/kg). The locomotor activity of males was reduced by quipazine. However, females responded heterogeneously to quipazine, i.e., the activity levels of some females were decreased while others were unaffected. Variance changes were evaluated by Levene's test for homogeneity of variance. Methysergide did not affect the locomotor activity of either sex. Although TVX Q 7821 administration did not affect saline-pretreated males, females again responded heterogeneously to the drug. This drug potentiated the effect of quipazine in males and the activity levels of females that received both drugs were uniformly decreased. Finally, TVX Q 7821 in combination with methysergide did not affect the locomotor activity of males, but this combination of drugs consistently decreased such activity in females. The discovery of sex differences in response to TVX Q 7821 was unexpected as was the fact that it frequently acted to potentiate the effects of other serotonergic drugs when not producing an effect of its own. Our results indicate that: (1) TVX Q 7821 may act as a partial or regionally specific serotonergic agonist and (2) its effects may interact with the hormonal constitutions of the animals.

126.6 LACK OF WITHDRAWAL EFFECTS IN MICE AFTER CHRONIC ADMINISTRATION OF THE NON-SEDATING ANXIOLYTIC CGS 9896. C. A. Boast, S. C. Gerhardt*, Z. L. Gajary* and W. N. Brown* (SPON: J. J. Welch). Neurosci. Res., Pharma Div., CIBA-GEIGY Corp., Summit, NJ 07901.

CGS 9896 is a pyrazoloquinoline with a nonsedating potential anxiolytic profile (Drug Dev Res, 4:75 , 1984). The drug is a mixed agonist/antagonist at benzodiazepine binding sites (JPET, 231:572, 1984) and might be expected to show reduced withdrawal effects, compared to diazepam, after chronic administration.

Male CF-1 mice were treated once daily with vehicle, diazepam (30 mg/kg p.o.) or CGS 9896 (30 mg/kg p.o.) for four weeks. Animals were tested behaviorally 1,3,8 or 15 days after this drug treatment. At each time period, groups of mice were treated with vehicle (spontaneous withdrawal), or the benzodiazepine inverse agonist CGS 8216 (30 mg/kg p.o.) (precipitated withdrawal). Thirty min following this treatment, movement time and distance travelled were recorded for 15 min in motor activity chambers (Omnitech Digiscan). At one hour, pentylenetetrazol (PTZ) (2.5 mg/ml i.v.) was infused in increments until each mouse showed seizure activity. The total dose (mg/kg) of PTZ required to produce the seizure was recorded.

Movement time was not altered in any treatment condition. Distance travelled was reduced in the 1-day and 3-day diazepam-treated, precipitated withdrawal groups only. PTZ threshold was reduced by CGS 8216. The diazepam-treated spontaneous withdrawal groups showed further threshold reductions at 1, 8, and 15 days of withdrawal. After 15 days of withdrawal, the diazepam-treated spontaneous withdrawal group also showed a lowered threshold compared to vehicle-treated mice. Animals treated chronically with CGS 9896, showed no differences in seizure threshold compared to controls at any time.

The present techniques appear to be useful for measuring withdrawal effects after drug-induced benzodiazepine binding site modulation, and indicate that chronic treatment with CGS 9896 does not induce any of these signs. This lack of withdrawal in mice following chronic CGS 9896 is in agreement with a previous study in baboons (Drug & Alcohol Dependence, 14:11, 1984) and suggests a major clinical advantage of this new nonsedating anxiolytic.

126.7 A NON-SEDATING ANXIOLYTIC, CGS 9896, IS DIFFERENTIATED FROM DIAZEPAM IN BEHAVIORAL TESTS OF TOLERANCE IN MICE. S. C. Gerhardt*, Z. L. Gajary*, W. N. Brown* and C. A. Boast. Neurosci Res., Pharma Div., CIBA-GEIGY Corp., Summit, N.J. 07901.

CGS 9896 is a recently described (Drug Dev Res, 4:75, 1984) pyrazoloquinoline with a nonsedating potential anxiolytic profile. The drug binds to benzodiazepine sites and consequently might be expected to show tolerance after chronic administration (e.g. Psychopharmacol., 62:61, 1979). To test this possibility, CF-1 male mice were injected once daily with vehicle, diazepam (3.0, 10 or 30 mg/kg p.o.) or CGS 9896 (3.0, 10 or 30 mg/kg p.o.) for periods of 1, 2, 3 or 4 weeks. Additional groups received drug acutely on the day of behavioral assessment. Thirty min after the last dose, movement time and distance travelled were recorded for 15 min. in motor activity monitors (Omnitech Digiscan). One hour after dosing, traction reflex was assessed, and pentylenetetrazol (PTZ)(10 mg/ml i.v.) was then infused in increments. The total dose of PTZ (mg/kg) required to produce a seizure was recorded.

Movement time was reduced by the acute 10 and 30 mg/kg doses of diazepam. After chronic diazepam, this reduction was no longer apparent. This parameter was not altered by CGS 9896 at any dose or time. Distance travelled was increased by 3 mg/kg diazepam, regardless of treatment duration. The 10 and 30 mg/kg doses of diazepam slightly reduced the distance measure when given acutely but increased it after chronic administration. CGS 9896 increased distance travelled after acute administration, but this effect was no longer apparent after 4 weeks of treatment. Traction was impaired after acute administration of diazepam. After chronic dosing, however, this impairment was considerably reduced. CGS 9896 did not impair traction at any dose or time. PTZ seizure threshold was increased after acute treatment with either drug. Chronic diazepam treatment reduced threshold relative to the acute values. After chronic CGS 9896, threshold was not different from acute values.

These data indicate that tolerance for the anticonvulsant and muscle relaxant properties of diazepam occurs after one week of daily dosing in the mouse. In contrast, CGS 9896 did not induce muscle relaxation and did not show tolerance for its anticonvulsant action. These differential effects may reflect subtle modulation of benzodiazepine receptors.

126.8 THE DIFFERENTIAL SEDATIVE/MUSCLE RELAXANT PROFILES OF SEVERAL CLASSICAL AND NOVEL ANXIOMODULATORS IN FOUR BEHAVIORAL PARADIGMS. C.L. Corradi, D.A. Bennett, J. Diverio* and D.E. Wilson*. Neurosci. Res., Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, N.J. 07901.

While classical benzodiazepine anxiolytic drugs are effective in alleviating anxiety, they also produce sedation and muscle relaxation which often interfere with normal functioning. Various novel anxiolytic compounds have been identified which induce fewer side effects. Preclinical profiling of the sedative/muscle relaxant properties of these compounds is very often a prerequisite for clinical evaluation.

In the present investigation, various anxiomodulators (benzodiazepine agonists, partial agonists and antagonists) were evaluated in four tests designed to predict sedative/muscle relaxant effects in rats: Rotorod performance, motor activity measures of distance travelled and movement time, potentiation of hexobarbital-induced sleep time or potentiation of alcohol-induced rotorod deficit. A profiling of these compounds and a comparison of the sensitivity of the various behavioral measures were obtained.

The results separate these anxiomodulators into four distinctive groups. Diazepam, chlordiazepoxide, alprazolam, CL 218,872 and pentobarbital showed sedative/muscle relaxant effects at low doses in each of the four test systems. A different profile emerged with halazepam, premazepam and quazepam. These compounds did not induce rotorod deficit and produced motor activity reductions only at high doses (100-300 mg/kg p.o.), if at all. Some deficit was noted in the drug potentiation studies with an increase of sleep time produced by quazepam (300 mg/kg p.o.), halazepam (100 mg/kg p.o.) and premazepam (10 mg/kg p.o.). Each of these drugs interacted with alcohol at relatively low doses (1-10 mg/kg p.o.). A third profile was exhibited by CGS 9896 and fenobam. These compounds were without effect in each of the test systems with the exception of alcohol interaction. Even here, the interaction of these compounds was only noted at doses of 30-100 mg/kg p.o. CGS 8216 and CGS 9895 formed a fourth profile as these compounds, producing benzodiazepine antagonist activity, did not induce sedation or muscle relaxation in any of the four behavioral paradigms. CGS 8216 actually increased some of the parameters in the motor activity study.

Of the behavioral assays used, the drug combination paradigms (hexobarbital and alcohol) appeared to be the most sensitive, although some of the interaction effects were only noted at relatively high doses. Rotorod and motor activity paradigms easily characterized sedative/muscle relaxant effects of the classical drugs and identified most of the novel compounds as free from side effects. For the most efficient evaluation of novel compounds, it appears that test systems measuring the effects of the drug alone in addition to test systems that investigate the potential potentiation of another drug effect should be conducted.

126.9 EVIDENCE THAT CGS 9896 AND DIAZEPAM DIFFER IN THEIR ACTIONS ON GABA TRANSMISSION. S.L. Fallon* and C.M. Sinton. Research Dept., Pharmaceuticals Division, Ciba-Geigy Corp., Summit NJ 07901.

CGS 9896 is a nonbenzodiazepine anxiolytic with potency approximately equal to that of diazepam in neurochemical and behavioral tests. Although it binds with high affinity to benzodiazepine receptors in vitro and in vivo, ^{3}H-Flunitrazepam binding and photo-labelling studies indicate that CGS 9896 may act via a mechanism distinct from that of benzodiazepine agonists like diazepam. To characterize further the differences in the central actions of the two compounds, we examined the effects of CGS 9896 and diazepam on the firing rate of single units recorded in the Substantia Nigra Zona Reticulata (ZR). This region is innervated by the GABA-ergic striatonigral tract and it possesses the highest density of GABA terminals in the brain. Neuronal activity in the ZR has been shown to be sensitive to GABA modulation, and any differentiation in response at this level is likely to reflect differences in action on GABA transmission. Rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and a glass microelectrode was lowered stereotaxically to the ZR. Units were identified on-line by their characteristic firing pattern and rate, and by their position just ventral to nigral dopamine cells. The two anxiolytics were also tested against the benzodiazepine antagonist CGS 8216. All drugs were administered i.v. through a tail vein - in control experiments, the vehicle was administered alone. Confirming previous studies, diazepam (1 mg/kg) produced prolonged reductions in ZR firing rates of up to 70 %. This effect was reversed by treatment with CGS 8216 (1 mg/kg). In contrast, CGS 9896 (1 mg/kg) produced only small ZR firing rate reductions which lasted for approximately 5 min. When diazepam was administered following CGS 9896, the inhibitory action of the former compound was blocked. Conversely, when CGS 9896 was administered following diazepam, CGS 9896 was without effect on the inhibition induced by diazepam. CGS 8216 alone increased the firing rate of ZR neurons. In conjunction with data from binding studies, these results indicate that CGS 9896 and diazepam may produce their anxiolytic effects by competitive action at overlapping recognition sites on the benzodiazepine receptor; both compounds can also be antagonized by CGS 8216. Despite these similarities, however, CGS 9896 and diazepam appear to differ in their action on the benzodiazepine-GABA receptor complex. Diazepam is known to act by increasing the opening frequency of the chloride ionophore in response to GABA, whereas the results reported here imply that CGS 9896 may have only a minimal effect on GABA transmission. These findings could have important implications for the dependence liability and adaptation effects that occur after prolonged administration of anxiolytics.

126.10 ANTICONFLICT ACTION OF MUSCIMOL IN THE LATERAL SEPTUM OF THE RAT. Jacqueline N. Crawley, Robert C.Drugan, Phil Skolnick, and Steven M. Paul. Clinical Neuroscience Branch, NIMH, and Laboratory of Bioorganic Chemistry, NIADDK, National Institutes of Health, Bethesda, MD 20205.

Several brain areas within the limbic system have been proposed to be critical in the modulation of anxiety and the facilitatory effects of the benzodiazepines on anticonflict behavior. GABA-mimetics as well as benzodiazepines have been shown to increase punished responding in a "thirsty-rat" conflict test when microinjected into the lateroposterior and basolateral nuclei of the amygdala (Scheel-Krüger, J. and Petersen, E., Eur. J. Pharmacol., 82:115-116, 1982). Intracerebroventricular (icv.) muscimol has also been demonstrated to have an anticonflict action (Cananzi, A., Costa, E., and Guidotti, A., Brain Res., 196: 447-453, 1980).

In the present study, we investigated the efficacy of musimol microinjected into the lateral septum on conflict behavior in the "thirsty-rat" conflict test. Rats were implanted with a stainless steel 24 gauge hypodermic cannula (A-P + 0.2, lat. - 0.7, vert 4.5 mm) into the lateral septum. One week later, rats were water deprived for 24 hours. Muscimol (0.1, 2, 5, 10, or 1000 ng) or vehicle (artificial CSF) was infused in a volume of 0.4 µl over a one minute period through a 31 gauge injection tube 5 minutes prior to the conflict test session. A dose-dependent increase in punished responding was observed, which reached significance at 5 ng of musicimol. However, at higher doses sedation and ataxia were observed, markedly reducing the number of approaches to the water spout.

The present data support the hypothesis of Gray (Gray, J. and McNaughton, N., Neurosci. Biobehav. Rev., 7:119-188, 1983) suggesting that the lateral septum may be a critical structure in anxiety-related behaviors. Moreover, the effective dose (5 ng) of muscimol used in the present study is much lower than those used in previous studies, e.g. 25 ng and 150 ng injected into the amygdala or ventricles, suggesting greater sensitivity of the lateral septum to the anticonflict actions of muscimol.

126.11 **ANXIOLYTIC TESTING OF BUSPIRONE IN RODENTS.** J. H. Porter, D. N. Johnson, and J. Y. Jackson* (SPON: G. H. DeVries). Department of Psychology, Virginia Commonwealth University and A. H. Robins Co., Richmond, VA 23261.

Buspirone, a dopamine antagonist, has been reported to have anxiolytic activity in humans. The drug was developed in the 1970s as a neuroleptic, but it produced only transient antipsychotic effects. Its anxiolytic potential was first realized during these early clinical trials; however, some have reported that it does not appear to possess the typical anxiolytic effects in animals. We have studied buspirone in a variety of anxiolytic testing procedures in rodents and compared it with both typical and atypical anxiolytics.

While both diazepam (ED_{50} = 1.0 mg/kg) and cartazolate (ED_{50} = 10.6 mg/kg) blocked seizures induced by electroconvulsive shock in mice (N = 5-8/group, buspirone was inactive in doses up to 100 mg/kg (IP). Similarly, buspirone (3.16, 10, and 31.6 mg/kg) failed to block seizures induced by picrotoxin; whereas, cartazolate (ED_{50} = 38.6 mg/kg) did block this chemically induced seizure. Other effects characteristic of most anxiolytics, such as muscle relaxation and sedation, were not seen with buspirone. In a disinhibition procedure (Poschel, Psychopharmacologia, 19:193, 1971) adult female rats (N = 5/group) were given access to a novel food (diluted, sweetened, condensed milk) for 4 hr. Chlordiazepoxide (20 mg/kg, PO; mean intake = 10.2 mL), but not buspirone (50 mg/kg, PO; mean intake = 5.2 mL), significantly increased the amount of milk consumed compared with control animals (vehicle; mean intake = 2.6 mL).

Finally, buspirone (IP) was assessed in a modified conflict procedure (Geller & Seifter, Psychopharmacologia, 1:482, 1960). Adult male rats (N = 34) were maintained at 80% body weight and tested in daily 30-min sessions on a multiple, fixed-interval 1-min (food reward only), fixed-ratio 1 (food plus scrambled foot shock) schedule. As shown in the table below, buspirone produced a significant increase in punished responding at the 3 lowest doses, but the magnitude of its antipunishment effect was much less than that for diazepam.

These findings are in agreement with recent reports (e.g., Riblet et al, Psychopathology, 17:69, 1984) that buspirone, unlike the benzodiazepines, lacks anticonvulsant, sedative, and muscle-relaxant properties. However, buspirone is active in the Geller-Seifter conflict procedure that is predictive of antianxiety activity.

Mean Punished Responding in the Conflict Test (% of Control Day)

	Vehicle	1.25	2.5	5.0	10	20 mg/kg
Buspirone	120.8	217.4*	317.2*	243.8*	111.1	46.9
Diazepam	127.1	2 mg/kg = 1451.1*		4 mg/kg = 2179.3*		

*Significantly different from vehicle ($p<0.05$).

126.12 BUSPIRONE AND MJ-13805 SELECTIVELY ATTENUATE FEAR AS MEASURED WITH THE POTENTIATED STARTLE PARADIGM. M. Davis, J.H. Kehne, and J.V. Cassella. Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

Benzodiazepines have been widely used in the treatment of anxiety in humans. Recently, buspirone, a compound structurally unrelated to benzodiazepines, has been reported to be anxiolytic with minimal muscle relaxant or sedative side effects. A useful model system for studying fear or anxiety in animals is the potentiated startle paradigm whereby the amplitude of acoustic startle is enhanced by eliciting the response in the presence of a light previously paired with a shock. Potentiated startle is reduced by anxiolytic drugs including benzodiazepines (i.e., diazepam). Given buspirone's reported clinical efficacy, the major aim of the present study was to investigate the effects of buspirone on the potentiated startle response.

Initially, rats were fear conditioned with multiple presentations of a light paired with a foot shock. Subsequently, rats were tested for potentiated startle by presenting the startle stimulus in the presence of the light that had previously been paired with shock. Subcutaneous injections of buspirone immediately before testing resulted in a dose-dependent blockade of potentiated startle. The ED-50 was approximately 0.7 mg/kg, in close agreement with clinically effective doses. Buspirone selectively blocked potentiated startle without reducing baseline startle amplitude. MJ-13805, a buspirone analog with a different neurochemical profile, has also been reported to have anxiolytic actions. In the present study, subcutaneous injection of MJ-13805 dose-dependently blocked potentiated startle without altering baseline. The ED-50 for MJ-13805 was approximately 2.0 mg/kg. In a control study, buspirone was compared with the antipanic/-antidepressant drug, imipramine. While imipramine is beneficial in treating panic disorder, it does not have anxiolytic properties. Neither acute (10 mg/kg ip injected 15 minutes before testing) nor chronic administration (10 mg/kg/day for 21 days) of imipramine reduced potentiated startle despite adequate plasma levels of the drug.

In summary buspirone and MJ-13805 showed potent and selective anxiolytic activity using the potentiated startle paradigm. Current studies are investigating the mechanism of buspirone's action on this model system.

126.13 BUSPIRONE DIFFERENTIALLY AFFECTS HYPER-RESPONSIVENESS TO APOMORPHINE AFTER BILATERAL OR UNILATERAL 6 OHDA LESIONS IN RATS. A. Baduel*, L. Stinus* and M. Le Moal (SPON :S. Laroche). INSERM U-259 Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

The mechanism of action of buspirone (Bus) a potent non-benzodiazepine anxiolytic drug is unknown. However binding studies and studies which reveal neuroleptic-like properties suggest an interaction with DA neurons. Since Bus exerts only subtle effects on spontaneous or drug-induced behaviors in control rats, we studied its effects on hyper-responsiveness to apomorphine (Apo) after 6 OHDA lesions of DA neurons.

In experiment 1, rats received bilateral 6 OHDA lesions of either n. accumbens or striatum. After DA mesolimbic denervation, Apo (50µg/kg sc) induced locomotor hyperactivity. After DA nigrostriatal denervation, Apo (500µg/kg sc) induced intense stereotyped behavior. In both cases, the delay between lesion and test was critical for the effect of Bus : at 4 weeks post lesion, Bus (1mg/kg sc) reduced Apo-induced behaviors and at 8 weeks post lesion, Bus totally blocked both behaviors. In experiment 2 we studied Apo-induced (50µg/kg sc) contralateral turning after unilateral 6 OHDA lesions of lateral hypothalamus. Bus reduced turning (25%) at 2 weeks post-lesion while it enhanced turning (60%) at 7 weeks post-lesion.

The opposing results obtained after bilateral and unilateral lesions are difficult to reconcile but may be explained by complex differences in neuronal state occurring after the two types of lesion. Our results indicate that in addition to its acute anxiolytic effect, Bus must have other pharmacological properties which are revealed only on a pathological substrate. The delay needed to obtain a robust effect of Bus suggests that this drug is acting on long term disturbances and thus could be of therapeutic use in psychopathological disorders. Experiment 1 indicates a neuroleptic-like action of Bus, however it does not act as a DA receptor antagonist as indicated by experiment 2. It may be that Bus acts on neurons post-synaptic to DA receptors. The present results may provide a pharmacological model for understanding the mechanisms of action of Bus.

126.14 BUSPIRONE EFFECTS IN A PRIMATE MODEL OF EXTRAPYRAMIDAL MOTOR DYSFUNCTION. D.A. Downs, S.E. Harrigan*, and T.G. Heffner. Department of Pharmacology, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48105

Buspirone is a novel anxiolytic which lacks affinity for benzodiazepine receptors but appears to have both dopamine agonist and antagonist actions. Because drugs which alter central dopaminergic function can cause or attenuate extrapyramidal side-effects in man, we tested buspirone in a primate model of extrapyramidal dysfunction (EPD). Cebus monkeys were sensitized to the acute dyskinetic effects of haloperidol by repeated doses over several weekly intervals. In these sensitized monkeys, acute haloperidol and other neuroleptics but not clozapine reliably induced EPD characterized by tonic extension of the limbs, twisting of the neck or upper torso, protrusion of the tongue, chewing, biting, or sucking movements, circling or thrashing about the cage, and other dyskinesias or dystonias. When given approximately four hours after haloperidol, low doses of buspirone (0.3 mg/kg i.m.) reversed haloperidol-induced acute EPD. Apomorphine, atropine, and clozapine produced similar reversals of haloperidol-induced EPD. However, the same or higher doses of buspirone (10-32 mg/kg p.o., 1.0 mg/kg s.c., and 0.3 mg/kg i.m.) when given alone caused dose-related EPD in the haloperidol-sensitized monkeys. Although the quality and diversity of signs elicited by buspirone resembled those caused by haloperidol, they were generally of shorter duration. In addition, tremors, salivation, and marked depression were seen with buspirone but not with comparably active doses of haloperidol. Like haloperidol and other neuroleptics, buspirone caused EPD at approximately the same doses which blocked conditioned avoidance responding in non-sensitized cebus monkeys, and these buspirone-induced EPD were reversed by apomorphine and atropine. These effects of buspirone are consistent with mixed agonist and antagonist actions on dopaminergic neurotransmission, and indicate, to the extent that the primate model is predictive, that high doses of buspirone may cause haloperidol-like extrapyramidal side-effects (EPS) in man.

127.1 **THE MECHANISM OF ACTION OF MPTP AND MPP ON DOPAMINE RELEASE FROM THE RAT CORPUS STRIATUM AND SUBSTANTIA NIGRA SUPERFUSED IN VITRO.** G.D.Chang* and V.D.Ramirez (SPON:G.Davis). Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

The action of N-methyl-4-pheynl-1,2,3,6-tetrahydropyridine (MPTP) and N-methylphenylpyridine (MPP) on dopamine (DA) metabolism was examined in the rat corpus striatum (CS) and substantia nigra (SN) using an in vitro superfusion system. Adult male rats were killed by decapitation between 10:00 and 10:40. The corpus striatum from both sides of the brain of one animal was dissected out and superfused with Krebs-Ringer Phosphate medium, pH 7.4 containing 0.18% glucose (KRP). For the study with SN, tissue from three animals was used for each superfusion chamber. Following a 30-min stabilization period, perfusate samples were collected on ice for seventeen 15-min intervals. For the experimental groups, 10^{-6}M and 10^{-5}M of MPP or 10^{-5} and 10^{-4} of MPTP was administered for 45 min starting from interval four. All groups received a 15 min infusion of 30 mM K^+ in KRP at interval 13. Twenty µl of untreated perfusate samples were then subjected to HPLC-EC analysis of DA and 3,4-dihydroxyphenylacetic acid (DOPAC).

The rate of DA release from control CS decreased significantly during the first two hours of superfusion and then reached a stable release rate of about 3.0 pg/mg/min while DOPAC release rate retained a constant value of 9.5 pg/mg/min one hour after superfusion. MPTP, at 10^{-5}M and 10^{-4}M, suppressed DOPAC release rate to 50 and 25% that of control, respectively. In addition, at 10^{-4}M, MPTP caused an increased in DA release from rat CS (7.04±1.31 pg/mg/min vs 2.93±0.86 pg/mg/min at interval 10). On the other hand, at 10^{-6}M and 10^{-5}M, MPP exerted very potent and rapid inhibition of DOPAC output and stimulated DA release in a dose-dependent manner. The peak DA release was noted 45 min following 10^{-5}M MPP (44.99±17.74 pg/mg/min vs 7.21±3.23 pg/mg/min) and 75 min following 10^{-6}M MPP (12.89±4.72 pg/mg/min vs 2.93±0.86 pg/mg/min). Furthermore, pretreatment of MPP markedly potentiated K^+-stimulated DA release from CS.

The release rates of endogenous DA and DOPAC from SN were much lower than those from CS. Unfortunately, 60 min after superfusion, output of DA from SN became barely detectable in control group. However, with 10^{-5}M MPP infused for 45 min, a significant increase in DA release was evoked (2.60±0.58 ph/mg/min at interval seven vs 0.64 pg/mg/min the lowest detectable value). Interestingly, DOPAC release from SN was not affected by 10^{-5}M MPP and the DOPAC release profiles from control and MPP groups were superimposable. The possible mechanism of action of these two drugs on DA metabolism in nigrostriatal neurons will be discussed.

127.2 EFFECTS OF 1-METHYL-4-PHENYL-PYRIDINIUM ON MONOAMINE OXIDASE AND ON NIGROSTRIATAL DOPAMINE NEURONS. M. L. Leavitt*, M. L. Gittings*, S. K. Hemrick-Luecke*, D. W. Robertson* and R. W. Fuller (SPON: W. E. Hoffman). Biomed. Sci. Dept., Southwest Missouri State Univ., Springfield, MO 65804 and Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

1-Methyl-4-phenyl-pyridinium (MPP+) is a metabolite of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) that has been implicated in the neurotoxic effects of MPTP on nigrostriatal dopamine neurons (Chiba et al, Biochem. Biophys. Res. Commun. 120:574, 1984). MPP+ iodide was synthesized, and some of its in vitro and in vivo effects were compared to those of MPTP.

MPP+, like MPTP, inhibited rat brain mitochondrial monoamine oxidase (MAO) in vitro. Like MPTP, MPP+ was a competitive inhibitor of MAO-A and a noncompetitive inhibitor of MAO-B. MPP+ (Ki = 2 µM) was more potent than MPTP (Ki = 9 µM) as an inhibitor of MAO-A, but was less potent than MPTP as an inhibitor of MAO-B (Ki values were 370 and 106 µM, respectively).

In mice, MPTP HCl injected systemically (20 mg/kg s.c. daily for 4 days) resulted in marked depletion of striatal dopamine, DOPAC (3,4-dihydroxyphenylacetic acid) and HVA (homovanillic acid) one week after the last dose. MPP+ iodide injected in the same way was lethal to 5 of 8 mice. In the 3 surviving mice and in mice treated in the same way with 10 mg/kg of MPP+, a nonlethal dose, there was no depletion of striatal dopamine at one week. The ineffectiveness of MPP+ probably indicates the inability of the quaternary nitrogen compound to cross the blood-brain barrier.

MPP+ was administered intranigrally to male Sprague-Dawley rats. Stainless steel guide cannula were chronically implanted with the tip extending 1 mm dorsal to the substantia nigra pars compacta. Following recovery, rats were infused daily for 3 days with either 10 µg MPP+ iodide or saline (5 µl) via an injector cannula that projected 1 mm beyond the guide. On the fifth day after intranigral microinjection, rats were observed subsequent to treatment with the indirect dopamine agonist, d-amphetamine (5 mg/kg). Two days later, striata from both sides of the brain were removed for analysis. Behavioral effects including contralateral circling were observed acutely after MPP+ administration. Amphetamine induced ipsilateral rotation. Marked reductions in the striatal concentrations of dopamine (55%), DOPAC (50%) and HVA (40%) were found in ipsilateral vs. contralateral striata.

The data suggest that MPP+ can be neurotoxic to nigrostriatal dopamine neurons and that MPTP administration may be a means of introducing MPP+ into the region of these neurons. MPP+ may release dopamine and inhibit its oxidation by MAO-A, but there is no direct evidence that these actions are essential to the neurotoxic effect of MPP+.

127.3 MPTP ALTERS BOTH MESO-LIMBIC AND NIGROSTRIATAL DOPAMINERGIC SYSTEMS IN MICE. M.Gupta, S.Y.Felten and D.L.Felten. Dept. of Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

N-Methyl-4-Phenyl-1,2,3,6-tetrahydropyridine(MPTP) alters nigrostriatal dopamine neurons in humans, monkeys, and mice. Some reports suggest that MPTP also affects other monoaminergic systems, perhaps paralleling well established neurochemical changes reported in humans with Parkinson's disease. The present study was undertaken to investigate the extent to which MPTP alters other monoaminergic systems in addition to the nigrostriatal dopamine system. Male Swiss-Webster mice were injected intraperitoneally with MPTP at doses of 3, 30 or 60mg/kg body weight for three consecutive days. Control animals received vehicle injections. All mice were sacrificed by decapitation 21 days following the last injection. The brains were removed and microdissected to permit neurochemical and neuroanatomical examination of individual nuclei containing cell bodies and terminal projections from nigrostriatal and meso-limbic dopaminergic systems, from noradrenergic systems, and from serotonergic systems. Dopamine, norepinephrine, epinephrine, and serotonin levels from these microdissected regions were analysed using high-performance liquid chromatography with electrochemical detection (LCEC). MPTP treatment depleted the nigrostriatal system of its dopamine content in a dose dependent manner. In addition, high dose treatment with MPTP also depleted dopamine content in nucleus accumbens and olfactory tubercle, the major terminal fields for the meso-limbic dopamine projection from the ventral tegmental area. However, dopamine levels in mediobasal hypothalamus were unaltered with MPTP treatment at all doses. Additional alterations in serotonin levels were found in specific nuclei. These findings suggest that both the nigrostriatal and meso-limbic dopamine systems are susceptible to toxic damage from MPTP administration. Since both of these dopaminergic systems show degenerative changes in humans with Parkinson's disease, we suggest that MPTP treatment in mice may provide a good animal model for investigating neurodegenerative phenomenon and potential therapeutic approaches that may apply to Parkinson's disease.

127.4 CHRONIC TREATMENT OF MICE WITH 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) RESULTS IN A DECREASE OF RETINAL DOPAMINE AND THE APPEARANCE OF LIPOFUSCHIN-LIKE MATERIAL.
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Retina catecholamines were studied in mice treated with MPTP by histofluorescence and HPLC. After treatment with MPTP, 10 mg/kg IP, two doses 16 hr apart and killing 4 hr after the second dose, there was a significant rise of dopamine content and a fall of its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC). Consistent with the biochemical findings, catecholamine fluorescence was considerably brighter in the cell bodies and neuronal processes indicating an increase of the amacrine cell content of dopamine.

After chronic treatment with MPTP, 30 mg/kg IP for 7 days and killing on the 8th day, there was a significant decrease of dopamine and DOPAC content of the retina. Histochemically there was a dramatic reduction in the number of fluorescent amacrine cells and neuronal processes. Although the number of catecholamine amacrine cells were reduced, those present were brightly fluorescent and well defined. Interestingly, there was a profusion of bright yellowish-orange lipofuschin-like granules of various size not found with acute MPTP treatment. These granules were located in a plane much deeper in the retina than the catecholaminergic amacrine cells, presumably in the pigment epithelium. The pronounced increase of the autofluorescent lipofuschin-like pigment in the retina with chronic MPTP treatment might be indicative of premature aging of the retina. Indeed, chronic MPTP treatment might be useful model for studying the biochemistry of neuronal aging.

127.5 STRUCTURE-ACTIVITY STUDY ON THE MECHANISM OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)-INDUCED NEUROTOXICITY. S.K. Youngster*, W.J. Nicklas and R.E. Heikkila, Dept. of Neurology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

MPTP selectively destroys nigrostriatal dopaminergic neurons in monkeys and in mice. The 1-methyl-4-phenyl-pyridinium ion (MPP^+) is the predominant metabolite of MPTP in both of these species. Inhibitors of monoamine oxidase-B (MAO-B) including deprenil prevent MPP^+ formation from MPTP, and MPTP-induced neurotoxicity (Markey, S.P., et al. Nature, 311:464, 1984; Heikkila, R.E., et al. Nature, 311:467, 1984). MPP^+ has a high affinity for the dopamine uptake system while MPTP has a much lower affinity. Furthermore, the dopamine uptake inhibitor mazindol blocks both the neostriatal uptake of MPP^+ (Javitch, J.A., et al. Proc. Natl. Acad. Sci. USA, 82:2173, 1985) and MPTP-induced neurotoxicity in mice. In addition, the stereotaxic injection of MPP^+ into the nigrostriatal pathway of rats causes destruction of dopamine neurons and MPP^+, but not MPTP, inhibits NADH-linked respiration in mitochondria. These findings, taken together, are consistent with the following mechanism for MPTP-induced neurotoxicity: MPTP is taken up by MAO-B containing cells (perhaps glial cells) and oxidized to MPP^+, which is then released. MPP^+ is subsequently accumulated by dopamine neurons, and reaches concentrations sufficient to inhibit mitochondrial respiration, which results in the death of dopaminergic neurons. To test this possible mechanism, we have synthesized analogs of MPTP and MPP^+ and studied their effects on dopamine uptake, their capacity to be oxidized by MAO, their capacity to inhibit mitochondrial oxidation, as well as their ability to damage striatal dopamine neurons. For example, we find that 1-methyl-4-cyclohexyl-1,2,3,6-tetrahydropyridine (MCTP) is a comparable substrate to MPTP for MAO. 1-Methyl-4-cyclohexyl pyridinium (MCP^+), the MAO oxidation product of MCTP, is, like MPP^+, a good substrate for the dopamine transport system and capable of inhibiting NADH-linked respiration in mitochondria. MCTP injected i.p. into mice produced reductions in dopamine and dopamine metabolite levels in the neostriatum as did MPTP. In contrast, although 1-methyl-4-benzyl-1,2,3,6-tetrahydropyridine (MBzTP) is a good substrate for MAO and its oxidation product 1-methyl-4-benzyl-pyridinium ($MBzP^+$) is capable of inhibiting mitochondrial respiration, $MBzP^+$ is not a good substrate for the dopamine carrier and MBzTP is not toxic to dopamine neurons in vivo. Only compounds capable of being oxidized by MAO to a pyridinium ion which is a substrate for the dopamine carrier and is capable of inhibiting mitochondrial respiration were found to be toxic in vivo. In conclusion, it appears as if all three of these above parameters are required for a compound to exhibit MPTP-like neurotoxicity.

127.6 PROTECTION AGAINST 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) NEUROTOXICITY WITH DOPAMINE UPTAKE INHIBITORS. R.A. Mayer* and R.E. Heikkila, Dept. of Neurology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

There is considerable evidence that the neurotoxicity of MPTP depends upon its metabolism by monoamine oxidase B to 1-methyl-4-phenylpyridine (MPP^+). MPP^+ is actively taken up by dopaminergic neurons, while MPTP is only weakly transported (Javitch, J.A., et al. Proc. Natl. Acad. Sci. USA, 82:2173, 1985). Moreover, MPP^+, but not MPTP, inhibits NADH-linked respiration in mitochondria. Based on these observations, it is possible that MPTP is accumulated by monoamine oxidase B-containing cells outside of the dopaminergic neuron and oxidized by MAO-B to MPP^+. After its release, MPP^+ is actively accumulated into dopaminergic neurons by the dopamine transport system and, once inside the cell, exerts its neurotoxic effect, perhaps through its action on mitochondria. The purpose of the present study was to examine the role of the dopamine transport system in MPTP-induced neurotoxicity. Specifically, we: 1) evaluated the in vitro release of ^3H-dopamine induced by MPP^+, 2) determined the degree to which a series of dopamine uptake inhibitors could prevent the MPP^+-induced release of ^3H-dopamine and 3) determined if compounds effective in blocking MPP^+-induced release might be able to prevent MPTP-induced neurotoxicity.

In tissue slices prepared from the neostriata of male Swiss-Webster (CF-W) or C57-black mice, MPP^+ caused a dose-dependent release of previously accumulated ^3H-dopamine. For example in CF-W mice, 2.5 μM MPP^+ caused a 31% release of ^3H-dopamine, and 10 μM MPP^+ a 57% release. We evaluated the capacity of several agents to prevent the ^3H-dopamine release induced by 10 μM MPP^+. The compounds tested included GBR-13069, amfonelic acid, mazindol, WIN-35,428, WIN-35,065-2, nomifensine, cocaine and desmethylimipramine. All of the drugs except desmethylimipramine, at 2.5 μM, significantly attenuated MPP^+-induced release. For example, MPP^+-induced release in the presence of mazindol was only 5%, compared to the 57% release induced by MPP^+ alone. Mazindol by itself, did not release ^3H-dopamine. In a representative in vivo experiment, MPTP, injected into C57 black mice at a dose of 30 mg/kg/day for 5 days, caused a 79% decrement in the neostriatal content of dopamine (2.5 μg/g compared to a control value of 11.8 μg/g). In contrast, mice treated with 10 mg/kg of mazindol 30 min prior to MPTP had a neostriatal dopamine content of 8.6 μg/g. Mazindol and other dopamine uptake inhibitors also protected against MPTP-induced toxicity in CF-W mice. These results, and other data on the effects of the uptake inhibitors on ^3H-MPP^+ accumulation, support the premise that the selective toxicity of MPTP is dependent upon the uptake of MPP^+ into dopaminergic neurons by the dopamine transport system.

127.7 MPP^+: BINDING AUTORADIOGRAPHY, REGIONAL UPTAKE, DOPAMINE NEUROTOXICITY, AND BEHAVIORAL PHARMACOLOGY FOLLOWING D2 PROLIFERATION. M. R. Marien, †R. E. Heikkila and C. A. Altar. Neurosci. Res., CIBA-GEIGY Corp., Summit, NJ 07901 and †Dept. Neurology, UMDNJ-Rutgers Medical School, Piscataway, NJ, 08854.

The dopaminergic neurotoxicity of MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been associated with its oxidation to MPP+ (N-methyl-4-phenylpyridine) (Markey et al, Nature 311:464, 1985). The autoradiography of [3H]MPP+ and the dopaminergic uptake, neurochemistry and behavioral effects of intracerebral MPP+ injection in the rat are presented here.

Quantitative autoradiography revealed a regional distribution of specific (MPP+-displaced) and saturable (Bmax = 138-965 fmol/mg protein) binding sites of high-affinity (Kd = 13-61 nM) for [3H]MPP+. Regions with the highest Bmax included nucleus accumbens (NA), interpeduncular nucleus, claustrum, subiculum/CA1 and lateral septum. Binding also occurred in the caudate-putamen (CP), VTA and SN. Binding was not displaced by benztropine (10 uM) nor was it reduced by dopamine neuron destruction with 6-hydroxydopamine. The Bmax values of [3H]MPP+ sites correlated ($r = +0.73$, $n = 11$, $p < 0.02$) with those of specific [3H]tryptamine binding sites (see L. L. Martin et al, This Volume).

Using the method of Javitch and Snyder (Eur. J. Pharmacol. 106:455,1985), we found that [3H]MPP+ was accumulated to about the same extent by homogenates of CP, NA and olfactory tubercle (OT) by a high-capacity (Vmax = 12.8-17.8 pmol/mg protein/8 min), benztropine-sensitive (75-77% blocked) mechanism. Injections of 10-25 ug MPP+ along the left medial forebrain bundle produced dose-dependent losses of DA (up to 95-98%) in the ipsilateral CP and NA. In these rats, no contralateral rotation to apomorphine (0.25 mg/kg) or carbidopa (20 mg/kg) was observed, but d-amphetamine (1.5 mg/kg) produced ipsiversive rotation. Quantitative autoradiography with [3H]spiroperidol showed that at 4 wk these lesions increased the Bmax of D2 sites in the CP by 42% without a Kd change and without affecting D2 sites in the NA. Whether disruption of descending pathways by MPP+ explains the inability of DA agonists to induce rotation and for the denervated NA to not proliferate D2 sites is under investigation.

We conclude that binding sites for MPP+ exist in rat brain. Consistent with MPP+ uptake into the CP, NA and OT and its ability to proliferate D2 sites in the CP, MPP+ is toxic to rat A9 and A10 neurons.

127.8 ^3H-MPTP BINDS TO NEUROGLIA IN THE RAT BRAIN. Arthur Hess, A. Bretschneider*, T. Sullivan* and P. J. Adamo*. Department of Anatomy, Rutgers Medical School, University of Medicine and Dentistry, Piscataway, NJ 08854.

MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) administration causes symptoms of Parkinson's disease in humans and degeneration of the dopaminergic nigrostriatal pathways in monkeys and mice.

Unilateral severe lesions of the medial forebrain bundle in rats produced by injections of MPP+ (1-methyl-4-phenylpyridinium iodide), a toxin produced after oxidation of MPTP by MAO B (monoamine oxidase B) (Markey, S. P. et al, Nature (Lond.), 311:464, 1984), resulted in drastic, virtually complete neuronal cell loss and massive gliosis in the zona compacta of the substantia nigra on the operated side. Histofluorescent studies revealed a severe loss of dopamine and radioautographic studies after in vitro incubation in ^3H-spiperone showed an increase in dopamine D2 receptors in the caudate nucleus on the operated side. Radioautographs after in vitro incubation in ^3H-MPTP revealed a substantial increase in binding of ^3H-MPTP in the zona compacta of the substantia nigra of the operated side, with minimal binding in the substantia nigra on the normal side. The large increase in ^3H-MPTP binding in areas with absence of neurons and intense proliferation of glia indicates that ^3H-MPTP binds largely, if not exclusively, to neuroglia.

It is suggested that MPTP is metabolized by glial cells and that the initial genesis of the toxic metabolites of MPTP occurs in neuroglia. The localization of MAO B in glial cells (Levitt, P. et al, Proc. natl. Acad. Sci. USA, 79:6385, 1982) allows MAO B to metabolize MPTP readily. Inhibition of MAO B in glia readily blocks metabolism of MPTP, also located in the same neuroglia, and probably accounts for the ability of MAO B inhibitors to block the toxic effects of MPTP on the catecholaminergic systems of the brain (Heikkila, R. E. et al, Life Sci., 36:231, 1985). The role of neuroglia should be considered further in attempts to understand the mechanism of MPTP-induced dopaminergic neurotoxicity.

The brain stem lesioned rats were provided by Dr. R. E. Heikkila and L. Manzino, Department of Neurology.

127.9 POSITRON EMISSION TOMOGRAPHY VISUALIZED BRAIN DAMAGE IN MPTP-INDUCED PARKINSONIAN MONKEYS BY USING FLUORINE-18 LABELED 6-F-DOPA. C. C. Chiueh, G. Firnau*, R. S. Burns*, C. Nahmias*, R. Chirakal*, I. J. Kopin, and E. S. Garnett*, NIMH/NINCDS, Bethesda, Maryland, 20205, U. S. A. and Department of Nuclear Medicine, McMaster Univ. Medical Centre, Hamilton, Ontario L8N 3Z5, Canada.

The degree of brain damage in patients suffering from Parkinson's disease depends upon clinical assessments and confirmation by postmortem examination. The object of the present study is to employ a newly developed positron emission tomographic (PET) procedure (Garnett et al., Nature, 305: 137, 1983) using a presynaptic ligand of 6-F-DOPA (Firnau et al., J. Nuclear Med., 25: 1228, 1984; Chiueh et al., Neurosci. Abs., 10: 883, 1984) to visualize and determine in vivo the degree of striatal damage in rhesus monkeys with MPTP-induced parkinsonism (Burns et al., Proc. Nat. Acad. Sci.,80: 4546, 1983). Such a PET procedure could prove useful for identifying patients with subclinical Parkinson's disease or other syndromes involving brain dopaminergic systems.

Following an intravenous injection of 1.5 to 2 mCi purified 6-^{18}F-DOPA (sp. act. 75 mCi/mmole), pentobarbital anesthetized monkeys were examined in the McMaster PET scanner (Nahmias, Nuclear Instru. Methods, 221:113, 1984). The total radioactivity attributed to 6-F-DOPA and its metabolites in a 17 mm horizontal brain slice along the orbitomeatal plane was measured for 2 to 3 hours. At various intervals, blood samples were drawn and assayed for 3-methylated 6-F-DOPA which was found to disappear within about 40 min. Each animal was sacrificed at the end of the experiment. Disc shape punches of tissue from the caudate nucleus and the putamen were assayed for dopamine and its biosynthetic enzymes.

By carefully titrating the MPTP dose (0.5 to 2.5 mg/kg i.v.), we were able to obtain monkeys suffering from different degrees of parkinsonism: subclinical, mildly affected and severely affected (Burns et al., Adv. Neurol., in press, 1985). L-DOPA therapy was required in animals which suffered more than 80% damage to the nigrostriatal system. Dopamine and its synthetic enzymes were absent from the striatum of the severely affected monkeys. In controls, the striatal ^{18}F-PET activity was maximum at 15 min and declined with a half-life of 5 hours. The turnover rate of ^{18}F activity was diminished 3 days after MPTP administration. Two to three weeks later, the accumulation of 6-^{18}F-DOPA and its major metabolite, 6-^{18}F-dopamine, was diminished in the striatum of the mildly affected monkey and absent in the severely affected animal. The striatal ^{18}F-dopamine activity calculated by PET in vivo correlated with the content of endogenous dopamine measured postmortem in each animal.

The present results suggest that the PET brain ^{18}F-dopamine imaging may be useful in providing an index of dopamine depletion in patients with Parkinson's disease and allied disorders.

ELECTROENCEPHALOGRAPHY AND EVOKED POTENTIALS

128.1 REFERENCE FREE CHARACTERIZATION OF EEG WAVEFORM SOURCES BY LAPLACIAN COMPUTED TOPOGRAPHIC MAPS. R. Coppola. Laboratory of Psychology and Psychopathology, NIMH, Bethesda, MD 20205

Computer color display capability has added to increased interest in the topographic mapping of scalp recorded EEG data. This interest comes in part from the desire to localize brain electrical activity with the accompanying hope that this activity can be correlated with neural function. Mapping requires that EEG be recorded from multiple electrode sites all referenced to the same point. Linked ears, a non-cephalic reference, or common average electrode have been among those used. The particular choice greatly affects the resulting map complicating the interpretation with respect to localization of acitivity. The source derivation method of Hjorth (Electroenceph. Clin. Neurophysiol., 50:282, 1980) has been suggested as a reference-free method. The source derivation was meant to be an approximation to the Laplacian of the potential field thus representing the current source density distribution. Most methods used to implement the derivation either require having electrodes placed in a special arrangement or are only a poor approximation using distance weighted values.

In order to achieve a better estimate to the Laplacian we have first developed a surface fit method to provide an accurate interpolation for potential values between electrodes. Each pixel in the display of the potential map is then treated as if it were an electrode and the Laplacian then computed on those values. This results in a very spatially sharpened source density map.

Maps of resting alpha rhythm activity computed in this manner reveal two distinct source locations in a normal population (N=30), one over parietal cortex and one over occipital cortex. Maps of abnormal EEG from epilepsy and tumor patients show source locations in very good agreement with clinical or surgical data relating to the underlying brain pathology.

128.2 Transluminal Intracranial Electroencephalography in Primates: An Endovascular Procedure for Localizing Temporal Lobe Epileptic Foci. J.M. Pile-Spellman*, B.B. Sandrew*, K. Baker*, G. Pransky*, S. Schachter*, N.T. Zervas and J.M. Taveras. Departments of Neuroradiology and Neurosurgery, Massachusetts General Hospital and Harvard Med. Sch., Boston, MA 02114.

The benefits of surgery for medically intractable epilepsy are denied most of the 175,000 Americans thus afflicted because of the difficulty in patient selection. While CT, MRI, and angiography may identify structural abnormalities related to the seizure activity, the electrophysiological assessment of patients with medically intractable complex partial epilepsy remains the critical data indicating surgery. A majority of patients considered for surgery require surgically implanted chronic depth electrodes. The inherent problems of such recording procedures prompted the development of a less invasive technique for localizing and evaluating epileptic foci.

In 4 primates, via a transcutaneous femoral approach, selective cerebral angiograms were performed. Using a 3.5 Fr. coaxial system, a specially designed 450um electrode was then passed into the intracranial vessels and electrically induced paroxysmal activity in the temporal lobe was successfully recorded. In each case, the transluminal intracranial electrode (TIE) recordings were of high fidelity, with little artifact in wither the ictal or interictal states. Of particular significance, TIE could be repeated several times in each animal without complication, consistently producing records superior to the dural electrodes and comparable to the depth electrodes in the same animal.

128.3 NEUROTRANSMITTER INFLUENCES ON HUMAN EVOKED POTENTIALS. E.J. Hammond*, B.J. WILDER, Neurology Service, Veterans Administration Medical Center, Gainesville, Florida 32602.

Although much work has been done on the localization of various components of human evoked potentials, almost nothing is known about their biochemical basis. We studied the effects of manipulation of CNS acetylcholine, a widespread excitatory neurotransmitter, and gamma aminobutyric acid (GABA), an ubiquitous inhibitory neurotransmitter, on click-evoked human auditory brainstem and pattern reversal visual cortical evoked potentials (check size: 50 min). We administered scopolamine, a centrally acting acetylcholine antagoinst, to 4 subjects in a physiologically active dose (0.4 mg i.m.) and made serial evoked recordings for 6 hrs. There was no change in either latency or amplitude for auditory wave I, II, III, IV, V or visual N70, P100 or N145 components. In contrast, a marked influence on the auditory cognitive P3 potential has been reported (Hammond et al, in press).

To study GABAergic effects, gamma vinyl GABA, a new anticonvulsant drug, which is a specific inhibitor of the GABA catabolizing enzyme GABA-T, was administered to 15 patients in a centrally acting dose (4 gm/day). Evoked responses were measured at monthly and bimonthly intervals for 1 yr, but no change in auditory brainstem or cortical visual evoked potentials could be detected.

These studies indicate a lack of effect of cholinergic and GABAergic manipulation on auditory brainstem and pattern reversal visual cortical evoked potentials.

128.4 FOCAL LESIONS OF VISUAL CORTEX: EFFECTS ON FLASH EVOKED POTENTIALS (FEPS),PAIRED FEP RECOVERY FUNCTIONS, AND PATTERN REVERSAL EVOKED POTENTIALS (PREPS) IN RATS. R.S. Dyer, K.F. Jensen and W.K. Boyes Neurophysiol. Branch, NTD, HERL, USEPA Research Triangle Park, NC 27711

FEPs, FEP recovery functions, and PREPs are used as indicators of visual system neurotoxicity, yet little is known about factors which alter specific peaks or the rate of recovery of these peaks in a paired stimulation paradigm. In this study we investigated the role of heat-induced focal destruction of cortical tissue in the vicinity of the electrode.

Adult male Long-Evans hooded rats (N=40) were implanted with chronic 00-90 screw electrodes at the following locations: ground - 2 mm anterior to bregma, 2 mm left of midline; reference - 2 mm anterior to bregma, 2 mm right of midline; visual cortex - 6 mm posterior to bregma, 4 mm on either side of the midline. All electrodes were connected by nichrome wires to a headplug. Visual cortex electrodes on the left side of the brain had, at the time of surgery, a hot soldering iron applied to them for about 0.5 sec, while those on the right side had no such treatment. One week later the rats were tested unanesthetized. For FEPs, averaged responses to 64 flashes were obtained simultaneously at the two visual cortex electrode sites for each animal. Similarly, averaged responses to 64 paired flashes were obtained at the two sites for interflash intervals of 200, 400, 600 and 800 msec, and the recovery function was constructed from ratios of second flash amplitude to first flash amplitude. For PREPs, averaged responses to 200 reversals of a square wave grating were obtained.

The FEP results indicated that all but the last negative peak (N3) were affected by the lesion. P1 was virtually eliminated by the lesion, and N2 amplitude was greatly reduced. N1 was enhanced by the lesion. The group average waveforms of the recovery function portion of the experiment indicated that recovery of P1N1 was greatly enhanced by the lesion, suggesting partial elimination of a normal recurrent inhibitory process. In the PREP recordings the group average waveforms indicated that N1 amplitude was enhanced by the lesion, but that N3 amplitude was depressed. Preliminary histological examination indicated that surface focal damage occurred in about a 1-1.5 mm radius and extended to a cortical depth as great as 1.4mm at the center. These findings demonstrate that while visual evoked potentials recorded from skull screw electrodes reflect activity from surrounding cortex as well as the cortex immediately beneath the electrode, damage to the immediately underlying cortex significantly alters waveform morphologies.

128.5 MIDLATENCY AUDITORY EVOKED RESPONSES: DIFFERENTIAL RECOVERY CYCLE CHARACTERISTICS. R. Erwin and J. Buchwald, Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

Recent studies have indicated that two middle latency response (MLR) components of the human auditory evoked potential, Pa (20-40 ms latency) and P1 (50-80 ms), may be analogous to the cat Wave 7 (12-15 ms) and Wave A (20-25 ms) respectively. Depth mapping and lesion procedures indicate that the cat Wave 7 reflects a generator in auditory cortex while the cat Wave A reflects a generator system extending through the mesencephalic reticular formation upward into the intralaminar thalamus (Hinman and Buchwald, Brain Res. 264: 573, 1983). Both the cat Wave A (Chen et. al., Neurosci. Abst. 10: 1141, 1984) and the human P1 (Erwin et. al., Neurosci. Abst. 10:1140, 1984) diminish and disappear during slow wave sleep, as compared to wakefulness, and reappear during active, rapid eye movement sleep. In contrast, the earlier auditory brainstem reponses (ABRs), cat Wave 7 and human Pa, do not vary in amplitude across states of sleep and wakefulness.

The present study attempted to extend these parallels by using a different experimental manipulation. In the cat the amplitudes of the ABRs and of Wave 7 do not change as rates of stimulation increase from 1 to 10/sec. In contrast, wave A disappears across this range of increasing rates (Buchwald et. al., Brain Res. 205: 91, 1981). Since the human Pa and P1 components have not been examined simultaneously for possible differential effects of stimulus rate, a parametric study of stimulus rate on waveform amplitudes was undertaken.

MLRs were recorded from a group of 7 normal adults in response to clicks (0.1 ms, 50 db HL) presented at 5 rates: 1/2 sec, 1/sec, 5/sec, 8/sec and 10/sec. Evoked potentials were recorded from the vertex (Cz) and bipolar eye electrodes were used to monitor eye movements. For each block of 500 stimuli at one rate, an evoked response average was generated.

Analyses of the MLR amplitudes indicated that the Pa amplitude did not vary systematically across rates, while the P1 amplitude decreased significantly at faster stimulation rate ($F(4,24)=10.21$, $p< .001$). These findings indicate that P1 is generated by a system which is physiologically different than the Pa generator system, i.e., the P1 system shows significantly longer recovery cycles. The similar recovery cycles characteristics responses of human Pa and cat Wave 7, and of the human P1 and cat Wave A across different stimulation rates provides additional evidence to support the hypothesis that these pairs of human-cat components reflect common generator substrates. (Supported by USPHS HD-05958 and HD-04612)

128.6 EFFECT OF A UNILATERAL PONTINE LESION ON HUMAN BRAINSTEM AUDITORY EVOKED POTENTIAL. D.H. York and C. Watts, Dept. of Physiology and Neurology, and Division of Neurosurgery School of Medicine, University of Missouri, Columbia, Mo. 65212.

The extensive crossing of auditory fibers at various levels through the brainstem and midbrain is frequently cited as the reason for the lack of detection of a unilateral brain lesion, especially in audiometric testing. This study describes a predominantly ipsilateral deficit observed in the brainstem auditory evoked potential (BAER) of an individual with brainstem infarct confined to one side of the dorsolateral pontine-midbrain region. The patient was a 21 year old male involved in a motor vehicle accident who was found unconscious at the scene and required ventilatory assistance. Pupils were equal and responsive to light upon admission. A BAER evaluation was done at 90 dB with 60 dB masking of opposite ear at gain of 10^4, bandpass 150-3000 Hz. The vertex (Cz) response to 2000 click stimuli at 17/sec showed only the presence of waves I, II and III on left ear testing. The latencies were 1.5, 2.7 and 3.7 respectively. The opposite ear showed the presence of waves I, II, III, IV, V with wave I at 1.6 msec, wave II at 2.8 msec, wave III at 3.7 msec, wave IV at 4.8 msec and wave V at 6.2 msec. The patient remained comatose for 39 days, whereupon he expired due to cardio-pulmonary failure. Brain pathology showed a localized infarct in the left dorsal mid-pons extending into the midbrain up to the substantia nigra. The lesion obliterated the lateral lemniscus on the left side. The presence of wave I-V at normal latencies on right ear testing suggests that crossing fibers in the trapezoid body may not play a major role in conducting click evoked responses. A decrease in amplitude of wave V on right ear test suggests only minimal involvement. The complete abolition of waves IV-V on left ear testing, ipsilateral to the lesion suggests that ipsilateral fibers of the lateral lemniscus are important in conducting auditory information evoked by click stimuli and may offer evidence of localizing value in lesions of the brainstem.

128.7 THE BRAINSTEM AUDITORY PATHWAY IN CAT AND MAN. J. K. Moore. Dept. of Anat. Sci., SUNY at Stony Brook, Stony Brook, NY 11794.

Interpretation of early auditory evoked potentials has been based on a combination of human clinical cases and experimental studies in mammals such as the cat. For this reason, cat and human brainstems have been subjected to identical histological processing, and stained sections have been used to produce graphic and computer-assisted reconstructions of the brainstem auditory pathway. The two- and three-dimensional images produced by these reconstructions provide quantitative information on the nuclei and tracts which are the neural generators of the early evoked potentials.

The human cochlear nuclei are more elongated than those of the cat, extending up to 8mm dorsally and 10mm medially in the lateral recess from the point of entry of the auditory nerve into the nuclei. Commissural axons from the nuclei converge to form a compact trapezoid body at the posterior edge of the pons. Other efferent axons from the nuclei run rostromedially in the acoustic striae and trapezoid body toward the ipsilateral superior olivary complex. In the human olivary complex, only one of the three principal nuclei identified in other mammals is well developed. This nucleus, the medial olivary nucleus, is a sheeo of bipolar neurons which extends 4mm rostrocaudally. A small lateral olivary nucleus is identifiable, and the complex is ringed by periolivary cell groups. The elongated rostral pole of the complex contains globular neurons which resemble cells of the nucleus of the trapezoid body in other species. Overall length of the human olivary complex is 13mm, compared to 4mm in the cat. Rostral to the olivary complex, the lateral lemniscus contains a ventral nucleus consisting of islands of small fusiform cells. This nucleus, 5mm in length, is longer than that of the cat, but cell density is less. A dorsal lemniscal nucleus, very similar in size to that of the cat, gives rise to apparent commissural axons.

In terms of overall length of the brainstem auditory pathway, an axon(or series of axons) passing from the cochlear nuclei to the centra of the opposite inferior colliculus travels a distance of 24mm in the cat, but courses approximately 42mm in man. Given the size of neuronal populations in human auditory nuclei, the largest component of the ascending pathwya in man should be direct axons from multipolar and fusiform cells of the cochlear nuclei to the contralateral inferior colliculus. A smaller, but still sizeable, component of the system should be cochlear nucleus spherical cells projecting through the medial olivary nuclei to the colliculus bilaterally. (Supported by the Deafness Research Foundation).

128.8 SOURCES OF THE FREQUENCY FOLLOWING RESPONSE IN MAN: AN ANALYSIS USING PHASE. R. Batra and S. Kuwada. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

Electrical potentials which follow the periodicity of low frequency tones can be recorded from the scalp of both animals and humans. Previous studies indicate that the source of this frequency following response (FFR) is from multiple generator sites. We demonstrate here that the FFR has at least two sources in man. Signals from these sources sum in different proportions depending on electrode configuration.

Continuous tones were used instead of the more traditional tone bursts as data can be collected more efficiently using the former. Moreover, continuous tones avoid transient responses produced by tone bursts which complicate the response pattern. Stimuli were delivered monaurally at 95 dB SPL via an electrically and magnetically shielded headphone. Potentials were differentially recorded between vertex and either ipsilateral or contralateral earlobe. They were amplified, averaged and assessed using Fourier analysis. Presence of a response was determined using a statistical criterion derived from background noise.

Responses recorded between vertex and ipsilateral earlobe exhibit the presence of at least two generators. Both amplitude and phase measurements show a sequence of peaks and valleys as a function of frequency. Responses above 600 Hz are nearly sinusoidal while those at lower frequencies have substantial harmonic content indicating a neural origin. In contrast, amplitudes of responses measured between vertex and the contralateral earlobe increase up to about 200 Hz and then decline. Responses are rarely seen above 600 Hz. The phase of responses below 300 Hz changes linearly as a function of frequency, implying a source with a latency of 8.2 ± 0.1 ms (mean ± standard deviation, 4 subjects). Phase analysis indicates that the FFR is generated by interference between at least two sources summing in different proportions when different electrode configurations are used. One has a latency of 0.25 to 2 ms and dominates at high frequencies while the other has a latency of about 8 ms and produces most of the response at lower frequencies. The former is consistent with a cochlear origin while the latter probably lies in the midbrain.

These results clearly demonstrate that the FFR recorded in man has at least two sources, the signals from which either sum or cancel one another depending on frequency. An implication for those using the FFR is the necessity of measuring the response at several closely spaced frequencies in order to identify these extremes. The frequencies at which such extremes occur and the invariant latency of the neural generator seen while recording between vertex and the contralateral earlobe may be useful for clinical diagnosis.

This work was supported by a NIH grant (NS18027, S. Kuwada) and the University of Connecticut Research Foundation (G.Leonard).

128.9 SCALP-RECORDED POTENTIALS TO AMPLITUDE MODULATED TONES: A METHOD FOR ASSESSING HEARING. S. Kuwada, R. Batra and V.L. Maher*. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

The early detection of hearing abnormalities is desirable so that remedial procedures may be implemented. The goal of electrical audiometry is to assess hearing in those unable or unwilling to participate in standard behavioral audiometric testing, such as infants and the mentally retarded. Most techniques involve the analysis of scalp-recorded potentials evoked by clicks or tone bursts. To date, the most fruitful approaches have been the analysis of short latency brainstem evoked responses, the frequency following response, and the middle latency response. However, none of these methods alone or in combination can assess frequency-specific hearing thresholds across the full audiometric range (250-8000 Hz). We report here that sinusoidally amplitude modulated (sine AM) tones evoke scalp-recorded responses which can be used to assess the sensitivities of hearing-impaired subjects on a frequency by frequency basis, across the whole audiometric range.

We tested adults with normal hearing as well as those with low, high and mid-frequency hearing losses. Sine AM tones were presented through an electrically and magnetically shielded headphone. The scalp-potentials were differentially amplified between vertex and the contralateral earlobe, averaged, and digitally filtered. A fast Fourier transform was used to measure the amplitude and phase of the response.

The largest responses were seen to modulation frequencies in the 30-50 Hz range, although potentials were detected as high as 400 Hz. The phase of the response from normal subjects indicates that the generator for this following potential has a latency of about 30 ms at modulation frequencies below about 70 Hz. At higher frequencies the latency is about 7 ms. In general, responses to 50 Hz amplitude modulation increased as a function of intensity and declined as a function of carrier frequency. In hearing impaired subjects, responses were recorded to a 50 Hz modulation at carrier frequencies between 250 and 8000 Hz, usually at intensities between 50 and 60 dB HL. In such subjects, there was a close correspondence between the amplitude of the 50 Hz potential and the subject's behavioral audiogram. At higher intensities this correspondence was not as close, presumably due to the spread of excitation along the basilar membrane. As the threshold for this following response is close to that of hearing, we were also able to determine absolute levels of hearing loss at each of the frequencies tested. Thus, it seems that scalp potentials to sine AM tones can be used to accurately evaluate hearing sensitivities on a frequency by frequency basis across the audiometric range.

This work was supported by a NIH grant (NS18027, S. Kuwada) and the University of Connecticut Research Foundation (G. Leonard).

128.10 Inversion of the Scalp-Recorded P300 in a Tumor Patient R. Johnson, Jr. and P. Fedio. National Institutes of Health, NINCDS/MNB, Building 10, Room 4N246, Bethesda, MD 20205.

In recent years, much effort has been expended in attempts to localize the neural generator of the P300 component of the event-related potential (ERP). Whereas the initial results placed the P300 generator in the amygdala, pes, uncus and anterior hippocampal areas, more recent work has demonstrated that these sources do not appear to contribute to the activity of the scalp-recorded P300. Despite the fact that a given scalp distribution cannot uniquely determine the sources of a particular ERP component, it does provide important information which must be accounted for by any proposed source. Therefore, if the scalp distribution of P300 is altered in either surgical or tumor cases, such data can provide constraints on the number of backward solutions for testing hypotheses about source localization.

The subject (S.M.) was a 34 year old right-handed white male with 15 years of education and an above average IQ. He had a papillary tumor located in the right lateral ventricle. The standard "Oddball" task, with 20/80 stimuli, was used to elicit the P300. The stimuli were presented in three modalities (auditory, visual, and somatosensory) either bilaterally or unilaterally in different series. The subject's task was to press one of two buttons depending upon which stimulus was presented. The EEG was recorded from FPz, Fz, Cz, Pz, Oz, F3, F4, C3, C4, P3, P4, O1, O2, T3, and T4, all referred to linked mastoid electrodes.

The averaged waveforms revealed that normal looking ERP components were elicited in all three modalities except for the P300 component. Although larger potentials were elicited by rare events than by the frequent events, the overall scalp distribution of P300 in S.M. was different from that usually observed. Thus, while P300 was distributed normally over the central and posterior areas of the scalp, P300 amplitude was notably smaller than usual at the F_z electrode. Most surprising was the activity at the FPz electrode site where the P300 component was completely inverted (i.e., negative going) relative to more posterior electrode sites. This phase reversal of the P300 at FP_z was in clear contrast to the normal polarities for all of the earlier components at the same electrode. These results were obtained in all three modalities, for both bilaterally and unilaterally presented stimuli. There were no hemispheric asymmetries in any of the ERPs which were associated with the presence of the tumor.

The data from this subject represent the first reported scalp-recorded polarity reversal of the P300 component. Although these data alone are not sufficient to specify completely the generator of P300, they do provide constraints which must be accounted for by any hypothesized generators.

128.11 THE EFFECT OF ALTERATION OF VASCULAR FACTORS ON RECOVERY OF SPINAL CORD CONDUCTION AFTER TRAUMA IN RATS. H. F. Martin III, J. G. Blackburn, and S. Katz. Dept. of Physiology, Medical University of S. C., Charleston, S. C. 29425.

Rats were anesthetized with pentobarbital and ketamine for surgical preparation then maintained by supplemental ketamine alone. Somatosensory evoked potentials (SEPs) were recorded bilaterally from stimulation of sciatic nerves, before and after calibrated spinal cord trauma. SEPs were monitored at regular intervals during 4-6 hours of acute recovery. At 4-8 g-cm trauma, SEPs fully recovered following initial loss. With 10-16 g-cm trauma, slow recovery occurred over a 2-4 hour period, frequently terminated by a secondary loss at 3½-5 hours. With 20-24 g-cm trauma, most animals had no recovery of SEPs. Since 24 g-cm is just above the level of variable recovery in untreated animals, this was used in subsequent trials. A set of untreated animals had recovered only 16% of pre-trauma SEP at 3½ hours post-trauma.

In order to reduce the contribution of the formed elements of the blood to vascular reactions after trauma, animals were transfused with perfluorocarbon emulsion (Oxyferol, Alpha Therapeutics) to Hct.<10%. This resulted in 54% SEP recovery at 3½ hours ($p<.002$). To block participation of prostaglandins in vascular response, animals were pretreated with the cyclooxygenase inhibitor Ibuprophen (Upjohn, 15 mg/kg). This resulted in 32% SEP recovery at 3½ hours followed by secondary loss. To block the vasoconstrictor and clotting effects of thromboxane A_2, animals were pretreated with the thromboxane synthetase inhibitor Dazoxiben (Pfizer, 30 mg/kg). This resulted in 60% SEP recovery at 3½ hours ($p<.005$). In order to increase blood volume and decrease viscosity, animals were transfused with 4cc of 10% Dextran-40. This hypervolemic hemodilution resulted in 90% SEP recovery at 3½ hours ($p<.001$). Identical hypervolemic hemodilution with normal saline produced no recovery.

These results suggest that alterations in hemodynamic factors play a role in recovery of spinal cord conduction after trauma. Also prostaglandins, especially thromboxane, may mediate part of the post-traumatic response. Current studies are under way to assess the role of other agents and correlate changes in spinal cord blood flow.

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DISEASES OF THE NERVOUS SYSTEM: ISCHEMIA, TRAUMA

129.1 PRETREATMENT WITH SCOPOLAMINE ACCELERATES RECOVERY OF LOCOMOTOR FUNCTIONING FOLLOWING CEREBRAL CONCUSSION IN THE RAT. C. E. Dixon, B. G. Lyeth, M. L. Giebel,* T. Yamamoto,* H. H. Stonnington,* D. P. Becker and R. L. Hayes. Division of Neurosurgery, Department of Psychology and Department of Rehabilitation Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0001.

Recent data suggest that increased functional activity of cholinergic systems located in the brain stem contributes to components of behavioral suppression following concussion (Hayes et al., Science, 233:301-303, 1984). Additionally, pretreatment with scopolamine has been shown to attenuate some acute (<60 min) neurological deficits following cerebral concussion produced by fluid percussive techniques (Lyeth et al., Neurosci. Abst., 1985). The present investigation examined the effects of pretreatment of scopolamine, a muscarinic, cholinergic antagonist, on the rate of recovery of locomotor functioning following concussion.

Prior to injury, 40 rats were trained on a beam-walking task similar to that employed by Feeney et al. (Science, 217:855-857, 1982) in which rats traversed a narrow beam to escape white noise and bright light. Baseline intervals to traverse the walking beam were obtained. Additionally, the rats' ability to traverse the beam was assessed by a rating scale (e.g. number of foot slips) made by independent observers. The interval to maintain balance on a separate balance beam task was also assessed prior to injury. Following injury rats were retested daily for 10 days. Rats were surgically prepared 24 hours prior to injury by chronically placing a hollow injury screw epidurally over a hole trephined along the sagittal suture, midway between lambda and bregma. Scopolamine (0.1, 1.0, or 10.0 mg/kg) or saline was injected i.p. 15 minutes prior to injury. Under methoxyflurane anesthesia, animals were concussed at 2.86 atm (an injury level associated with transient areflexia and more enduring motor dysfunctions) using a fluid percussion method modified for rats in our laboratories (Dixon et al., Neurosci. Abst., 1984). Results indicated that scopolamine (1.0 mg/kg) facilitated the rate of recovery of locomotor functioning as indicated by a significantly more rapid return to baseline of walking-beam traversal times ($p<0.5$), rating scale scores ($p<0.5$), and balance beam times ($p<0.5$) when compared to saline treatment. These findings indicate that the observed, seemingly beneficial effects of pretreatment with scopolamine associated with acute neurological recovery may also be associated with an accelerated rate of recovery of more enduring motor deficits. These findings lend further support to the hypothesis that the components of behavioral suppression associated with concussion are not necessarily related to nonspecific disorganization of brain functions or histopathology but to an active cholinergic inhibitory system. Supported by NIH Grant #NS 12587.

129.2 NEUROLOGICAL DEFICITS FOLLOWING EXPERIMENTAL CEREBRAL CONCUSSION IN THE RAT ATTENUATED BY SCOPOLAMINE PRETREATMENT. B. G. Lyeth, C. E. Dixon, R. J. Hamm, T. Yamamoto,* M. L. Giebel,* H. H. Stonnington,* D. P. Becker and R. L. Hayes. Department of Psychology, Division of Neurosurgery, and Department of Rehabilitation Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0001.

Recent data from our laboratory suggest that some consequences of cerebral concussion may be mediated by increased activity within an endogenous cholinergic system located in the brain stem and capable of suppressing responses organized both supraspinally and at the level of the spinal cord (Hayes et al., Science, 233: 301-303, 1984). The present investigation examined the effects of systemic preinjections of a cholinergic antagonist on acute features (<60 min) of behavioral suppression following concussion.

Sixty rats were surgically prepared under sodium pentobarbital anesthesia 24 hours prior to injury by chronically placing a hollow injury screw epidurally over a hole trephined along the sagittal suture, midway between lambda and bregma. Rats were assigned to one of four groups: scopolamine 0.1 mg/kg, 1.0 mg/kg, 10.0 mg/kg, or saline (equal volume). Drugs were injected i.p. 15 minutes prior to injury. Experimenters were uninformed of drug and dosage. Animals were anesthetized five minutes prior to injury with methoxyflurane. Animals were concussed at 2.86 atm (saline pressure pulse, 18 ms duration) with a fluid percussion injury device (Dixon et al., Neurosci. Abst., 10:998, 1984). Animals were assessed neurologically for 60 minutes immediately following injury. The durations of suppression of righting, pinna, corneal, and auditory startle reflexes were scored. Spontaneous locomotion, ability to support the head, tail and hind paw flexion, proximal tail pinch, and organized escape from tail pinch were also assessed. Scopolamine (1.0 mg/kg dose) produced significantly shorter ($p<.05$) durations of suppression of certain spinally mediated behavioral responses: paw flexion, escape, and proximal tail pinch. Scopolamine had no effect on other response measures. Mortality for the 1.0 mg/kg scopolamine group (23%) was significantly lower ($p<.05$) than for the saline group (72%). Scopolamine administered prior to injury appears to improve behavioral recovery after cerebral concussion and decrease mortality. Our findings lend further support to the hypothesis that the components of neurological disruption associated with concussion are not necessarily related to nonspecific disorganization of brain functions or histopathology but to an active cholinergic inhibitory system. Supported by NIH Grant #NS 12587.

129.3 **ATTENUATION OF PROGRESSIVE BRAIN ISCHEMIA FOLLOWING EXPERIMENTAL SUBARACHNOID HEMORRHAGE BY LARGE I.V. DOSES OF METHYLPREDNISOLONE.** M.A.Travis and E.D.Hall, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The purpose of this study was to examine the ability of a large i.v. dose of methylprednisolone sodium succinate (MPSS) to affect acute post-subarachnoid hemorrhage (SAH) cerebral vasospasm and the associated fall in local regional blood flow. MPSS was chosen for study due to its previously disclosed action to enhance chronic neurological recovery after SAH in dogs (Chyatte and Sundt, J. Neurosurg. 59:925, 1983).

Experimental SAH was produced in alpha-chloralose-anesthetized cats by slow injection through a butterfly cannula of 0.5 ml/kg of autologous arterial blood into the cisterna magna. Blood flow (CBF) was measured by H_2 clearance in the caudate nucleus before, at 5 min post-SAH, and every 30 min thereafter until 3 hrs. MPSS (Solu-Medrol® Sterile Powder) or its buffered aqueous vehicle was administered by i.v. bolus at 2 min prior to the 30 min post-SAH blood flow.

In all experimental groups, the SAH produced a transient hypertension and increase (40-50 mmHg) in intracranial pressure (ICP). In the vehicle-treated group, a two-phase decrease in CBF was observed with an initial rapid (20.9%) drop by 5 min followed by an additional, but slower, 37.0% decrease over the ensuing 3 hrs (see Table 1). Although the mean arterial pressure (MAP) remained stable at a normal level, the ICP increased slowly from about 5 up to 15-20 mmHg, leading to a decrease in the cerebral perfusion pressure (CPP; MAP-ICP). Associated with the fall in CBF was a slow elevation in the cerebral vascular resistance (CVR; CPP/CBF). In contrast, in animals treated with 30 mg/kg MPSS following SAH, CBF and CVR were stabilized. In fact, a partial restoration of both parameters toward the pre-SAH levels was observed even though the progressive increase in ICP and decrease in CPP were unaffected. In the 15 mg/kg MPSS-treated group, only a slight attenuation of the decrease in CBF and increase in CVR following SAH was seen.

TABLE 1. Effect of vehicle or MPSS on CBF, CVR, ICP and CPP at 3 hrs after SAH (values = mean ± S.E.) *$p<0.05$ by t-test versus vehicle.

Group	N	% Decrease in CBF	ICP (mmHg)	CPP (mmHg)	CVR (CPP/CBF)
V	5	57.9±4.4	17.5±2.5	93.3± 6.2	5.7±1.2
15 mg/kg	4	47.7±9.7	18.7±8.5	100.4± 9.3	4.3±1.3
30 mg/kg	4	23.0±1.4*	17.0±5.0	88.0±10.0	2.8±0.5*

These results demonstrate that MPSS in high doses can antagonize the development of acute post-hemorrhagic ischemia. Although the specific mechanism(s) by which acutely administered MPSS is able to stabilize blood flow following SAH has yet to be elucidated, a decrease in hemorrhage-induced microvascular lipid peroxidation is a possible contributing effect. A similar conclusion has been reached in previous studies concerning the prevention of post-traumatic spinal cord ischemia by MPSS (Hall et al., J. Neurosurg. 61:124, 1984).

129.4 PAROXYSMAL FIRING IN HIPPOCAMPAL SLICES DURING THE EARLY PHASE OF HYPOXIA. M. Balestrino* and P.G. Aitken
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Paroxysmal firing is the repeated, synchronous discharge of large numbers of neurons not time-locked to stimulus pulses. Such firing can be caused in CA1 of hippocampal slices by delivery of stimulus trains to afferent pathways (Aitken, P.G., Neurosci. Abstr., 10:547, 1984). We report here that paroxysmal firing can also be triggered in CA1 by a hypoxic insult.

Transverse hippocampal slices 400μ thick were maintained at 35-36°C in an interface chamber superfused with artificial cerebrospinal fluid (pH=7.35; K^+=3.5mM; Ca^{++}=1.2mM; Mg^{++}=1.2mM). The gas phase above the slices was warmed, humidified 95% oxygen - 5% carbon dioxide. Hypoxia consisted of replacing the gas phase with warmed, humidified 95% nitrogen - 5% carbon dioxide, flowing at the same rate. Extracellular electrical activity was monitored in stratum pyramidale of CA1 with a micropipette electrode inserted to the depth for maximum amplitude responses to Schaffer collateral stimulation. DC-coupled low gain and AC-coupled high gain records were made on tape recorder.

All slices responded to hypoxia with negative shifts of the extracellular sustained potential (SP). This SP shift occurred in two phases: an initial, slow shift of 2-8mV beginning 135-210 sec after the start of hypoxia, followed by a larger, rapid shift of 15-30mV occurring 140-263 sec after the start of hypoxia. Unsynchronized single unit firing often occurred shortly before or during the slow SP shift.

Paroxysmal firing was seen in approximately 30% of hypoxic episodes. This firing consisted of a 1.2 - 4.1 sec burst of compound action potentials (population spikes), with each population spike having 1-6mV amplitude and 3.0 - 6.5msec duration. Paroxysmal firing could occur either shortly before or during the slow SP shift. We cannot rule out the possibility that this paroxysmal firing was a direct consequence of hypoxic metabolic changes. It is suggestive, however, to think that it was a consequence of the hypoxia-induced cellular depolarization. This latter possibility is consistent with earlier suggestions from this laboratory that en masse depolarization of CA1 pyramidal cells, from whatever cause, results in an increase in electrotonic and/or ephaptic communication between neurons; this in turn could result in synchronization of normally unsynchronized neural activity.

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129.5 EFFECTS OF VINPOCETINE IN A RAT MODEL OF CEREBRAL ISCHEMIA. G. A. King, D. Narcavage, L. Romanski, D. Hannig, and A. Shieh. Ayerst Laboratories Research Inc., CN 8000, Princeton, NJ, 08540.

Vinpocetine is an eburnamenine derivative which has been reported to have beneficial effects in acute focal cerebrovascular disease (P. Kalvach, et al., Cs. Neurol. Neurochir. 45:380, 1982). We have examined the effects of vinpocetine and other drugs in a rat model of cerebral ischemia. Male, Fisher (F-344) rats were anesthetized with halothane, and the carotid arteries were doubly ligated and cut bilaterally. Following recovery from anesthesia the animals were observed for 12 hr. In drug-free animals, seizures began as early as one hour post-surgery, and by 4 hr post-surgery approximately 80% of rats experienced one or more seizures. These animals died at a rate that was linear with respect to time post-surgery, and less than 10% of the animals remained alive after 12 hr. Drugs were administered i.p. either acutely, BID, once immediately post-surgery and again 6 hr later, or subchronically once a day for five days, the last injection being given immediately post-surgery. On BID administration, vinpocetine (50 and 100 mg/kg) and a structurally related drug vincamine (50 and 100 mg/kg) significantly delayed the latency to seizure onset, while the anticonvulsant drug, phenytoin (12.5 and 25 mg/kg), had no effect. On subchronic administration, neither vincamine nor phenytoin altered the latency to seizure onset nor the latency to death, as compared to control rats. Subchronic administration of vinpocetine (25 and 50 mg/kg) significantly delayed seizure onset, and at 25 mg/kg the drug significantly delayed time to death. Therefore, vinpocetine exhibited protective effects in this model of cerebral ischemia, and its potency and efficacy were increased by subchronic administration. While vincamine had effects similar to those of vinpocetine when given acutely, its activity was not maintained after repeated administration. Vinpocetine's effects in this model were probably not due to an anticonvulsant action, since phenytoin at anticonvulsant doses was inactive in this test.

129.6 INFLUENCE OF TISSUE-TYPE PLASMINOGEN ACTIVATOR ON BLOOD FLOW DURING EVOLUTION OF THROMBOTIC STROKE. B.D. Watson*, W.D. Dietrich, R. Busto*, M.D. Ginsberg and C.F. Hoyng* (SPON: J. N. Barrett). Cerebral Vascular Disease Research Center, Univ. Miami School of Medicine, Miami, FL 33101 and Genentech Inc., San Francisco, CA 94080

The recent synthesis by recombinant techniques of human tissue-type plasminogen activator (rt-PA) has facilitated thrombolysis of isolated coronary arterial occlusions in animals and in man, without inducing systemic fibrinogenolysis. Cerebral thrombolysis with rt-PA has not been reported, however. Recently we have developed in the rat a reproducible model of photochemically induced thrombotic stroke. In this model we have observed primary endothelial injury which likely arises from singlet oxygen-induced lipid peroxidation reactions initiated by the photochemical reaction. Platelet adhesion is stimulated, followed by degranulation and formation of occlusive aggregates in small vessels throughout the depth of the cortex. The purpose of this study was to determine whether rt-PA infusion following the initiation of infarct formation would alter the progressive decline in cerebral blood flow (CBF) previously demonstrated in this stroke model.

Alterations in CBF were assessed autoradiographically at 30 minutes and 4 hours following photochemically induced cortical infarction. The infarction was produced by irradiating the brain through the intact skull for 20 minutes with green light following the systemic injection of the photosensitizing dye rose bengal. At 10 minutes following the irradiation period, saline or rt-PA (15 ug/kg/min) in saline was infused for 20 minutes into the external carotid artery. CBF was determined either immediately following the infusion period or following a 4-hour survival period. Thirty minutes following irradiation, autoradiograms demonstrated a focus of reduced CBF (less than 0.02 ml/g/min) in saline-infused rats. In contrast, rats infused with rt-PA and studied 30 minutes following irradiation demonstrated no such focal hemodynamic defect. Both saline and rt-PA-infused rats at 4 hours demonstrated a zone of severely reduced CBF extending to the level of the subcortical white matter. These results indicate that the early infusion of rt-PA appears to inhibit vascular occlusion by fibrin-stabilized platelet aggregates within the irradiated cortex. Once rt-PA infusion is discontinued, however, aggregation appears to proceed and by 4 hours this results in a focus of severely depressed flow. Studies in progress will determine the duration of rt-PA infusion needed irreversibly to inhibit platelet aggregation.

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129.7 CEREBRAL EMBOLISM THERAPY WITH TISSUE PLASMINOGEN ACTIVATOR. J.Zivin, M.Fisher*, U.DeGirolami, J.Stashak*, C. Hemenway*. Neurology Dept., Univ. Of Massachusetts Med. Center, Worcester, MA 01605

Embolic stroke is potentially treatable by thrombolytic therapy, but use of streptokinase or urokinase has been complicated by frequent hemorrhages. Tissue plasminogen activator (tPA) can lyse existing thrombus without producing widespread hematological abnormalities and has been effective in treatment of coronary artery thrombosis. We used a newly developed embolic stroke model which does not depend on prediction of lesion size and location to demonstrate efficacy of tPA in reducing cerebral ischemic damage.

New Zealand white rabbits were anesthetized. In each animal one external carotid artery was ligated, a cannula was placed in the common carotid artery, and the incision was closed so that the cannula was accessible externally. The rabbits were allowed to recover from anesthesia. Blood was drawn from a donor animal and allowed to clot at 37°C for 2 hr. The clot was fragmented with a Polytron and the fragments were suspended in Dulbecco's PBS buffer. The fragments were then sized between screens of 104 μm^2 and 240 μm^2. The rabbits were restrained and predetermined amounts of clot particles were injected into the carotid artery cannula. Intravenous infusion of tPA* was begun 1 min after clot injection. The total dose of tPA was 1 mg/kg; 20% was given immediately and the remainder infused over 30 min. Controls were similarly treated but saline replaced tPA.

When 42 to 72 mg of clot fragments were injected into control animals, 7 of 12 were grossly encephalopathic (signs included lethargy, weakness, ataxia, and nystagmus) or dead at 24 hrs. Of 11 tPA treated animals given identical amounts of clots, 1 died and the remainder were normal. This difference is significant by Fisher's Exact Test (p=0.027). External examination of the brains of tPA treated animals did not reveal evidence of hemorrhage.

This in vivo study demonstrated that tPA was able to prevent major neurological deficits when the drug was given after emboli entered the cerebral circulation. The tPA appears to be safe at doses used in this model.

(*a gift from Genentech, Inc., So. San Francisco, CA)

129.8 CEREBRAL METABOLISM DURING KAOLIN-INDUCED HYDROCEPHALUS IN THE RAT: A 2-DEOXYGLUCOSE STUDY. E.H. Elowitz*, D.L. Dow-Edwards, and T.H. Milhorat. Lab. of Cerebral Metabolism, Dept. of Neurosurgery, SUNY, Downstate Medical Center, Brooklyn, NY 11203.

The model of kaolin-induced hydrocephalus has been shown to produce alterations in cerebrospinal fluid (CSF) physiology and brain morphology. Following cisternal injection of kaolin, the outlets of the fourth ventricle become obstructed leading to a temporary rise in intracranial pressure and a permanent dilatation of the ventricular system. While chronic low-pressure hydrocephalus is clinically associated with dementia and ataxia, little is known about the metabolic derangements caused by hydrocephalus. To better understand the functional and metabolic changes during kaolin-induced hydrocephalus in the rat (Hochwald, G.M. et al., Z.Kinderchir., 34:403-410, 1981), the quantitative autoradiographic 2-deoxyglucose (2DG) method (Sokoloff, L. et al., J. Neurochem., 28:897-916, 1977) was employed.

Male Sprague-Dawley rats (350-400g) were anesthetized with IP pentobarbital and fixed in a stereotaxic frame. Under sterile conditions, a 23G needle was percutaneously inserted into the cisterna magna and 0.1 ml of CSF was withdrawn. Either 0.1 ml of sterile normal saline or sterile kaolin (250mg/ml saline) was then injected over one minute. The animals were allowed to recover for a period of four weeks. During the first two weeks all kaolin animals lost about 30% of their body weight and almost half died. The remaining animals regained their original body weight by the fourth week when the 2DG method was performed. Catheters were inserted into the femoral artery and vein of each rat under halothane-N_2O anesthesia and the animals were allowed to recover. ^{14}C-labeled 2DG was injected into the venous catheter. Timed arterial blood samples were collected. At 45 minutes, the animals were killed with pentobarbital. Local cerebral glucose utilization was determined from autoradiographic analysis and the integrated arterial curve for 2DG and glucose.

The kaolin produced a variable degree of hydrocephalus with some animals having massive ventricular dilatation. Kaolin was seen in the basal cisterns, around the cerebellum and into the fourth ventricle. Preliminary analysis of data indicate little alteration in cerebral metabolism. Only parietal cortex appears to have decreased glucose utilization during experimental hydrocephalus.

129.9 NODAL AND PARANODAL STRUCTURAL CHANGES IN EARLY WALLERIAN DEGENERATION. J. Ishise* and J. Rosenbluth (SPON: V. DeCrescito). Depts. Physiology and Rehab. Medicine, New York University School of Medicine, New York, NY 10016.

Ultrastructural changes in the nodal and paranodal regions of myelinated nerve fibers during early stages of Wallerian degeneration were studied in freeze-fracture replicas and thin sections. Frogs (Rana pipiens) were anesthetized with ethyl carbamate; the optic nerve was exposed on the right side through the palate and then transected under microscopic observation, sparing the opthalmic artery. The eye was not removed. Animals were fixed by vascular perfusion of aldehydes from one to eight days after the date of the surgery (day 1), and the degenerating optic nerve and contralateral normal nerve were removed from each animal. There was no sign of regeneration across the gap between proximal and distal segments in any case. The observations below apply to the segment ~ 1-2 mm from the eye. However, degenerative changes developed at widely different rates among different fibers in this segment, and it was therefore impossible to time the sequence of events precisely. The earliest changes appeared on day 3. Thin sections showed disorientation of axonal microtubules and some detachment of paranodal loops of myelin immediately adjacent to the node. In freeze-fracture replicas irregularities appeared in the paranodal axolemma, and detachment of myelin loops adjacent to the node was also apparent in some cases, but there was no change in the distribution of nodal particles. On day 5 some axons showed accumulations of mitochondria and dense lamellar inclusions in the nodal region. Widening of nodes became more apparent as a result of detachment of myelin loops from the paranodal axolemma. In some axons the "undercoating" along the nodal surface appeared patchy in thin sections, and in replicas a corresponding mixture of particle clusters and particle-free areas appeared in both P- and E-faces of the axolemma at some nodes. Demyelinated paranodal regions showed few node-like particles at this stage. In addition, many new "lakes" appeared between shrunken paranodal loops, but unlike those in the normal paranodal axolemma they contained few E-face particles. On day 8 the concentration of E-face particles was lower than normal at some nodes (~ 600/μ2 vs. 1350/μ2) and higher than normal in the adjacent demyelinated region of the paranodal axolemma (~ 250/μ2 vs. 25/μ2). Since the distal axon was no longer connected to the cell body, it is not likely that the increase in paranodal particles resulted from insertion of new particles. It is concluded from these results that Wallerian degeneration in the optic nerve is associated with detachment of lateral loops of myelin lamellae from the axolemma beginning adjacent to the node and that nodal E-face particles subsequently redistribute into these formerly junctional paranodal regions. Supported by grants from the NIH (NS 07495) and National Multiple Sclerosis Society.

129.10 Recovery of the Hippocampal Slice Preparation Following Hypoxia is Profoundly Influenced by the Physiological Buffer. John Grigg and E.G. Anderson Dept. of Pharmacology, Coll. of Medicine, Univ. of Illinois at Chicago, IL 60612.

Studies were initiated on the ability of synaptic activity in the hippocampal slice to recover after significant periods of anoxia. Slices (400 μm) were prepared from 150 gm rats, and immersed in 1 ml of physiological buffer infused at a rate of 2 ml/min. Bath temperature was held at 37°C. Recordings were made with a glass microelectrode placed near the cell bodies in the CA_1 region. Synaptic activity was assessed by the size of the population spike evoked by stimulating the Schaffer and commissural afferents. Pyramidal Cell viability was tested by antidromic stimulation of their axons in the alveus. Hypoxia was induced by switching to infusion media which had been bubbled with 95% N_2 and 5% CO_2. Oxygen electrode measurements showed a 90-95% reduction in O_2 content.

These experiments were initfated using an artifical cerebral spinal fluid (ACSF) containing: Na^+, 152.24; K^+, 2.5; Ca^{2+},2.4; Mg^{2+}, 1.3; Cl^-,133.3; HCO_3^-,25; HPO_4^-, 1.24; SO_4^{2-}, 1.3; glucose 11 (in mM). Under these conditions, all detectable synaptic acitvity disappeared within 2-3 mins of initiating hypoxia. The fiber spike and antidromic spike remained throughout the anoxic period. Following exposure to 20 to 90 minutes of hypoxia, reperfussion with oxygenated buffer resulted in rapid reappearance of the population spike. After 20 minutes of hypoxia reoxygenation resulted in reappearance of the population spike within 2.5 to 5 minutes and recovery reached 95-125% of the control spike within 7.5 minutes.

Kass and Lipton (J. Physiol (Lond) 332:459-472, 1982) using a comparable protocol have reported irreversible damage to hippocampal slices after 10 mins. of anoxia. The ACSF used in their experiments had a higher K^+ (5.4 mM) and lower glucose (4 mM). Using this buffer we found that a hypoxic medium induced failure of the population spike within 2 to 3 mins. From 4 to 6 mins during hypoxia a brief period of erratic recovery occurred followed by total failure. The antidromic and fiber spike then proceeded to fail. After 20 minutes of hypoxia, irreversible damage had occurred since return to oxygneated buffer failed to restore any observable activity. Initial experiments using ACSF with 6.1 mM K^+ and 10 mM glucose (Dunwiddie, Dev. Neurosci 4:165-175, 1981) showed recovery after 20 mins of hypoxia. Experiments are in progress exploring the role of low glucose in facilitating irreversible damage by hypoxia.

129.11 CARDIOVASCULAR SYSTEM TOXICITY ASSOCIATED WITH LOCAL ANESTHETIC INJECTIONS IN THE CENTRAL NERVOUS SYSTEM. R.D. Thomas, M.M. Behbehani, D.E. Coyle, D.D. Denson (sponsor: N. Sperelakis), Departments of Physiology and Biophysics and Anesthesiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45267-0576.

Numerous in vivo and in vitro studies of bupivacaine (BUP) cardiotoxicity have recently appeared. However, none of the studies to date have addressed the issue of concomitant cardiovascular system (CVS) and central nervous system (CNS) toxicity. This study was designed a) to evaluate the potential contribution of the CNS to CVS toxicity associated with amide local anesthetics, and b) to clarify the relative influence of the C1 area of the medulla versus the tractus solitarius (NTS) on the maintenance of resting mean arterial pressure (MAP).

Rats were anesthetized with chloral hydrate (400 mg/kg). In all experiments, the MAP and electrocardiogram (ECG) were continuously monitored. Next, 1 ul 2% or 4% BUP or 1.6 ul 2% lidocaine (LID), equimalor with 4% BUP, were infused into C1, NTS, or the cervical spinal cord (thus, only affecting the C1 fibers). Also, single unit recordings of C1 neurons and the effect of BUP on the firing rate of these cells were examined. Control injections of 0.9% saline was completed on each animal.

The results of these experiments showed that saline injections caused no change in heart rate (HR) or MAP. When injected into C1, 4% BUP and 2% LID caused a drop in MAP (-42.6 ± 18.2 vs -17.5 ± 3.5 mm Hg) and slight bradycardia, but neither drug produced a change in any of the ECG intervals. Recordings of C1 cells showed that 4% BUP produced an increase in the cell firing rate of 18.0 ± 5.2 Hz (a 58% increase), but iontophoretic application of BUP at currents varying from 80 nA to 200 nA produced no changes in the firing rate. Also, 4% BUP infused at the cervical spinal cord produced a drop in MAP of -51.2 ± 30.6 mm Hg. Infusion of 2% or 4% BUP or 2% LID on NTS produced bradycardia (-15.3 + 4.2 vs -61.7 + 48.2 vs -25.4 + 9.2 bpm) and hypotension (-13.3 ± 2.3 vs -31.7 + 15.9 vs -13.0 + 4.4 mm Hg). When 4% BUP was infused on NTS, the PR interval lengthened, the T wave either disappeared or became inverted, and arrythmias usually resulted.

Results obtained in these experiments indicated that 1) LID and BUP act on axons and not on nerve cell bodies; 2) the C1 region has a tonic excitatory influence on resting MAP and NTS influences both resting MAP and the electrogenic conduction in the heart; 3) direct placement of local anesthetics within the CNS can result in arrythmias and hypotension similar to those reported following a massive intravenous injection. These data, therefore, suggest that the CNS plays a role in mediation of CVS toxicity with local anesthetics.

129.12 HIGH-FIELD PROTON NMR OF HUMAN CEREBROSPINAL FLUID. RK Yu, OAC Petroff*, T Ogino*.
Departments of Neurology and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

Human CSF samples were obtained from the clinical chemistry laboratory of Yale-New Haven Hospital. The medical records of the patients involved were reviewed for clinical information including the reasons the CSF samples were obtained and the results of the clinical laboratory's analysis. Samples were analyzed near or after concentration by lyophilization. The latter samples were resuspended in 100 mM neutral phosphate in deuterium oxide buffer with a known amount 3-trimethylsilylproprionate (TSP) added as an internal standard. Spectra were obtained at 500 or 360 MHz at 25°C using a 30° pulse and a 5 or 10 second repetition rate. Low power pre-saturation of the water resonance was used as needed.

Preliminary assignments were made on the basis of pure compound spectra and literature values. These compounds include beta hydroxy butyrate (1.21 & 2.36 ppm), lactate (1.33 & 4.12 ppm), acetate (1.92 ppm), glutamine (@ 2.1 & 2.4 ppm) and creatine (3.06 ppm). The region between 3.2 and 4.0 ppm contained multiple resonances from choline containing compounds, glucose, fructose, and polyols. The alpha and beta conformers of glucose showed resonances at 4.65 and 5.24 ppm, respectively. Quantitation of these glucose resonances using the TSP standard showed good agreement with the measurements made by the clinical laboratory on the same samples. Values calculated for lactate, creatinine, and glutamine were within the range listed in the literature.

129.13 LACK OF DYNORPHIN, BUT NOT ENKEPHALIN OR SUBSTANCE P, IMMUNOREACTIVITY IN THE DORSAL GLOBUS PALLIDUS IN GILLES de la TOURETTE'S SYNDROME. S.N. Haber, N.W. Kowell, J.P. Vonsattel, E.D. Bird and E.P. Richardson. Department of Anatomy, University of Rochester School of Medicine, Rochester, NY 14642, and Departments of Neurology and Pathology, Harvard Medical School, Boston, MA 02114.

The normal distribution of enkephalin (ELI)-, dynorphin (DLI)- and substance P (SPLI)-like immunoreactivity have been described in detail in the human forebrain.[1] We report here the peptide distribution patterns in the forebrain of a patient diagnosed as having Gilles de la Tourette's syndrome (TS), a disease which has, to date no known associated pathology.

The brain was removed less than 4 hours post-mortem. Tissue to be processed for pathological analysis was immediately placed in buffered formalin and blocks were divided for routine stains used for pathological analysis and for immunohistochemical studies. Peptide distribution patterns from this brain were compared to five brains from neurologically normal patients and from three patients diagnosed as having Huntington's Chorea.

ELI, DLI and SPLI normally appear in a unique pattern in the globus pallidus (and substantia nigra), now termed woolly fibers. All three peptides appear densest in this form and levels in the globus pallidus and nigra are the highest in the brain.

The routine pathological examination of the TS brain did not reveal a specific anatomical locus of pathology to account for the patient's illness. Both enkephalin and substance P-positive woolly fibers were very densely stained and their distribution appeared normal in the TS brain. DLI, however, appear to stain lighter than normal throughout the brain. The most striking observation was the complete lacking of dynorphin-positive woolly fibers in the dorsal globus pallidus. Very faint staining was noted in the ventral pallidum. These results, indicating a decrease of dynorphin in striatal fibers projecting to the pallidum, are intriguing, and represent the first suggestion of a possible pathology in TS.

Supported by NIH 5R23-NS20467, The Scottish Rite Schizophrenia Program and NIH MH-NS31862.

[1]Haber, S.N. and Watson, S.J., 1984. The comparative distribution of enkephalin, dynorphin, and substance P in the human globus pallidus and basal forebrain. Neuroscience, in press.

129.14 CHOLINERGIC PROTEINS IN ALZHEIMER'S DISEASE S.G. Younkin, J. Katz*, D. Nafziger*, L.H. Younkin, Depts. of Pathology and Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106

Candy et al. (J. Neurol. Sci. 54: 59: 277, 1983) have reported that in Alzheimer's disease (AD) the decreases in choline acetyltransferase (over 90%) and acetylcholinesterase (79%) in the nucleus basalis of Meynert (nbM) are more extensive than the loss of cholinergic perikarya (35%). Their observations suggest that altered metabolism of cholinergic proteins in nbM neurons may contribute to the loss of cholinergic proteins observed in Alzheimer's disease (AD). To evaluate this possibility, we assayed choline acetyltransferase (ChAT) and the several molecular forms of acetylcholinesterase (AChE) and counted cholinergic neurons in alternating 24 um cryostat sections through the nbM in 17 AD and 8 age-matched control brains. ChAT and AChE forms were also assayed in cerebral cortex (Brodmann area 21). Only a small fraction of the nbM blocks and cryostat sections prepared from each brain were required to make these measurements. Thus we have accumulated a "library" of frozen nbM blocks and cryostat sections that are well characterized and available for additional biochemical and histological analysis. In cases similar to those described by Candy et al. in which there was a subtle decrease in the number of cholinergic nbM neurons, the ChAT/ cholinergic neuron decreased significantly as previously described. The seven cases in this category showed a decline in nbM cholinergic neurons to 68% of normal (282-461 neurons/section vs 357-836 neurons/section in control brains), a decrease in nbM ChAT to 51% of normal, and a decline in cortical ChAT to 51% of normal. In those cases in which there was a large decrease in the number of cholinergic nbM neurons, we observed a significant five-fold increase in mean ChAT/cholinergic neuron. The nine cases in this category showed a marked decline in nbM cholinergic neurons to 22% of normal (26-201 neurons/section vs 357-836 neurons/section in control brains) no change in nbM ChAT, and a marked decline in cortical ChAT to 22% of normal. The cases with a low ChAT/cholinergic neuron and relative preservation of cholinergic neurons and cortical ChAT could be explained by postulating reduced ChAT synthesis with relative preservation of ChAT transport. The cases with a high ChAT/cholinergic neuron are most easily explained by postulating severe disruption of ChAT transport. It is currently unclear if the differences observed represent different stages in the evolution of a single disease process or are due to fundamentally different disease processes.

129.15 THREE-DIMENSIONAL DISPLAY OF REGIONAL CEREBRAL BLOOD FLOW USING EMMISION COMPUTED TOMOGRAPHY. D.S. Schlusselberg, W.K. Smith, T.R. Simon*, D.J. Woodward. University of Texas Health Science Center at Dallas, and Dallas VA Medical Center, Dallas, TX.

Emission tomography provides quantitative information within a 3D volume, but current display techniques are limited to serial cross-sectional slices or rotating projection images. We have developed new algorithms which provide computer-generated 3D images of radiopharmaceutical activity from analysis of tomographic data. Regional cerebral blood flow data is provided by intravenous injection of radioactive iodoamphetamine. A rotating gamma camera provides projection images which are used to compute serial transverse sections by backplane projection algorithms.

Combinations of manual perimeter tracing and computed edge-detection are used to create 3D regions of interest from the serial slices. The 3D regions of interest can either be selectively displayed with overlying activity removed, or enhanced within a transparent volume. Other imaging strategies include: surface models, color-coded circumferential histograms, and transparent slices. Multiple imaging strategies can be combined in a single image to enhance anatomic localization of abnormal areas. The images can be displayed on most raster-based display devices currently used to examine serial tomographic sections.

These techniques provide a new tool for assessment of regional cerebral blood flow abnormalities in normal aging humans and patients with neurological disorders.

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129.16 STRUCTURAL AND FUNCTIONAL CORRELATES OF PEDOPHILIA. B. Graber, S.E. Hendricks, C.J. Golden* and K. Hartmann* Dept. of Psychiatry, Univ. Nebr. Med. Ctr., Omaha, NE 68106

Diagnosed pedophiles detained as mentally disordered sex offenders were compared to a control group and established norms utilizing several neurodiagnostic techniques. Measures obtained included regional cerebral blood flow (rCBF), computerized tomographic (CT) brain scan, and neuropsychological assessment. We estimated rCBF using a Xenon-133 inhalation technique. Eight detector probes were distributed over each hemisphere. Blood flow was estimated for both white and gray matter. CT scans were examined to select three slices reflecting cortical frontal lobe activity. The mean density of each hemisphere was determined. Neuropsychological assessments we obtained using the Standardized Luria-Nebraska Test Battery. Pedophiles were found to differ from controls and/or normative data with respect to all of these measures. Findings are consistent with an interpretation of pedophilia being associated with atypical CNS structure function.

129.17 IMPAIRMENT IN RECOGNITION OF FACES, MIMIC AND GESTURES IN PATIENTS SUFFERING FROM SCHIZOPHRENIA OR UNILATERAL BRAIN LESIONS. O.J. Grüsser, K.Berndl*, M. von Cranach* and R.Kiefer*.Dept.Physiology, Freie Universität Berlin and Landeskrankenhaus Kaufbeuren (Germany-West).

A test film consisting of 12 different silent movie scenes, each of 10 sec duration, was used to test elementary abilities in perception and recognition of mimic and gestural expressions. 10 verbal and non-verbal multiple choice tests were applied to each scene (fig. 2), all tests being very easy for normals (fig. 1).

(a) In patients suffering from a unilateral left- or right - hemisphere lesion a reduction in all tests was found with an expected additional increase in errors when verbal tests were applied in aphasic patients. Patients suffering from lesions in the temporo-occipital region tended to have the highest error scores.

(b) A surprisingly severe impairment of mimic and gesture recognition was found in a second group of 81 schizophrenics (fig. 1). For more than 90 percent of the schizophrenic patients the test results were outside of the range found in a group of age-matched normals. Error score was slightly correlated with schizophrenic defect.

(c) In a third group of adolescent schizophrenics the same impairment was found, indicating that long-term hospitalization, drug treatment etc. are not essentially responsible for this cognitive defect. Since many schizophrenic patients suffer from severe behavioural impairment in the social field, our data might indicate the underlying cause of this disturbance.

Supported by a grant of the Deutsche Forschungsgemeinschaft (Gr 161).

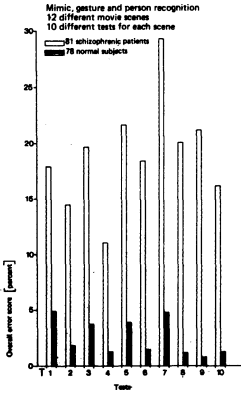


Fig. 1

Fig. 2

Recognition of:

Test 1 : Person
Test 2 : Expression (5x same person)
Test 3: Expression (5x other person)
Test 4 : Expression and person
Test 5 : Person with other expression
Test 6: Verbal description
Test 7 : Sketches
Test 8 : Word selection (reading)
Test 9: Word selection (hearing)
Test 10 : = Test 3

129.18 AN ISCHEMIC STROKE MODEL IN THE RAT WITH CONSISTENT LARGE CORTICAL INFARCTION. S. T. Chen*, C. Y. Hsu, E. L. Hogan and J. D. Balentine*. Departments of Neurology and Pathology, Medical University of South Carolina, Charleston, SC 29425.

We have developed a reproducible model of ischemic stroke in the rat by graded interruption of the collateral circulation to the occluded middle cerebral artery (MCA). Three groups of animals were investigated in the process: Group 1, ligation of the right MCA; Group 2, ligation of the right MCA and ipsilateral common carotid artery (CCA); Group 3, ligation of the right MCA and CCA plus clip compression of the left CCA for 1 hour. Sham operated animals received isolation of bilateral CCAs, and passage of needle underneath the right MCA without ligation. The relative surface blood flow of the cortex supplied by the right MCA was determined by laser Doppler flowmetry (Periflux, Perimed, Sweden). MCA ligation produced a fall of blood flow to 62 $\pm$ 11% of the baseline (mean $\pm$ SEM, n=5). Additional occlusion of ipsilateral CCA and bilateral CCAs reduced the flow to 48 $\pm$ 10% and 18 $\pm$ 4% respectively. The reduction in the relative surface blood flow after each successive ligation was significant by paired-t test ($p<0.005$). Morphological studies conducted 3 days post surgery showed grossly visible infarcts in cerebral cortex supplied by the right MCA in all group 3 animals (n=10), but only 3 in Group 2 (n=10) and none in Group 1 (n=10). The maximum cross-sectional area of infarction was 10.4 $\pm$ 3.5 mm^2 (mean $\pm$ SEM, n=10), 4.8 $\pm$ 1.9 mm^2 (n=10) and 1.7 $\pm$ 0.8 mm^2 (n=10) in Group 3, 2, and 1 respectively. In Group 3, the surface area of infarction measured on a Videoplan interactive image analysis system (Carl Zeiss, West Germany) was 100 $\pm$ 6 mm^2 (mean $\pm$ SEM, n=10). In sham operated animals pathological changes were restricted to a surface lesion of the surgical site (1-4 mm diameter). Using the Group 3 rat as a stroke model we have followed the post-ischemic changes up to 14 days and noted the progressive accumulation of calcium and degredation of neurofilament proteins corresponding to evolving histopathological findings. This model thus offers the advantages of a consistent large cortical infarct, satisfactory long term survival and a relatively low mortality rate of 7% (n=70).

(Supported by NS 00792, NS 12044, NS 11066 and MUSC Research Fund).

129.PO TRIMETHYLTIN (TMT) INDUCED NEUROPATHOLOGICAL CHANGES IN THE NEONATAL RAT. L.W. Chang. Dept. of Pathology, Univ. of Ark. for Medical Sciences, Little Rock, AR 72205.

Trimethyltin (TMT) as a potent neurotoxicant, especially on the limbic system, is well recognized. Our previous study indicated that the developing nervous system was even more sensitive to TMT than that of adult animals. A single injection of TMT between postnatal day (PND) 11-15 produced total destruction of the Ammon's horn within 3-4 days. Our present study is to provide a more detailed time-course evolution of this rapid pathological action of TMT. Neonatal rats (Sprague-Dawley) were injected (i.p.) with a solution of trimethyltin chloride at a dose of 6.0 mg/kg b.w. on postnatal day 13 (PND 13). Control animals were similarly injected with equal volumes of saline solution. Animals, in groups of 5, were sacrificed at 8 hour intervals following TMT administration. At sacrifice, animals were perfused intracardially with saline solution followed by 2.5% buffered glutaraldehyde. Brains were carefully removed. Left hemispheres of the brains were further fixed in 10% buffered formalin and prepared for light microscopy examination. The hippocampus from right hemisphere of the brains were dissected out and processed for electron microscopy. No remarkable light microscopic histopathology was observed at 8 and 16 hours after intoxication. By 24 hours post-injection, significant neuronal changes, including neuronal swelling and necrosis, were found in the olfactory (OC), pyriform (PC), and entorhinal (EC) cortices. The large pyramidal neurons of the Ammon's horn, particularly those in field CA_3, also showed progressive neuronal necrosis. Involvement of the $CA_{1,2}$ neurons of the Ammon's horn was also evident but was considerably less than those in field CA_3. Despite the rapid destruction of the OC, PC, and EC neurons as well as the Ammon's horn, involvement of the granule cells in the fascia dentata was only moderate and subsided rapidly. Such degenerative pattern resulted in total destruction of the Ammon's horn with a relatively intact fascia dentata by 72 hours post-injection. Electron microscopy revealed extensive accumulation of lysosomes in the pyramidal neurons as early as 16 hours after intoxication with increasing and progressive neuronal degeneration and necrosis with time. The present study demonstrated that the neonatal rat limbic system is extremely sensitive to TMT toxicity, particularly during the third week of postnatal life. The overall sensitivity among the limbic neurons to TMT may be rated as: OC $\geq$ PC/EC $\geq$ CA_3 > $CA_{1,2}$ > granule cells of fascia dentata. It is of interest to note that the septotemporal gradients for TMT-induced lesion, as those observed in adult animals, also existed. The overall sensitivity for Ammon's horn pyramidal neurons, particularly those in field CA_3, was septal > temporal and for the fascia dentata granule cells was temporal > septal. Neurons in the dorsal hippocampal formation are by far more sensitive than those in the ventral hippocampus.

STAINING AND TRACING TECHNIQUES

130.1 A SURVEY OF CARBONIC ANHYDRASE HISTOCHEMICAL ACTIVITY IN THE NERVOUS SYSTEM. V. Wong, C.P. Barrett, E.J. Donati, and L. Guth (SPON: T.H. Oh). Dept. of Anatomy, Univ. of Maryland Sch. Med., Baltimore, MD 21201.

While it is generally believed that neurons do not contain carbonic anhydrase, a recent histochemical study has demonstrated that a subpopulation of dorsal root ganglion (DRG) neurons contain the enzyme (J. Histochem. Cytochem. 31:292-300). Such observation raises questions on the role of carbonic anhydrase in neurons. In the present study, we describe a global histochemical survey of carbonic anhydrase activity in the nervous system, including structures derived from the neural crest and non-neural crest ectoderm. We found that carbonic anhydrase histochemical activity is present in many neurons of neural crest origin, including dorsal root, trigeminal, nodose and celiac ganglia, myenteric plexus, and glial cells throughout the CNS. Quantitative study revealed that, in first order sensory neurons, the enzyme is found only in neurons of large (50 um or above) and medium (20-50 um) size. Small neurons (< 20 um) did not show reactivity. In the DRG, the reactive neurons constitute about 35-40% of the total neurons; the distribution between heavily and moderately stained cells are 23% and 16%, respectively. The action of the enzyme is hydration of carbon dioxide; however, since carbon dioxide can be the by-product of many metabolic pathways in the nervous system, the specific function of carbonic anhydrase in each of these cell types may be different. We speculate that carbonic anhydrase in neurons of the dorsal root and trigeminal ganglia is responsible for maintaining a carbon dioxide tension necessary for optimal monosynaptic reflex function. While further experimentation is necessary to confirm this, we believe that the histochemically demonstrable amount of neuronal carbonic anhyrase is related to physiological functions of these neurons.

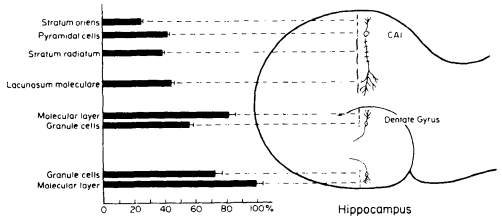
130.2 REGIONAL HETEROGENEITY OF NEUROFILAMENTS REVEALED BY MONOCLONAL ANTIBODIES. M. Vitadello, C. Triban*, M. Fabris*, R. Zanoni*, A. Gorio and S. Schiaffino*[1]. Fidia Research Laboratories, 35031 Abano Terme, (PD), Italy and [1]Institute of General Pathology, University of Padua, Padua, Italy.

A library of monoclonal antibodies directed against neurofilament protein was obtained immunizing Balb/c mice with a crude neurofilamnet preparation (Wood J.N. and Anderton B.H., Bioscience Reports, 1:263-268, 1980). These antibodies were characterized by indirect immunofluorescence technique on adult rat cerebellum cryosections and by immunoblotting procedures on whole protein homogenates from rat PNS and CNS. The distribution of neurofilament protein recognized by the different antibodies was checked on cryosections from different regions of rat nervous system and results showed that immunoreactivity was present exclusively in neuronal structures. The distribution of neurofilament antigens was in general identical with the different antibodies. However, certain neurofilament populations, such as those in dendrites and cell bodies of motor neurons were not labeled by some antineurofilament antibodies. These results suggested that in some locations, like motor neuron cell bodies and dendrites, neurofilament have a reduced or even absent complement of the different subunits. An alternative possibility is that different isoforms of the neurofilament subunits exist in different regions of the nervous system.

130.3 A VOLTAGE MAP OF THE HIPPOCAMPUS USING CYANINE VOLTAGE-SENSITIVE DYE AS A HISTOLOGICAL MARKER. R. M. Dasheiff. Epilepsy Center, VA Hospital; and Dept. of Neurology, Univ. Wis., Madison, WI 53705

It is desirable to know the electrical properties of large neuronal populations when studying epilepsy and seizure activity. Current electrophysiologic techniques lack the ability to sample large anatomical areas. Although histologic techniques allow the entire brain to be examined, existing methods could not produce a direct record of membrane potential. Voltage-sensitive dyes (VSD) have been successfully used to monitor membrane potential in living tissue. However, all previous work with VSD has required direct optical access to the tissue, and the recordings performed in real-time. We now show how cyanine VSD can be used as a histologic marker of membrane potential.

DiO-C_2-(5) was injected into the common carotid artery of awake rats. The dye (10^{-4}M) was mixed with a 30% concentration of mannitol, 5% EtOH, to open the blood brain barrier. Two ml were injected at 6.8 cc/min. Electrodes implanted into each hippocampus showed minimal or no changes during the 20-sec injection. Awake animals were induced to have a seizure by either bicuculline (0.25 mg/kg in dimethylsulfoxide injected into the jugular vein) or kainic acid (12 mg/kg, i.p.). The injection of dye during a seizure did not abort the seizure but sometimes caused a reduction in the voltage of the waveforms on the EEG. Animals were decapitated immediately after dye injection, their brains frozen in 2-methylbutane, 10-μ thick sections cut on a cryostat and cover-slipped with permount. I have discussed the concept and validity of using the rate of dye uptake into tissue as a measure of membrane potential (J Neurosci Methods 13:199-212, 1985). A computerized fluorescent microscope scanned the septal hippocampus and quantitatively assessed dye content. Absolute voltage could not be calculated. Instead, data from each slide were normalized to the molecular layer of the inferior blade of the dentate gyrus. The normalized voltage map obtained from 130 sections from 4 control rats is shown below. Error bars are S.E.M. ANOVA yields $P < .001$. Voltage maps from seizing animals differed from control and will be displayed.



130.4 HISTOFLUORESCENT METHOD FOR HIPPOCAMPAL ZINC. M.L. Blockus*, J.D. McCourt*, E.J. Kasarskis, and C.J. Frederickson. Lab. for Neurobiology, University of Texas at Dallas, Richardson, TX 75080 and Department of Neurology, University of Kentucky, Lexington KY, 40536.

Quinoline derivatives form fluorescent chelates with zinc and have been used successfully for fluorometric assays and visualization of zinc in somatic tissues and fluids (Smith et al., J. Histochem. Cytochem., 1969,17:749). In search of a micro histochemical technique for studying zinc in brain tissue which would be (1) quantitative, (2) of high-resolution, and (3) reasonably efficient, we have examined the usefulness of quinoline fluorescent methods for studies of zinc in the hippocampal mossy fiber neuropil in the mouse.

Both intravital and post-mortem procedures have proved acceptable for fluorescent labeling of the mossy fiber zinc with 2-methyl-8-hydroxy quinoline. With the intravital method, strong fluorescence is obtained when animals are sacrificed 5 - 20 minutes after systemic injection of acidic aqueous quinoline solution, and brains are either cryotomed fresh or after a brief perfusion with buffered aldehydes. For post-mortem staining, unfixed cryostat sections, as well as aldehyde-fixed sections and JB4-embedded sections, have all been found to yield a fluorescent reaction in the mossy fiber neuropil. Besides the mossy fiber tissue, other zinc-rich tissues including the amygdala, pancreas, and adult male mouse submandibular gland also fluoresce after the quinoline staining procedures.

When excited with 360 nm ultraviolet light, the fluorophore emits the characteristic yellow-green zinc-quinoline fluorescence spectrum which is approximately symmetrical with an emission peak at 520 nm. With the intravital method, the intensity of emisions found in the tissue increases monotonically with low doses of quinoline, but plateaus at about 500 mg/kg, suggesting that fluorescence intensity at the higher doses is quantitatively determined by the concentration of endogenous tissue zinc. The results indicate that quinoline fluorescence may be a useful tool for histoanalytic studies of zinc in the brain.

130.5 LONG-TERM PRODUCTS OF NEURAL DEGENERATION IN PRIMATE VISUAL SYSTEM. Alfredo A. Sadun and Betty M. Johnson, Dept. of Ophth., USC School of Med., L.A., CA. 90033.

There is a long held dictum that axonal products of degeneration in the CNS are transitory. Conventional wisdom suggests that metabolic degradation and phagocytic processes in the mammalian brain remove degenerated axonal elements. Successful silver impregnation of degenerating axons produced by a lesion usually require a survival period of 3-7 days from the beginning of Wallerian degeneration. Despite this limitation, which precluded many investigators from attempting to trace degeneration in the human brain, Mesulam (1979) and Grafe (1980) have recently demonstrated the persistence of argyrophilic degenerating elements in the human CNS with survival periods in excess of one year.

Most neuroanatomic tracing techniques are not suitable for studies in man. We recently addressed this problem and developed the PPD staining method. This method reliably stains degenerated axons and axon preterminals in postmortem human brains even with very long (years) survival periods. Application of this method on 22 human necropsy brains has permitted us to histologically confirm retinofugal pathways to LGN and pretectum, and significantly, to demonstrate retinal projections to the superior colliculus, pulvinar, and 3 hypothalamic nuclei in man (Sadun, 1982-1985).

PPD-method studies suggest that products of degeneration persist for years in some pathways in the human brain. Ultrastructural confirmation of long-term products of degeneration is difficult in the human brain because of the poor quality of fixation. We therefore examined twelve brains from monkeys who had uniocular enucleation one month to one year prior to sacrifice by perfusion. Tissue from the optic nerves, optic tracts, and LGN was examined by the PPD technique and by EM. We compared the results of these two methods and demonstrated, by ultrastructural criteria, products of degeneration in the primate visual system one year following injury to the retinal efferents.

Differences in degeneration rates have been shown to exist in different fiber systems and in different species. We feel the present evidence for long-term degeneration in the primate visual system provides a basis for the direct study of human visual neuroanatomy by techniques such as the PPD method.

130.6 NOVEL STAINING PROPERTIES OF EPIDERMAL MERKEL CELLS AND THEIR MECHANOSENSORY INNERVATION. C.A. Nurse. Dept. of Biology, McMaster University, 1280 Main St. W., Hamilton, Ontario L8S 4K1.

In mammalian hairy and glabrous skin, the epidermal Merkel cells (MCs) aggregate in well defined patterns at locations concerned with tactile sensation. These MCs contain large dense cored granules and are frequently innervated by large myelinated sensory fibers. Vital staining with the fluorescent dye quinacrine (QC) has been used to study the distribution of MCs in the vertebrate integument (Nurse et al., 1983, Cell Tiss. Res. 228: 511-524) and the role of sensory nerves in their postnatal development in rat hairy skin (Nurse et al., 1984, Neurosci. 11: 521-533). I now report that MCs can also be stained with low concentrations (0.01 mg/ml) of another vital dye Neutral red (NR), following ½ hr incubations of epidermal tissues in buffered physiologic salt solutions. This NR-staining reveals similar cell patterns to that seen with QC-fluorescence in various rat tissues including the vibrissal follicle and the split epidermis of the whisker pad, the (glabrous) footpad and the touch dome. Further, in monolayers of dispersed vibrissal (outer root sheath) cells, NR stains a select cell population that was previously QC-positive; the latter cells were previously shown to be MCs by ultrastructural criteria (Nurse et al., 1983). Preliminary studies on developing skin tissues suggest however that quantitatively more cells are labelled by QC than by NR; this may simply reflect differences in the sensitivity of the two methods, especially with respect to immature MCs. This NR-staining of MCs appears general since it also occurs in the split epidermis of adult salamander (hind limb) skin where the MCs are irregularly distributed; in this tissue the NR-stained cells were also QC-positive and occurred typically with a density of 180-300 cells/mm^2 and a size of 14-18 μm. It is of interest that though several monoamine containing cells possessing large dense cored granules can also be vitally stained with NR (Stuart et al. 1974, Cell Tiss. Res. 153: 55-61), the MCs so far appear unable to synthesize or accumulate the common biogenic monoamines. The innervation of the MCs in rat skin is also being studied using a zinc iodide-osmium procedure; in 10-20 μm frozen sections, sensory nerve fibers and their terminations are readily stained including those in the MC-rich regions of the upper vibrissal follicle and of the overlying epidermis. More recently, I have successfully used the fluorescent dye 3,3'-diethyloxadicarbocyanine iodide (see Yoshikami and Okun, 1984, Nature 310, 53-56) for vital staining of sensory nerves in rat skin. Since this dye fluoresces in the red wavelength and can be used in combination with quinacrine (which fluoresces in the green), the double labelling procedure appears potentially useful for studying the innervation of MCs by a vital staining technique. (Supported by NSERC Canada; studies were initiated under PHS grant NS 15592-02 to J. Diamond.)

130.7 THE USE OF SUPER GLUE IN ISOLATING TRACER PLACEMENT IN PERIPHERAL TARGETS. T. C. Bour* and A.O. Humbertson, Jr. Department of Anatomy, The Ohio State University, Columbus, Ohio 43210.

Retrograde tracers have been used to identify neurons of the central nervous system that contribute to the innervation of peripheral structures. A major difficulty in using retrograde tracers for this purpose is that inaccurate labelling may be caused by the diffusion of peripherally applied tracers into surrounding tissue. Tracer diffusion can be especially compromising when examining visceral innervation due to the close proximity of visceral structures. Accordingly, it is of importance to develop techniques that can be used to apply retrograde tracers without tracer diffusion. This abstract will report one such technique.

Six adult white rats received a visceral application of the fluorescent tracers True Blue and Diamidino Yellow. The tracers were separately and exclusively applied to pairs of visceral organs, such that if True Blue was used for one organ of the pair (eg., stomach), Diamidino Yellow was used for the other (eg., pancreas). Following blunt dissection of the epithelium, crystals of the tracer were applied directly to the exposed wall of a given organ. The opening and the crystals were then covered with Super Glue. After an appropriate survival time, the animals were sacrificed and all tissues surrounding the tracer application sites, as well as the brainstem and spinal cord, were removed, sectioned, and examined for tracer diffusion and labelling. Examination of visceral tissue revealed no tracer diffusion; the glue contained the tracer at the application site. Furthermore, cells of the dorsal motor nucleus of the vagus nerve and of the nucleus ambiguus were labelled by the tracers in a pattern similar to that obtained by other investigators, indicating that the glue did not interfere with retrograde transport. The results of this study suggest that Super Glue may be effectively used to isolate the placement of retrograde tracers in peripheral targets.

T. Bour was supported by the Samuel J. Roessler Fund of the Ohio State University College of Medicine.

130.8 ORIGIN AND IDENTIFICATION OF FIBERS IN THE CRANIAL NERVE IX-X COMPLEX OF *XENOPUS LAEVIS*: LUCIFER YELLOW BACKFILLS *IN VITRO*. H. Blair Simpson*, M. Tobias and D. B. Kelley. Dept. of Biol. Sciences, Columbia Univ., New York, N. Y. 10027

Neurons of cranial nerve nucleus (n.) IX-X in *Xenopus laevis* are sexually dimorphic in cell size, number and dendritic extent. Golgi-Cox impregnations which reveal dendritic morphology do not permit precise identification of motor neuron muscle targets. We have therefore developed a Lucifer Yellow backfill method for use in identifying motor nuclei and sensory projections of cranial nerves in an isolated brain preparation. Male and female frogs were anesthetized, chilled and the brain anterior to the first spinal nerves gently removed and placed into a Hepes-buffered Ringer's solution. A Histoacryl-vaseline cup was built around the nerve root of interest and a drop of 4%LY-Ch applied for 1-2 hours. The brain was then fixed in 4% buffered paraformaldehyde, cryostat sectioned at 60μ, dehydrated and viewed with a fluorescent microscope. Lucifer Yellow filled laryngeal motor neurons were recognized by retrograde labelling with horseradish peroxidase (HRP). For double label experiments, 1-2μl of HRP was injected into the larynx. Following a two day survival, frogs were saline-perfused, LY-backfilled as above and fixed in 0-1% buffered paraformaldehyde/0.1% glutaraldehyde.

Neurons of cranial nerve nucleus (n.) IX-X send axons to the periphery through the most posterior root (4) of the IX-X nerve complex. In some preparations, a small population of anterior n. IX-X cells exit the medulla with root 3. Root 4 also contains axons of autonomic preganglionic neurons whose cell bodies are located medial to n. IX-X. Double label (LY-HRP) studies indicate that the majority of n. IX-X cells project to the bipennate muscles of the larynx via root 4. Comparison of male and female root 4 fills reveal cells of three morphological classes (triangular, ovoid and elongate) in both sexes. Males have more large n. IX-X neurons than females; females more small n. IX-X neurons than males. Primary and secondary dendrites of n. IX-X neurons are well filled in both sexes. Root 3 contains afferent fibers projecting to five anatomically distinct sensory projection zones; these include cutaneous afferents from the shoulders and trunk and presumed somatosensory fibers projecting to the dorsal column nuclei. Root 2 has sensory fibers terminating in the nucleus tractus solitarius and axons of lateral line efferents. The most anterior root (1) contains lateral line afferents terminating in the lateral line lobe.

With this LY-backfill method, spinal or cranial nerve roots can be filled and the location of sensory terminal fields and efferent cell bodies rapidly mapped with good resolution. In combination with HRP tracing, the origins or targets of LY-filled central projections can be determined. We anticipate that this method may, with modifications, be useful for combined anatomical and electrophysiological studies of laryngeal motor neuron activity underlying the generation of vocal behavior in male and female *X. laevis*.

Supported by HD 00493.

130.9 DIFFERENTIAL LABELLING OF IDENTIFIED NEURONS AND SEROTONERGIC FIBERS IN THE STOMATOGASTRIC GANGLION OF DECAPOD CRUSTACEA. P.S. Katz and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853

We have developed a double label protocol using the established peroxidase visualization techniques of o-dianisidine (DIA, Sigma) and diaminobenzidine (DAB, Sigma II) to differentiate intracellularly injected horseradish peroxidase (HRP, Sigma VI) from peroxidase tagged antibodies at the light microscope level. Using this technique, we are able to map the distribution of dendritic processes of single cells in the stomatogastric ganglion, which appear light brown due to the DIA chromagen, and serotonergic (5-HT) afferents which are dark brown or black due to the DAB reaction product.

Physiologically identified neurons in the pyloric CPG of the lobster, Homarus americanus, and the crab, Cancer irroratus were pressure-injected with HRP (5% in filtered 0.2M KCl) Fast Green FCF (2mg/ml) was added to allow visual confirmation of cell fills. After allowing one hour for the HRP to diffuse, the ganglia were fixed in 0.2% glutaraldehyde, 0.5M NaCl for one hour followed by paraformaldehyde-lysine-periodate fixative (McLean and Nakane, J. Histochem. Cytochem., 22:1974) for 16 hours. The ganglia were soaked in 0.1M $NaBH_4$ to remove excess aldehydes. The HRP was then developed using a modified DIA procedure (Colman et al., Brain Res., 102:1976) to produce a light brown reaction product. The tissue was soaked in 0.3mg/ml DIA in 0.1M phosphate buffered saline (PBS) containing 1% nitroprusside (NP). After 30 minutes, 33μl of 1.2% acidified H_2O_2 was introduced for each ml of solution. The reaction was stopped after 5 minutes with 2 changes of 3% NP followed by PBS. The ganglia were soaked in 20% sucrose for at least 24 hours prior to cryostat sectioning. For immunocytochemistry, sectioned material on slides was rinsed in PBS + 0.3% Triton-X100 + bovine serum albumin (1mg/ml). 150μl of 1:200 anti-5-HT antibody (New England Nuclear) was placed on the sections for 90 minutes in humidity chambers. The sections were rinsed in PBS-Triton + 10% normal goat serum. The 5-HT antibody was then labelled with peroxidase using either Avidin-Biotin (Vecta Stain) or peroxidase-anti-peroxidase (Polysciences) with a goat-anti-rabbit bridge (Sternberger-Meyer). The peroxidase-labelled antibody was developed using a standard DAB reaction to produce a dark brown product. (Freshly made 0.5mg DAB/ml PBS to which 0.1ml 3% H_2O_2 was added for each 100ml of solution.) The slides were rinsed, counterstained and coverslipped.

This technique provides a convenient method for examining the anatomical connections of physiologically well-studied invertebrate systems. Supported by HATCH Grant NYC191410 and NIH NS17323.

130.10 A FRAGMENT OF "FAST BLUE" BUT NOT "FAST BLUE" ITSELF IS TRANSPORTED BY AXONS. G.F.Alheid. Dept. of Behavioral Medicine and Psychiatry, Univ. Virginia Sch. of Med., Charlottesville, Va 22908.

The retrograde fluorescent tracers have been a powerful tool for identifying long axonal pathways in the brain, and when used in the correct combination may allow tracing of collaterals to two different targets in the brain. Furthermore, identification of the potential transmitter used by neurons projecting to a particular area may be accomplished by using retrograde fluorescent tracers combined with immunofluorescence for an antigen specific to a particular transmitter system.

Of the available fluorescent tracers, "FAST BLUE" [trans-1-(5-amidino-2-benzofuranyl)-2-(6-amidino-2-indoyl) ethylene dihydrochloroid; Bentivoglio et al. Neurosci. Lett. 18:25, 1980] is one of the more interesting since in our experience it appears to be transported retrogradely better than most of the other available tracers, fluorescent or otherwise, and it apparently also is transported anterogradely.

After receiving a new sample of "FAST BLUE" we found that, using the same concentration and volume of the compound, transport was minimal. The new sample differed superficially from the old sample in color; the new sample was a yellow powder (YFB), while the old sample was a brown powder (BrFB). Using mass spectroscopy we verified that the new sample was indeed "FAST BLUE" while the old sample contained a major contaminent which differed from the peak for YFB by 17 daltons. Presuming the contaminent is a by-product of the synthesis of YFB, the difference may be accounted for by a missing amine group. Since there are two amine groups on YFB, the contaminent could be one of two compounds either of which would have a reactive cyano-group on one end.

This result suggests that the reason that BrFB is transported so avidly is that a reactive (non-aminated) end of BrFB could readily bind with amine groups in vivo, thus attaching itself to pre- and post-synaptic cell membranes. This same logic may apply to other related fluorescent tracers, so that more avidly transported compounds might be designed in a variety of colors.

This work was supported by NIH grant R NS1774303. Harvey Laboratories Inc., Charlottesville, Va. generously performed the mass spectroscopy.

130.11 SULFORHODAMINE 101 AS A FLUORESCENT PROBE FOR INTRACELLULAR DYE-FILLS, DIFFUSION BACKFILLS, AND DOUBLE LABELING WITH LUCIFER YELLOW. J.W. Pendleton* and H. Keshishian (SPON: E.R. Roederer). Biology Department, Yale University, New Haven, CT 06511.

Lucifer Yellow (LY) has proved invaluable as an intracellular dye for revealing the anatomy of individual neurons. A second fluorescent probe with qualities similar to LY but fluorescing red would be very useful for double labeling purposes. Here we describe the use in both vertebrate and invertebrate preparations of sulforhodamine 101 acid chloride (aka Texas Red, TR) as well as its similarities to and differences from LY. TR absorbs maximally at approximately 584nm and at that wavelength its fluorescent emission maximum is at approximately 600 nm. Neurons filled with TR fluoresce brilliantly red when excited with conventional rhodamine filter sets. The cells are essentially invisible when viewed using FITC optics. Because of these spectral characteristics, TR is suitable for double labelling experiments, for example in indirect FITC immunocytochemistry.

Giant axons in the larval lamprey spinal cord may be filled with Texas Red (5% in saline or distilled water) by diffusion from a spinal transection. Stained axons remain clearly visible in preparations 10 days after injection of TR into a spinal transection, demonstrating that TR is a vital and long-lasting fluorescent stain. The dye may also be intracellularly iontophoresed into neurons using hyperpolarizing current. The microelectrodes had tips filled with 5% TR in distilled water, with 3M KCl in the shafts. TR proved to be an excellent intracellular dye in cells ranging from small (5 micron) interneurons and efferents in Drosophila to giant bulbar cells in the lamprey. TR is retained within the cells during the impalement, and does not leak across the cell membrane. It diffuses rapidly, revealing fine neuritic processes, as well as filopodia on growth cones. TR appears to be a vital dye when not excited; filled neurons exhibited action potentials and retained stable resting potentials. The dye is fixed using the same protocols as for LY, i.e. 4% paraformaldehyde. In an effort to test whether the extent of the intracellular TR dye-fills equalled that observed using LY, we examined the anatomy of a set of motoneurons (the AM cells) of the terminal ganglion of grasshoppers using both intracellular dyes. As these neurons comprise an identified cluster of cells with essentially indistinguishable CNS arborizations, the two dyes could be easily compared in a single ganglion. We did not notice any significant difference in the extent or detail of the arbors revealed by both dyes. Given the small molecular weight of TR (MW = 625), we tested whether it can cross junctions is a manner similar to LY, using systems that previously have been showed to be dye-coupled. Intracellular fills of the columnar ectodermal epithelium of the grasshopper limb-bud revealed a rapid transfer of the dye between cells. Similar dye-coupling was demonstrated between afferent neurons (the Ti1 pioneers) in the embryonic grasshopper leg.

130.12 EFFECTS OF RETROGRADELY TRANSPORTED RICIN ON VAGAL NEURONS: TIME COURSE OF RICIN TRANSPORT, INHIBITION OF NEURONAL PROTEIN SYNTHESIS AND MORPHOLOGIC CHANGES. R.G. Wiley. Neurology Dept., Vanderbilt U. Medical School and VAMC Nashville, TN 37203.(SPON: S. Wall)

Ricin, the toxic lectin from castor beans, injected into peripheral nerves is taken up by axons in that nerve and retrogradely transported to their parent cell bodies resulting in destruction of those neurons. This 'suicide transport' strategy has been used to ascertain the cellular localization of substance P receptors in brainstem and spinal cord and to investigate reorganiza- of somatosensory cortex responses to mechanoreceptor stimulation after sciatic nerve ablation with ricin. However, sophisticated application of the suicide transport technique requires detailed knowledge of the effects of retrogradely transported ricin on motor and sensory neurons. In the present study, 300 ng of ricin-horseradish peroxidase conjugate (RCA-HRP; E-Y Labs, San Mateo,CA) dissolved in 300 nl of PBS was pressure microinjected into the cervical vagus nerves of adult male rats. RCA-HRP was demonstrable by TMB histochemistry for peroxidase within ipsilateral nodose ganglion sensory neurons in less than 9 hours after injection and within ipsilateral dorsal motor nucleus of the vagus neurons within 11 hrs. Transganglionic labelling of vagal sensory neuron terminals in the nucleus tractus solitarius was never seen. Application of vincristine to the vagus nerve proximal to the injection site for ricin prevented labelling of vagal neurons for 24 hrs but not 48 hrs. Appearance of cytotoxic cell changes was also delayed for a similar period. Light microscopic cytotoxicity (loss of Nissl substance) was normally evident by 15 hrs post vagal ricin injection. Electron microscopy localized RCA-HRP to cytoplasmic membranes and revealed that the loss of Nissl substance was due to disaggregation of polyribosomes with dissolution of the rough endoplasmic reticulum into numerous small vacuoles. Autoradiography using ^{3}H-leucine (2 mCi injected i.p. 4-6 hrs before sacrifice) revealed profound inhibition of protein synthesis limited to vagal sensory and motor neurons within 18-24 hrs after vagal ricin injection. Lastly, coinjection of ricin and wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) gave abundant retrograde labelling of vagal neurons, but the prominent transganglionic (anterograde) labelling usually seen with WGA-HRP was decreased. These results indicate: 1 - ricin reaches cell bodies via fast axonal transport; 2 - ricin produces the same morphologic and biochemical changes in neurons as it does in other cell types; 3 - the initial metabolic and morphologic effects occur within a few hours after ricin arrives in the cell body; 4 - within the time interval studied, these effects of ricin are limited to neurons sending axons through the cervical vagus nerve injection site; and 5 - fast axonal transport fails rapidly after arrival of ricin in the cell body. (Supported by VA Merit Review Award.)

130.13 CONCENTRATION GRADIENTS AT AN INTRACEREBRAL INJECTION SITE. M.F.Dubach*, Y.Nievergelt* and D.M.Simmons* (SPON: P. Prinz). Regional Primate Research Center, University of Washington, Seattle, WA 98195; Department of Mathematics, St. Olaf College, Northfield, MN 55057; Salk Institute for Biological Research, La Jolla, CA 92037.

The concentration gradients of an intracerebrally injected substance can be estimated in three dimensions by applying a mathematical model to punch-scintillation data. The model, in the form of a series of computer programs in BASIC, will be presented along with results of its application to dopamine injection sites in Macaca fascicularis.

Tritiated dopamine was injected at six striatal sites in the monkey, at time intervals before perfusion of 120, 60, 30, 20, 10, and 2 minutes. Sites were selected to avoid the ventricles and white matter, to satisfy the basic assumptions of the model. The brain was perfused with 4% paraformaldehyde and the portion bearing the injection sites was frozen on a copper plate packed with dry ice. Thick sections (760 um) were cut while frozen, and punch samples were taken by pressing a 5 x 5 grid of sharpened pieces of 22-ga stainless steel needle stock through the region of each injection site, as it appeared in each of several sections. Each sample was treated and counted separately in a scintillation vial.

The model is based on the assumption of an isoplethic distribution of the substance, such that for a given injection site its concentration at any given point is a function of the three-dimensional location of that point relative to the center of the site. This assumption implies that for a given concentration between maximum and background, the locus of points characterized by that concentration will take the form of an ellipsoidal shell, and that the relative proportions of the three principal axes will be the same for all such shells. The model first considers the average shell, finding the center of the site in each section and determining the axes for this shell that best fit the punch-sample counts. The computer then uses the ratios of principal axes and the center of the injection site determined by this best-fit analysis, to project the location of each sample onto a single axis, so that counts can be considered as a function of location along this axis. The points of the gradient, so derived, fit an exponential equation that describes the gradient quantitatively. Best-fitting coefficients for three such equations describe the distribution of the label at the injection site.

Comparison of the time-staged injection sites suggested that the injectate reached approximately 70% of its ultimate extent during the injection itself, driven by pressure from the needle, and that diffusion over the next two hours carried amounts of label above background only slightly further. Comparison of distribution along the three axes suggested that the bevel and orientation of the injection needle influenced the shape and orientation of the injection distribution.

The interpretation of anatomical and behavioral studies based on intracerebral injection requires an understanding of the physical and temporal dimensions of the affected region. The method presented here provides a highly quantitative approach to this problem.

130.14 Somata of Rat Facial Nucleus: Comparison of Techniques for retrograde labeling. Kyoi Horikawa* and Ervin W. Powell, Department of Neurosurgery, University of the Rukyus, Okinawa, and Dept. of Anatomy, University of AR for Medical Sciences, Little Rock, AR 72205.

Comparisons of procedures are usually stimulated by uncertainties regarding specific features of their overall descriptions. This study of retrograde tracers was undertaken to select the procedures resulting in the greatest number of clearly labeled neurons. Horseradish peroxidase (HRP, 10%), wheat germ agglutinin (WGA, 1%), WGA-HRP conjugate (1%), and cholera toxin subunit B (CHB, 1%) are compared. The vibrissal area of the snout of 15 anesthetized adult rats was injected with 50µl of one of the four tracers listed. The animals were killed 48 hrs later. Perfusion of the fixative (800 ml) was preceded by a quick saline wash (300 ml) and followed by 800 ml of cold sucrose (20%) in phosphate buffer (PB). The brain was removed from the skull and stored overnight in 20% sucrose in PB. Thirty micron thick sections were cut through the facial nucleus. The avidin-biotin-peroxidase (ABC) immunocytochemical method was used along with the proper antibodies for localization of the transported substances. Both diaminobenzidine (DAB) and amino-ethylcarbazole (AEC) were used as chromogens. Cobalt chloride was used for intensification of the transported HRP. Labeled neuronal somata of the facial nucleus were counted and the counts were compared relative to changes in the procedures. The WGA-HRP Congugate (Vector Labs) and CHB (using anti-CHB; List Bio. Labs) labeled twice the number of neurons as did 10% HRP (Sigma VI). The greatest number of neurons was labeled when WGA (using anti-WGA) was used as a tracer. Glutaraldehyde in any concentration greater than 0.5% reduced the number of neurons labeled for all tracers, with number and intensity of the HRP-labeled neurons being least affected by this fixative. The most satisfactory fixative was 3.8% paraformaldehyde (pH 7.4), which provided the best fixation without detrimental effects to the visualization of the label. The ABC immunocytochemical technique was equally effective for CHB or WGA (same number of facial neurons labeled) with either the DAB or AEC procedures.

130.15 A NOVEL PROCEDURE FOR THE SIMULTANEOUS IMMUNOPEROXIDASE DEMONSTRATION OF TWO TISSUE ANTIGENS. A.I.Levey[1], D.B.Rye[1], J.P.Bolam[*2], and B.H.Wainer[1]. [1]Univ. Chicago, Dept. Pharm. and Phys. Sciences, Chicago IL, 60637; and [*2]Oxford Univ., Dept. of Pharm., Oxford.

Procedures for the simultaneous visualization of two tissue antigens have focused on employing immunoperoxidase and enzymatic visualization with diaminobenzidine (DAB), followed by immunolabeling of a second antigen with i)other enzyme/chromogen combinations; or ii)colloidal gold conjugated reagents. While these methods have proven useful, their light level analysis is limited by submaximal differentiation of the two chromogens, or by a difficulty in resolution of the second marker. In the present study, tissue incubated with a polyclonal rabbit-anti-tyrosine hydroxylae (TH) antisera, a goat-antirabbit bridging antisera, and rabbit PAP was employed to establish optimal conditions for the visualization of peroxidase with benzidine-dihydrochloride (BDHC). A 6-8min. incubation in .01M sodium phosphate buffer (pH=6.6) containing 0.01% BDHC, 0.002% hydrogen peroxide, and 0.02% sodium nitroprusside resulted in the labeling of TH-positive cells and processes with a coarse blue granular reaction product that was also visible with darkfield illumination. In the rat brainstem, choline acetyltransferase (ChAT) and TH were demonstrated in the same tissue employing visualization of ChAT with a monoclonal rat-antiChAT, goat-antimouse bridging antisera, rat PAP and DAB histochemistry, followed by the immunoperoxidase visualization of TH as described above. The resultant diffuse brown (DAB/ChAT) and granular blue (BDHC/TH) reaction products contrasted sharply, and allowed for a clear demonstration of topographic relationships between cholinergic and catecholaminergic cells and processes in the brainstem. Appropriate controls for residual peroxidase and antibody cross-reactivities were negative. In summary, we report here the utility of BDHC as a new, sensitive, blue chromogen in immunoperoxidase staining, alone, and for the simultaneous visualization of two tissue antigens.
Supported by the McKnight foundation and USPHS 5T32-GM07281, HD-0453, and NS-17661.

130.16 LOWICRYL METHODS FOR POSTEMBEDDING IMMUNOELECTRONMICROSCOPY. D.A. Crumrine* and K.L. Valentino (SPON: K.Buckley). Dept. Physiology, UCSF, San Francisco, CA 94143.

We have recently reported methods for rapidly embedding CNS tissue into Lowicryl K4M and using postembedding immunogold techniques (Valentino et al., J. Histochem. Cytochem., in press). We have since made several variations in these methods, in order to increase sensitivity and improve fixation.

For glutaraldehyde sensitive antigens, and especially for peptide antigens, the addition of 15% picric acid (final concentration) to 4% paraformaldehyde in (0.1 M Na cacodylate buffer), gives good ultrastructural preservation with or without the addition of glutaraldehyde. Following overnight fixation, the fixative is carefully washed out prior to en bloc uranyl acetate staining (2% aqueous uranyl acetate for 1 hour). Dehydration and embedding in Lowicryl proceed as previously described.

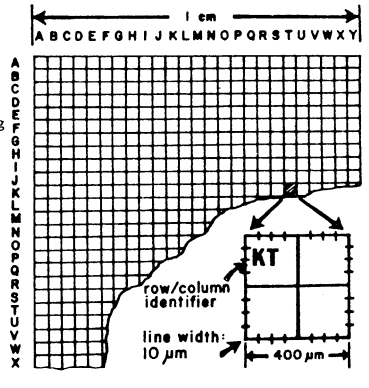
While immunogold detection of antibodies on ultrathin Lowicryl sections proves to be a useful method for many antigens, it does suffer from a lack of sensitivity. Sensitivity can be increased by variety of methods, including using an unlabeled bridging antibody, followed by colloidal gold conjugated IgG, and also by using immunoperoxidase methods. For immunoperoxidase detection, the grids are incubated in first antibody, followed by incubation in biotinylated second antibody (Vector, 1:500, 15 minutes), and incubation in streptavidin-horseradish peroxidase complex (Enzo, 1:100, 10 minutes). The reaction was developed in a solution of diaminobenzidine and H_2O_2 for 2 minutes, and grids were stained with 2% aqueous osmium tetroxide for 10 minutes and 2% aqueous uranyl acetate for 5 minutes. The HRP reaction product can be visualized easily at low magnification, and is specific.

These methods have been used to localize a large number of antigens in a wide variety of tissues, including cultured cells, and are easily adapted to double labeling studies.

Supported by Runyon-Winchell Fellowship DRG-611 and NIH grants NS16033 and NS07067.

130.17 CELL RELOCATION SLIDES FOR TISSUE CULTURE AND CLINICAL MICROSCOPY. J.B. Kirkpatrick, J.H. Lucas*, W. Wen*, L.E. Czisny* and G. Gross. Baylor College of Medicine, Houston, TX 77030; Texas Woman's University, Denton, TX 76201; Polytronix, Inc., Richardson, TX 75083.

Many investigations in neurobiology and in hospitals require specific identification of individual cells by light microscopy. Graduated stages, expensive machine-scored slides, or ink spots have served this purpose until now. These methods are cumbersome and inexact. We have produced a new type of glass cell relocation slide, using techniques from the computer industry. A coat of indium tin oxide (ITO) 300 A thick is deposited on a glass plate. This is heavier and darker than ITO used for liquid crystal displays. A pattern mask of 1 sq cm ruled into squares 200 x 200 um is projected on the ITO plate, then the extra ITO is etched away. The small squares are lettered alphabetically for specific identification. For tissue culture, one 1 cm square pattern is placed in the center of a 2 x 2 in glass plate. For clinical microscopy, the 1 cm square is repeated 2 x 4 times to fill the useful portion of a 1 x 3 in glass slide. The resulting grid survives washing and manipulations in tissue culture. It can be fixed and stained by the usual methods except for harsh basic solutions used in some silver stains. Tissue slices can be mounted over the grid. The grid is transparent, yet can be easily seen through the tissue. The ITO does not interfere with polarized light. The grid lines do not alter the spreading of cells in tissue culture, hematology or cytology, but cells adhere preferentially to the ITO unless the slide is coated with polylysine or collagen. We have found the slides useful for sequential observations in tissue culture, and for teaching and quantitative studies in the clinical setting.



130.18 Circumvention of Dehydration-Induced Morphological Artifacts in Intracellularly Stained Neurons by a Rapid DMSO Clearing Process. A. A. Grace & R. Llinas, Dept. of Physiology & Biophysics, New York Univ. Med. Ctr., New York, NY 10016. †Present Address: Depts. Psychology & Psychiatry, Univ. Pittsburgh, Pittsburgh, PA 15260.

In order to observe the morphology of intracellularly stained neurons in slices at the light microscope level, the tissue must be cleared of opaque matter. This clearing process involves first dehydrating the slice, typically by passing it through a series of increasing concentration alcohol solutions. The lipid components are then cleared by organic solvents such as xylene. Although this dehydration step is necessary to allow the xylene to have access to the lipids, it results in a large amount of slice shrinkage. Indeed, we found that the dehydration process reduces the size of guinea pig frontal cortical brain slices to 46% ± 6% of the control slice in area (mean ± SD). Furthermore, the shrinkage is not uniform over different brain regions.

We then investigated how this shrinkage affects the morphology of stained neurons contained in the slice. This was done by observing how the morphology of well-stained neurons in uncleared slices was altered at each stage of the clearing process. The dehydration process was found to induce artifacts in cellular morphology which were dependent on the type of staining technique used. Thus, cells stained with Lucifer yellow were found to decrease in size as the slice shrank, resulting in neurons less than 2/3 of their original dimensions. In contrast, cells filled with the HRP-polymerized diaminobenzidine (DAB) were apparently incapable of shrinking as the slice decreased in size. Thus, the cell soma remained its original size, but the dendrites were compressed due to slice shrinkage. This resulted in an alteration of dendritic morphology in which the previously long, straight dendritic processes assumed a bent, helical appearance. Consequently, neurons stained with HRP appeared to encompass a much larger proportion of the dehydrated slice, in addition to demonstrating an abnormally curled dendritic morphology.

In order to find a slice clearing technique which circumvented these artifacts, we investigated solvents which were capable of clearing the membrane lipids in an aqueous environment and thus obviating the need for slice dehydration. This was accomplished by using the dipolar aprotic solvent DMSO. Fixed slices placed in 100% DMSO for 15 minutes were rapidly and completely cleared of opaque matter without undergoing shrinkage. As a result, no observable morphological artifacts were noted in the stained neurons contained within the slice regardless of whether HRP or Lucifer yellow filled neurons were studied. Thus, DMSO effectively cleared the tissue slices to allow detailed morphological observations of intracellularly stained neurons without the morphological artifacts induced by the dehydration process.
(supported by USPHS NS13742 & NS07124)

131.1 GABA-TRANSAMINASE-DEHYDROGENASE ACTIVITY IN THE RAT MYENTERIC PLEXUS. A. Krantis and R.K. Harding, Dept. of Physiology, Univ. of Ottawa, Ottawa, Ont., K1H 8M5.

A number of the important criteria (Orrego, F., Neuroscience, 4: 1037, 1979) for γ-aminobutyric acid (GABA) to be classified as a neurotransmitter of the mammalian enteric nervous system (ENS) have now been satisfied, including the presence of GABA and its enzyme of synthesis (glutamic acid decarboxylase, GAD) as well as high affinity uptake sites for ^{3}H GABA, in the myenteric plexus (Jessen et al., Nature, 281: 71, 1979; Kerr, D.I.B. and Ong, J., Br. J. Pharmac., 83: 169, 1984; Krantis, A. and Kerr, D.I.B., Neuroscience Let., 23: 263, 1981.; Taniyama et al., 29: 53, 1982).

Recently, the primary enzyme for GABA degradation, GABA-transaminase (GABA-T) has been found in the myenteric plexus of humans and other mammals including the rat (Miki, et al., J. Neurochem., 40(3): 861, 1983) however the disposition of the sites of GABA degradation in the myenteric plexus have not been determined.

In this study, the presence and distribution of the degradative enzymes for GABA, GABA-T (4-aminobutyrate: 2-oxoglutarate aminotransferase)-SSADH (succinic semialdehyde dehydrogenase), in the rat myenteric plexus was investigated histochemically (using the tetrozolium salt, Nitro-BT) in laminar stretch preparations of the small intestine. Individual segments (laminar preparations) of the proximal ileum, and jejunum were incubated for 30-60 min in light proof containers with the reaction mixture: GABA (5 mg/ml), α-ketogluturate (5 mg/ml), NAD^+ (2 mg/ml), malonate (0.05 mg/ml), phenozine methosulphate (PMS, 0.2 mg/ml), Nitro-BT (1 mg/ml), in AMP_2 buffer (pH 7.6, 37°C), final volume 1 ml. In some experiments, aminooxyacetic acid (AOAA) .01 mg/ml or gabaculine 0.2 mg/ml, inhibitors of GABA-T, were present in solution throughout the equilibration and incubation period.

Strong blue-diformazan staining resulting from reduction of Nitro-BT by the transaminase step in the GABA-T-SSADH degradation of GABA was present to a scattered population of ileal and jejunal myenteric ganglion cells. By contrast, such staining was prevented in laminar preparations treated with GABA-T inhibitors. Tissues incubated in GABA free or NAD^+ free reaction mixture also remained unstained. Where ganglion cells were strongly stained, emergent processes were on occasion seen, coursing only within the ganglia. The stained cells generally comprised two types: those with a relatively round, smooth soma and eccentrically placed nucleus (usually with only one stout process and, on occasion, displaying a few smaller emergent processes); or cells with a more elongate soma and a single stout process.

These results indicate that GABA is enzymically degraded at specific sites in the rat enteric nervous system.

131.2 **SIMULTANEOUS DETERMINATION OF HOMOVANILLIC ACID (HVA), VANILMANDELIC ACID (VMA) AND 3-METHOXY-4-HYDROXY PHENYLGLYCOL (MHPG) IN HUMAN PLASMA BY HPLC WITH ELECTROCHEMICAL DETECTION.** C.J. Drebing*, G.A. Gerhardt and R. Freedman* (SPON: M. Reite). Dept. of Pharmacol., University of Colorado Health Sciences Center and Medical Research, Denver VA Medical Center, Denver, CO 80262.

Methoxylated metabolites of the catecholamine neurotransmitters in human plasma have received considerable attention in recent studies of schizophrenia and affective disorders. In addition to the measurement of the dopamine metabolite HVA, it would be advantageous to measure both MHPG and VMA, to help evaluate central and peripheral noradrenergic activity simultaneously. However, no method currently exists for the simultaneous determination of metabolites of both dopamine and norepinephrine. We now report a sensitive and reliable method for the simultaneous determination of HVA, MHPG AND VMA in human plasma by HPLC with electrochemical detection. After a simple plasma extraction, the three compounds are separated by a solvent gradient reverse phase HPLC system with subsequent dual-electrode coulometric electrochemical detection.

A portion of the proteins are removed from the human plasma samples by dilute acid precipitation and centrifugation. The supernatant is extracted with several aliquots of ethyl acetate over NaCl. These fractions are combined, evaporated to dryness and the residue is resuspended in a small volume of pH 5.0 buffer. A 20 uL portion of the sample is then directly injected into the HPLC system.

All separations are performed using an Altex 15 cm Ultrasphere-ODS 5u reverse phase column. The HPLC sytem consists of a Beckman 332 gradient liquid chromatography system, with a 420 control module, 504 autoinjector and an ESA 5100A dual-electrode coulometric eletrochemical detector. The firt electrode of the dual-electrode system is set at +.15V and is used for detection of more easily oxidizable species and initial cleanup of the eluting compounds. The second detector is set at +.36V and detects HVA, VMA and MHPG. Eluent A (pH 3.9 citrate-acetate buffer + octyl sodium sulfate) is used to first separate and detect VMA and MHPG. The gradient system is then used to change over to eluent B (eluent A with 10% methanol) for separation and detection of HVA. The gradient process has been optimized for complete sample throughput and re-equilibration of the system in 45 minutes.

The sensitivity of this method is currently better than 2 pmol/ml of plasma for all three of the species of interest. This is a considerable improvement in sensitivity as compared to many of the previously reported methods. (Supported by MH-38321).

131.3 A616U - A SELECTIVE INHIBITOR OF MAO-A WITH NEGLIGIBLE TYRAMINE-POTENTIATING ACTIVITY AND POTENTIAL ANTIDEPRESSANT ACTIVITY. H.L. White, M. Harfenist*†, O. Beek*, F. Soroko*, B.R. Cooper and R.A. Maxwell*, Depts. of Pharmacology and Organic Chemistry†, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

A616U is a novel, reversible, and selective inhibitor of monoamine oxidase-A (MAO-A) with a competitive mechanism of inhibition vs the substrates serotonin and tyramine (Ki = 0.016 μM using serotonin as substrate for MAO-A of human or rat brain). Following oral administration of A616U to rats, MAO-A inhibition in brains and livers was maximal between 3 and 7 hours after dosing and was negligible by 24 hours. Inhibition of MAO-A was completely reversible by dialysis, and no significant inhibition of MAO-B was observed. The selectivity and reversibility of the MAO-A inhibition were maintained after 10 days of chronic oral dosing.

In order to test whether the unique inhibitory mechanism of A616U might eliminate the danger of hypertensive crises in response to dietary tyramine, an animal model of this syndrome was developed in which blood pressure response to orally-administered tyramine was measured in conscious, unrestrained rats. To compare effects at equipotent and relevant antidepressant doses, A616U and other MAO inhibitors were given at oral doses which produced approximately 80% inhibition of brain MAO-A and at least 90% inhibition of liver MAO-A at the time of tyramine administration. Under these conditions A616U caused negligible potentiation of mean arterial blood pressure elevations induced by tyramine (15-90 mg/kg p.o.), while phenelzine increased the responsiveness to tyramine 3- to 10-fold.

A616U (12.5-100 mg/kg p.o.) showed dose-dependent activity in the tetrabenazine (sedation) and Porsolt models of depression in rodents, while no overt symptoms occurred after single oral doses of 1000 mg/kg. Thus A616U is a potential MAO-A inhibiting antidepressant not likely to produce a limiting side-effect observed with conventional MAO inhibitors.

131.4 **DOPAMINERGIC NEUROTRANSMISSION IN MALE RAT NUCLEUS ACCUMBENS (NA) AND CAUDATE (CN): EFFECT OF PROLACTIN.** J.C.Chen* and V.D.Ramirez (SPON:J.Kemnitz). Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We published that prolactin (PRL) increases either in vitro dopamine release or in vivo DOPAC output from corpus striatal dopaminergic terminals. Here we report: (1) the spontaneous in vivo activity of mesolimbic dopaminergic terminals of the NA in freely moving rats, and (2) the mechanism by which PRL modifies the activity of dopaminergic neurons innervating either the CN or the NA. To examine the in vivo activity of the NA, adult male rats were implanted with push-pull cannulas (PPC) and perfused within 30d post-implantation. DOPAC, HVA and 5-HIAA were determined with HPLC-EC every 20 min. The basal output of DA was non-detectable through the perfusion session (1120-2100h) while DOPAC output showed a 2-3 time increase from low afternoon values (60 pg/min) to high evening values (160 pg/min). The output of HVA oscillated between 80 to 120 pg/min while 5-HIAA remained unchanged with a mean value of 40 pg/min. This pattern was observed only in animals bearing PPC in the central region of the NA. To examine whether PRL will affect the synthesis rate of DA, an inhibitor of Dopa decarboxylase, NSD 1015 was employed. Animals were injected with ovine PRL (0.25 or 2.5 μg/kg, s.c. in the neck 30 min before NSD 1015 (100 mg/kg, i.p.) in each group and all animals were decapitated 30 min after drug injection. NSD 1015 specifically blocked decarboxylase activity in the CN and NA. Non-detectable values of Dopa in the saline groups rose to 15.4 and 19.6 ng/mg protein in the CN and NA of rats injected with the drug; whereas DOPAC levels decreased 92% and 91%, and HVA decreased 75% and 77%, respectively. Dopa concentration in rats treated with the 2.5 μg PRL were significantly lower than the control group (11.9±1.0 vs 15.4±1.2ng/mg protein) while DOPAC levels from either PRL groups (0.25 or 2.5 μg/kg) were higher (1.9±0.3 and 2.3±0.2 vs 1.0±0.2 in controls). Similar results were also found in the NA. To investigate whether PRL-evoked DOPAC increase is due to enhanced tyrosine hydroxylase (TH) activity, NSD 1015 was administered 30 min before 2.5 μg PRL and animals were then decapitated 30, 60 or 90 min afterwards. No significant differences were detected among all the groups in either CN or NA. These results indicated that PRL-evoked DOPAC stimulation was not due to an increase in CN or NA TH activity.

The spontaneous in vivo rise in DOPAC output from mesolimbic dopaminergic terminals in the NA suggests an increase in DA neurotransmission during the evening hours similar to those demonstrated earlier in the CN. It is possible that PRL may play a role in the activity of these two dopaminergic systems through changes in the release phase of DA from nerve terminals.

131.5 EFFECT OF AGE ON BILATERAL DOPAMINE METABOLISM. C.A. Taylor and T.P. Jerussi, Dept. of Pharmacology and Toxicology, Rutgers Univ., P.O. Box 789, Piscataway, NJ 08854 and Anaquest/BOC Health Care Group, 100 Mountain Ave., Murray Hill, NJ 07974.

Bilateral dopamine (DA) metabolism was investigated in two groups of female Sprague-Dawley rats approximately 10 and 16 weeks of age. Rats were tested twice for one hour, a week apart, for amphetamine (1.omg/kg, ip)-induced rotation. One week later, animals which had at least 10 full rotations/hour were decapitated at 0, 5, 10 or 20 minutes following pargyline (50mg/kg, ip) administration. DA, homovanillic acid (HVA) and 3,4 dihydroxyphenylacetic acid (DOPAC) concentrations were determined by HPLC-EC in the left and right corpora striata, olfactory tubercles (TO) and frontal cortices (FC).

There were no significant steady state left-right differences in the striata of both groups. However, each group had significantly greater DOPAC turnover in the left striata. In addition, in the 16 week old rat DOPAC turnover and steady state levels were significantly greater in the striatum ipsilateral to the direction of rotation.

Steady state DA, DOPAC and HVA were significantly greater in the TO of the 16 week old animals. Moreover, steady state DA and DOPAC levels and DOPAC turnover were significantly greater in the right than the left TO of the 16 week old animals, but no left-right differences were not evident in the younger rat. In contrast, ipsilateral-contralateral differences were not apparent in the TO of the 16 week old animals, yet DOPAC turnover in the younger rats was significantly greater in the ipsilateral TO.

Steady state DA and HVA levels were significantly greater in the FC of the 16 week old rat. Significantly greater DOPAC turnover was evident only in the left FC of the older rats, and there were no ipsilateral-contralateral differences in the FC of both groups.

It is evident from these data that the brains of older (16 weeks) rats are more lateralized with respect to dopamine metabolism, and the asymmetrical development of dopaminergic systems appears to develop at different rates.

131.6 IMIPRAMINE METABOLISM IN RATS IS INFLUENCED BY AGE AND GENDER. M.A. Wilson and E.J. Roy. Neural & Behavioral Biology Program and Dept. of Psychology, University of Illinois, Champaign, IL 61820.

Previous studies have suggested that both age and gender can influence the metabolism of the antidepressant imipramine. Studies from our laboratory examining changes in hypothalamic imipramine binding sites following chronic imipramine treatment in rats strongly suggested that age altered the neural retention of imipramine metabolites following such treatment (Wilson and Roy, 1984).

In the present study we examined the effects of age and gender on the levels of imipramine metabolites in the hypothalamus-preoptic area (HPA) and serum of rats 24 hours after the cessation of chronic (14 day) imipramine treatment. To examine whether the effects of age and/or gender were the result of differences in the initial metabolism of imipramine, age and sex were also analyzed for their influences on levels of imipramine (IMI) and its metabolite desmethylimipramine (DMI) in the HPA and serum one hour after a single imipramine injection. IMI and DMI were measured using high pressure liquid chromatography.

Age significantly increased the levels of DMI remaining in the HPA and serum following chronic imipramine treatment (CIT). Hypothalamic levels in middle-aged females were increased 220% compared to juvenile females (1.5 months old). HPA and serum also showed sex differences in the level of DMI remaining after CIT, with females having higher levels.

Repeated sampling of serum from individual juvenile and middle-aged animals showed contrasting profiles of serum imipramine metabolites after a single imipramine injection. Juvenile animals showed a sharp rise in DMI and a rapid decline in total metabolite levels; middle-aged females showed a prolonged elevation of serum DMI levels. This pattern was confirmed by the HPA tissue concentrations of IMI and DMI one hour after a single imipramine injection. Juvenile animals furthermore exhibited a sex difference in the metabolite profile, indicating more rapid metabolism of imipramine in males at this age.

The results indicate that in rats, female animals and older animals both show a reduction in the initial metabolism of imipramine which leads to an enhanced accumulation of DMI in the brain and serum during chronic imipramine administration. These changes in imipramine metabolism may be important to the neurochemical changes observed after chronic imipramine treatment in animals and, perhaps, the clinical efficacy of such antidepressant treatments in humans.

131.7 EXPOSURE TO 60-HZ ELECTRIC AND MAGNETIC FIELDS ALTERS BIOGENIC AMINE METABOLITE CONCENTRATIONS IN NON-HUMAN PRIMATE CEREBROSPINAL FLUID. R. F. Seegal. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201.

A major concern of exposure to extremely low frequency (ELF, 1-300 Hz) electric (E) and magnetic (B) fields has been the delineation of possible adverse effects on the central nervous system (CNS). Our results suggest that exposure to 60-Hz sinusoidal E and B fields significantly decreases cerebrospinal fluid (CSF) concentrations of biogenic amine metabolites in the non-human primate, Macaca nemestrina (pig-tailed macaque).

We have exposed six M. nemestrina to uniform 60-Hz E and B fields of up to 30kV/m and .9 gauss (g) for 63d. The monkeys are exposed to either sham or actual E and B fields for 18 h/day. The first 21d period consists of sham exposure to determine baseline neurochemical values. Animals are then exposed to 3, 21d intervals of E and B fields of 3kV/m and .1g, 10kV/m and .33g and 30kV/m and .9g. The final 21d interval consists of a post-exposure sham period to determine if possible alterations in CNS function induced by exposure to E and B fields persist. At the end of each 21d interval homovanillic acid (HVA), a metabolite of dopamine and 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, were determined in lumbar CSF of M. nemestrina following pretreatment with intravenous probenecid (100 mg/kg). Concentrations of HVA and 5-HIAA were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ECD).

Results are expressed as a percentage of the concentration of the biogenic amine metabolites determined during the initial pre-exposure period. No animal demonstrated an increase or lack of change during exposure. E and B field exposure resulted in a 20-30% decrease in CSF-HVA with the greatest change observed at the 30kV/m and .9g exposure level. CSF-5-HIAA concentrations decreased by approximately 10-15% with no discernible dose-response relationship.

These findings are important because: (1) this study is among the few that have reported significant, repeatable neurochemical changes following exposure to E and B fields; (2) the study was undertaken in a species that is neurochemically similar to man and (3) they suggest significant decreases in the activity of either central biogenic amine neurons or the degradatory enzymes that inactivate the neurotransmitters dopamine and serotonin.

Supported by the Power Authority of State of New York.

131.8 CARBACHOL STIMULATION OF POLY-PHOSPHOINOSITIDE TURNOVER IN [^{3}H]-INOSITOL-LABELED RAT BRAIN SYNAPTOSOMES. H-M. Huang* and G.Y. Sun. Biochem. Dept. and Sinclair Comparative Med. Res. Farm, Univ. of Missouri, Columbia, MO 65203.

Although muscarinic cholinergic stimulation of polyphosphoinositide breakdown by the phosphodiesterase has been well-demonstrated in brain and other tissues, most studies have been carried out with intact tissue slices preincubated in a medium containing the labeled precursor. Studies of synaptosomal phosphoinositides metabolism have been confronted with difficulties, mainly because synaptosomes lack the essential ingredients for active de novo biosynthesis of membrane phospholipids. Our present study describes a procedure for labeling the phosphoinositides (PI, PI-P and PI-P_2) with ^{3}H-inositol through the CMP and Mn^{2+}-dependent PI:myo-inositol exchange route as described by Berry et al. (Biochem. Biophys. Res. Commun. 112:817-821, 1983). Incubation of synaptosomes under this condition resulted in large incorporation of [^{3}H]-inositol into PI, but PI-P and PI-P_2 were only slightly labeled. After the prelabeled synaptosomes were washed, they were resuspended in Krebs-Ringer bicarbonate (KRB) buffer supplemented with glucose and ATP. Incubation of the prelabeled synaptosomes in this medium alone elicited a time-dependent decrease in labeled PI but the radioactivity was not recovered in any of the water soluble inositol phosphates (i.e., IP, IP_2 and IP_3). The decrease in label may be attributed to the Ca^{2+}-dependent phospholipase A_2 which utilizes PI and PC as substrate. When prelabeled synaptosomes were incubated in the KRB medium containing LiCl (10 mM), addition of carbachol elicited an obvious increase in labeled IP which accumulated with time of incubation. There was a small increase in IP_2, but practically no label was recovered in the IP_3 fraction under stimulated or non-stimulated conditions. Very little change in labeled PI-P and PI-P_2 was observed after carbachol stimulation, but there was a decrease in labeled PI concomitant with the increase in labeled IP. Results indicate that carbachol stimulated the turnover of the entire phosphoinositide cycle. The inositol-labeled synaptosomes provide a feasible system for studying the receptor-mediated poly-PI turnover mechanism and subsequently drugs which may affect this metabolism. (Supported in part by AA06661 from NIAAA.)

131.9 PHOSPHORYLATION OF HUMAN CHOLINE ACETYLTRANSFERASE
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Choline acetyltransferase (ChAT, EC 2.3.1.6), the terminal biosynthetic enzyme for the neurotransmitter acetylcholine, is located in the cytosol of the pre-synaptic cholinergic neurons. In recent years the study of cholinergic function has taken on a new interest in relation to Alzheimer's disease with ChAT being implicated in both the etiology (Perry, et al. [1982] Neurosci. Lett. 33, 311-315) and the possible therapy (Hefti, et al. [1984] Brain. Res. 293, 305-311) of this disorder. However the control of cholinergic function is poorly understood although mounting evidence shows an important part of neuronal control to be achieved through protein phosphorylation. Thus both neurotransmitter synthesizing enzymes (tyrosine hydroxylase) and neurotransmitter receptors (acetylcholine receptor) are now known to be controlled by phosphorylation. We report here, for the first time, that human ChAT can be phosphorylated in vitro.

Human ChAT was purified from placenta using immuno-affinity purification. This placental enzyme has been shown to be identical to the human brain enzyme (Bruce, et al. [1985] Fed. Proc. 44, 1634). The purified human ChAT was phosphorylated in vitro by kinase/s present in a cell free extract of rat brain. This phosphorylation was shown to be both time and concentration dependent, with maximum phosphorylation achieved after a 5 min incubation. A study of the subcellular location of the rat brain kinase/s involved in this phosphorylation showed both a membrane and cytosolic location. Phosphorylation was not stimulated in the presence of either cAMP or cGMP (final conc = 1 μM) but was completely inhibited in the presence of EGTA (final conc = 1 mM). Amino acid analysis of the phosphorylated ChAT showed serine to be the exclusive phospho-amino acid. The role of this phosphorylation in relation to the function of ChAT will be discussed.

131.10 ASSEMBLY OF MONOMERIC AChE INTO TETRAMERIC AND ASYMMETRIC FORMS
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A pulse-chase experiment was performed in embryonic rat myotube cultures to examine possible precursor-product relationships between the various forms of AChE. Cultures were first treated with 5 mM methanesulfonyl fluoride (MSF) to irreversibly inactivate almost all (98%) of the active AChE present in the cells. The MSF was washed from the cultures and medium containing 0.1 μM paraoxon was applied to the cultures for a period of 55 minutes. The paraoxon inactivated AChE synthesized by the myotubes during this labelling period by diethylphosphorylating the enzyme at its active site. Diethylphosphorylated AChE can be reactivated by treating it with 1-methyl-2-hydroxyimino-methylpyridinium (2-PAM). Labelled AChE then is defined as AChE that regains enzyme activity following treatment with 2-PAM. At the completion of the labelling period the paraoxon was washed off the cultures. MSF (20 mM) was again applied to the cultures immediately before they were harvested to inactivate any unlabelled AChE present that could interfere with the measurement of labelled AChE. Cells were harvested at 0 and 2 hours after the labelling period. AChE was sequentially extracted to separate globular forms from asymmetric forms. Samples were assayed before and after treatment with 2-PAM in order to determine the amount of labelled AChE present in the samples. Samples were also applied to sucrose gradients in order to separate the various forms of the enzyme. The results of four such pulse-chase experiments show that the amount of labelled G_1 AChE activity (expressed as picomoles of acetylcholine hydrolyzed per minute per culture dish) decreased from 336 to 150 ($p<.01$) during the chase period, while labelled G_4 AChE increased from 14 to 19 ($p<.05$) and labelled asymmetric forms increased from 14 to 19 ($p<.025$). These results indicate that complex forms of AChE are assembled from globular precursors. Additional experimentation indicates that the A_{12} form, in particular, is assembled from precursor forms. Since a pulse-chase experiment is required to demonstrate hypothesized precursor-product relationships, this data constitutes the first definitive proof that more complex forms of AChE are assembled from simpler precursor forms.

DISEASES OF THE NERVOUS SYSTEM: GENETIC DISEASES, MODELS

132.1 **Tremor Measurement in an Inherited Feline Tremor.**
G.A.Hegreberg, K.P.Hines*, J.M.Frame*, D.E.Norby*, and M.H.Ratslaff*. College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Inherited feline tremor (IFT) is a progressive neurodegenerative disorder transmitted as an autosomal recessive trait. Tremors, the earliest clinical manifestation, are observed in kittens as early as 2 weeks of age. The tremors are apparent in all affected kittens by the age of 4-5 weeks. Initially, the tremors are fine and of low amplitude involving the head, tail, and limbs. The tremors progress in severity with age and at the clinical apogee (8 to 16 weeks of age) are throbbing, rhythmical, and of high amplitude. Classified on the basis of appearance, the tremors are both action and postural. The tremors are absent when the kittens are completely at rest or asleep. Other neurologic and behavioral changes occur as the disease progresses, including posterior paresis, generalized seizures, limb rigidity, visual impairment, unprovoked agitated behavior, affective response, inadequate grooming practices, repetitious activity, and catatonia.

The tremor frequency was measured in 9 affected kittens ranging in age at the time of measurement from 21 to 44 days. The tremors were measured using a tremor plate devise consisting of a box resting on piezoelectric crystal sensors. Signals were recorded on a TEAC data cassette recorder. Hard copies of the data were obtained from a Honeywell oscillographic recorder. The tremors in all kittens were similar and the frequencies ranged from 5.8 to 6.9 Hz. The mean and 1 standard deviation of the tremor frequencies were 6.3+0.4 Hz. The types of tremor (action and postural) and the frequency of the tremor (approximately 6 Hz) in this feline disorder are similar to essential tremor and Parkinsonism of people.

This study was supported in part by NIH grant RR 00515, the WSUCVM locomotion laboratory, and Washington State University.

132.2 PREDICTIVE TESTING FOR HUNTINGTON'S DISEASE AND GENETIC COUNSELING: SCIENTIFIC AND ETHICAL CONCERNS.
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Huntington's disease (HD) is a serious and debilitating hereditary disease. It is an autosomal dominant disease with a late onset. Offspring of those with HD have a 50-50 chance of inheriting it, or, if they carry the gene, of passing it on. Until disease onset at approx. 40 yrs., those who carry the gene cannot be detected. Several surveys indicate that, in spite of the absence of preventive treatments, a safe, accurate and reliable test for HD is sought by many at risk because of the information it would provide for personal decisions regarding career, family planning, etc.

Recent research using recombinant DNA technology has identified a marker genetically linked to HD (Gusella et al., 1983, Nature 306:234). There are limitations to a predictive test based on this work, as well as a number of ethical and policy concerns associated with genetic screening in general. Yet it is likely that a testing program for HD based upon a genetic marker can and will be developed in the near future.

A marker-based test has some limitations. Because it requires samples of DNA from relatives of those at risk, and is dependent upon the form of the marker carried by those relatives, it would not be universally available or informative. In addition, because of the possibility of genetic crossover, the test can only provide a more accurate prediction of the probability of inheriting the disease, not a definite answer regarding gene-carrier status. Unlike other predictive tests for HD, the marker-based test could be done prenatally.

Given the substantial emotional impact and the significance of test results, what are the essential components of a predictive testing program? What are the appropriate roles of scientific and health care professionals, lay organizations, and effected individuals and their families in the development and implementation of such a program? Prenatal testing coupled with abortion raises issues associated with the abortion of a fetus that has the potential for several decades of normal life before the onset of disease. What are the attitudes and assumptions of health care professionals and others that may affect the decisions individuals feel able or compelled to make? These ethical and policy issues will be explored and discussed.

132.3 LATE ONSET MOTONEURON DEGENERATION IN A NEW MUTANT MOUSE. Anne Messer, Norman L Strominger, Mary Zotta* and Lorraine Flaherty. Wadsworth Center for Labs and Research, N.Y. State Dept. of Health, Albany, N.Y. 12201; Dept. of Anatomy, Albany Medical College, Albany, N.Y. 12208.

A late-onset, hereditary neurodegenerative disease has recently been identified in a C57Bl/6 substrain, in the mouse colony of the immunogenetics laboratory of Dr. L. Flaherty. Affected animals have a progressive deterioration of motor function (stiff-legged gait, abnormal limb placements and grasping, and finally paralysis), with a variable age of onset (beginning at 5 - 11 months of age), and moving from rear to forelimbs. Few survive beyond 12 months. Preliminary neuropathology shows dramatic degeneration of spinal cord motoneurons, more severe in the lumbar-saccral than in the cervical and thoracic regions, as well as some pathogenesis in hypoglossal and dorsal vagus nerves, red nucleus, restricted areas of the cerebral cortex and dorsal root ganglia. The mutation affects both sexes, and all affected animals seem to be fertile, although the motor dysfunction restricts breeding after 7-8 months of age. The late age of onset, symptoms and pathology all suggest that this mutant is a good model of amyotropic lateral sclerosis (ALS).

The pathology is consistent with severity of the symptoms, irrespective of the age of onset. Severely affected animals showed loss of many motoneurons, paticularly in the lower spinal cord, with substantial pathology obvious in most of the remaining large neurons. Mice with only slight ataxia showed some clearly pathologic cells, but many more healthy motoneurons than the cases above. Finally, 2 totally asymptomatic 3-month-old mice of the same substrain showed clear evidence of isolated pathological neurons in the lower spinal cord, with one case more prominent than the other. Controls were normal in all cases. Overall, there seems to be an ascending progression of the pathology, from anterior horn cells of the lower spinal cord to highest brainstem motor nuclei. Electron microscopy to further examine the nature of the pathology is in progress.

The mutation is on a C57Bl/6 background which carries a recombinant 17th chromosome. It appears that this mutation arose at the same time or shortly after the recombinational event, which affects the major histocompatibility region. Thus, the recombination site and the mutation may be closely linked, although the presence of an independent mutation cannot be ruled out. Since either symptoms or pathology have been evident in every mouse of this strain examined to date, it appears that the gene is homozygous. We cannot distinguish between recessive and semi-dominant inheritance, however, until the obligate heterozygotes are older.

This work was supported in part by a grant from the Muscular Dystrophy Association.

132.4 PRIMARY AND MODIFIER GENE ASSIGNMENTS IN JOSEPH DISEASE. A.I. Grossman,* L.A. Warmoth,* P.M. MacLeod* and R.N. Rosenberg (SPON:R.M. Stewart) Dept. Neurology, UTHSC, Dallas, TX 75235; Dept. Pediatrics, Queen's University, Kingston, Canada K713N6.

Joseph disease (JD) is a distinct autosomal dominant neurological disorder presenting either symptoms of spasticity, rigidity, and dystonia in the first to third decades of life or dysarthria, cerebellar ataxia, and amyotrophy in the fifth to seventh decades of life. Both symptoms and neurological findings of neuronal death and glial proliferation, most notably in the cerebellar cortex, irreversibly progress to death after 10-20 years of illness. Analysis of a large number of JD families revealed that there are two genes which determine the number of affected descendents in a family. They are the JD gene which actually causes the disease and the modifier (Mo) gene which reduces severity of JD or totally represses the expression of the JD gene. The JD gene has been preliminarily assigned on the short arm of chromosome 1 using protein and blood type polymorphisms (C.J. Forster-Gibson et al., Abstract. Amer. Soc. Hum. Genet., 1984, N.E. Simpson et al., Abstract. Human Gene Map, 1985). Using cDNA probe for Amy gene (chromosome 1, 1p21) we found a cosegregation of particular haplotypes with JD patients in two families. The existence of a number of pedigrees with a "skipped generation" supported a hypothesis of the presence of the Mo gene in some families. This gene was tentatively assigned to short arm of chromosome 2 near EAP-1 locus (2p23). The DNA hybridization of POMC probe (chromosome 2, 2p23) with genomic DNA's of one family indicated that the Mo gene might be situated closer to EAP gene than to POMC gene. Activity of mitochondrial and supernatant glutamate dehydrogenase (GDH) (localization is unknown) and malate dehydrogenase (MDH) (chromosome 2, 2p23) from cerebellar cortex of JD patients and dominantly inherited OPCA have been compared with corresponding fractions of enzymes from clinically normal patients. A significant increase of GDH activity was found in the supernatant fraction of JD and OPCA patients compared with controls and in OPCA patients compared with JD. A significant decrease of MDH activity was found in both mitochondrial and supernatant fractions of OPCA patients compared with control and JD patients. The differences in GDH and MDH activities among JD, OPCA and control patients did not appear to result from altered K_m's, but rather was consistent with the amount of available total enzyme activities.

132.5 PRENATAL DYSMYELINOGENESIS IN CAPRINE β-MANNOSIDOSIS. K.L. Lovell, M.Z. Jones, N.K. Ames*, and C. Blaze*. Depts. of Pathology and Large Animal Clinical Science, Michigan State University, E. Lansing, MI 48824

Caprine β-mannosidosis, an autosomal recessive deficiency of glycoprotein catabolism, is associated with a deficiency of β-mannosidase activity, tissue accumulation of oligosaccharides, and cytoplasmic vacuolation in many cell types in the nervous system and viscera. Severe neurological deficits present at birth include an intention tremor, ataxia and inability to stand. Morphological changes previously reported in neonatal animals, in which myelination is nearly complete, include severe myelin paucity in the brain, with consistent regional variation. In the present study, fetuses at 124/150 days gestation were obtained to investigate prenatal development of morphological changes in β-mannosidosis.

An obligate carrier doe was artificially inseminated with semen from an obligate carrier male. The presence of triplets was determined by radiography. At 124 days gestation, Cesarian section was performed under halothane anesthesia. After each fetus was removed from the uterus, heart blood was obtained for measurement of β-mannosidase activity and perfusion was initiated via the heart with glutaraldehyde/paraformaldehyde solution. Sections from the brain were taken for embedding in paraffin and Epon-Araldite. Measurement of plasma and kidney β-mannosidase activities demonstrated that one of the three fetuses was affected and thin layer chromatography of kidney extracts showed the accumulation of oligosaccharides only in the affected animal. Gross examination of brain sections indicated ventricular enlargement and a deficiency in white matter in the affected animal, sufficient to serve as a diagnostic indicator in these fetuses.

Light microscopic examination of white matter in selected brain regions of the two unaffected fetuses revealed varying degrees of myelination, consistent with that expected for 124 day goat fetuses. Substantially less myelin was present in the affected fetus than in the control fetuses. In addition, glial cell morphology was abnormal in the affected fetus, even in regions where myelination had not yet begun. These results indicate that the pathogenetic process leading to cellular abnormalities and myelin deficits in β-mannosidosis has been initiated prior to 124 days gestation. Further analysis of the prenatal CNS lesions will contribute to a better understanding of the pathogenesis of dysmyelinogenesis in this genetic disorder.

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133.1 THE EARLY DEVELOPMENT OF THE OLFACTORY PLACODE IN XENOPUS LAEVIS EMBRYOS. H.Kim*, P.P.C. Graziadei and G.A. Monti Graziadei. Department of Biological Science, Florida State University, Tallahassee FL 32306-3050

The olfactory organ originates from the so-called olfactory placode. However, the early stages of the development of this structure are not well known. We have used the amphibian Xenopus laevis to study in some detail the structural and ultra= structural development of the organ and the beginning of its ana= tomical connections with the brain. We have been primarily conce= rned with the morphological differentiation of the sensory neurons their initial growth and maturation patterns, the growth of their axons and the contacts of that these axons establish with the primitive prosencephalic vesicle before and after the development of the glomerular structures.

Xenopus laevis embryos, stages 23 to 37/38 were fixed by immersion in 1% glutaraldheyde and 0.4% paraformaldheyde in buffer and post-fixed in OsO_4. Following Araldite 506 embedding, the preparations were sectioned at 1 micron thickness and inter= mediate thin sections were also obtained for ultrastructural obser= vation.

We have been able to confirm that neurons originate exclusive= ly from the nervous layer of the ectoderm while the supporting cells originate from the non nervous layer of the ectoderm. The two populations of cells commingle by stage 28, the first axons originate from the placode by stage 29/30 and the first axons penetrate the C.N.S. by stage 32. The modalities of axonal growth and the first contacts with the neuronal populations of the forebrain will be illustrated at both LM and TEM.

(This work was supported by a Grant from NSF, BNS 8006803 to PPCG).

133.2 NEUROGENESIS IN THE RAT PRIMARY OLFACTORY CORTEX. Shirley A. Bayer, Indiana-Purdue Univ., Indianapolis, IN 46223.

Neurogenesis in the rat primary olfactory cortex was examined with ^{3}H-thymidine autoradiography. The experimental animals were the offspring of pregnant females given an injection of ^{3}H-thymidine on two consecutive gestation days. Nine groups of embryos were exposed to ^{3}H-thymidine on E13-E14, E14-E15,....E21-E22, respectively. On P60, the percentage of labeled cells and the proportion of cells originating during 24 hour periods were quantified at selected anatomical levels of the dorsal peduncular cortex (DPC), anterior piriform cortex (APC), and posterior piriform cortex (PPC). Neurons in the DPC originate between E14-E20 in an older caudal to younger rostral gradient of neurogenesis. Caudal parts of the DPC also have an older lateral to younger medial neurogenetic gradient. The most lateral part of the DPC does not have the typical older deep to younger superficial gradient seen throughout the rest of the DPC. Neurons in the APC, lying lateral to the caudal anterior olfactory nucleus and olfactory tubercle, are generated mainly between E14-E18 in an older caudal to younger rostral neurogenetic gradient. Neurons in the PPC, lying lateral to the caudal olfactory tubercle and the entire amygdala, are generated mainly between E14-E17 simultaneously along the rostrocaudal plane. Throughout the entire piriform cortex, deep cells are generated earlier than superficial cells. Superficial cells in the piriform cortex have some additional neurogenetic gradients: In the APC, ventromedial cells originate earlier than those located dorsally and laterally. In the PPC, younger neurons are located at middle dorsoventral levels while older neurons lie above and below. The neurogenetic gradients in the primary olfactory cortex, along with patterns of neurogenesis throughout the olfactory peduncle can be related to the termination patterns of afferents from the main olfactory bulb.

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133.3 Tactile stimulation during postnatal olfactory experience facilitates the subsequent neural and behavioral response of rats to that odor. R.M. Sullivan* and M. Leon. Dept. of Psychobiology, University of California, Irvine Ca 92717.

Rat pups develop a behavioral attraction to an odor previously associated with tactile stimulation which mimics maternal licking. Those experiencing the odor without such stimulation do not develop an attraction to the familiar odor (Sullivan, Hofer & Brake, submitted). Rat pups also have an enhanced neural response in specific olfactory bulb glomeruli to odors that were experienced during such stimulation (Coopersmith & Leon, 1984). The present study determined whether this enhanced neural response is dependent simply on odor familiarity, or if the odor must be accompanied by stimulation.

Olfactory experience consisted of 10 min per day from postnatal day 1 to 18 of either: 1) peppermint odor and vigorous stroking of the back with a brush to mimic maternal licking, 2) peppermint odor only, 3) stroking only, and 4) neither stimulus. On day 19, all pups were injected with 14C-2-deoxyglucose (200uCi/kg) and given a 45-min test exposure to peppermint in an apparatus which recorded pup respiration patterns. The developed autoradiographs were then analyzed with the aid of a computer-based image processing system which permits quantitative analysis and comparison of specific olfactory bulb area.

Pups that experienced peppermint simultaneously with tactile stimulation demonstrated both a behavioral attraction and enhanced 2-DG uptake in specific olfactory glomeruli (1.5-2.1mm from rostal pole of the olfactory bulb) upon subsequent presentation of peppermint. Pups in the remaining three groups demonstrated neither the behavioral attraction nor an enhanced 2-DG uptake to peppermint.

These results indicate that odor familiarity alone is not sufficient to induce an enhanced neural response. Rather, it requires association of odor experience with stimulation of the type normally experienced in the nest.

133.4 THE ENHANCED NEURAL RESPONSE INDUCED BY POSTNATAL OLFACTORY EXPERIENCE IS ODOR-SPECIFIC AND LONG-LIVED. R. M. Coopersmith* and M. Leon. (SPON: E. DEMET) Dept. of Psychobiology, University of California, Irvine CA 92717.

Norway rat pups develop a preference for familiar odors which is accompanied by an enhanced response in their olfactory systems. After 18 daily exposures to peppermint odor, the olfactory bulbs of the odor-familiar pups show an increased uptake of 14C-2-deoxyglucose (2-DG), in three groups of glomeruli on the lateral aspect of the bulb, 1.5-2.1 mm from its rostral pole. The enhanced response is not due to increased respiration. We now report that the enhanced response is long-lived, and can be induced by familiarity with other odors.

To measure the duration of the enhanced response, pups were exposed to either peppermint odor or clean air on postnatal days 1 to 18. Pups were weaned on day 25 and were not disturbed until day 85, when we implanted catheters into their jugular veins. On day 90, animals were injected with 2-DG (150 uCi/kg, i.v.) and given a 45-min test exposure to peppermint. Just as in 19-day old animals, three complexes of laterally located glomeruli showed significantly higher uptake in the peppermint-familiar animals than in the peppermint-unfamiliar animals. Thus, the enhanced neural response induced by neonatal olfactory experience persists for least 90 days.

On postnatal days 1 to 18, rat pups were exposed to either cyclohexanone odor (cyclohexanone-familiar) or clean air (cyclohexanone-unfamiliar). On day 19, all pups were injected with 2-DG (200 uCi/kg) and given a 45-min test exposure to cyclohexanone in an apparatus that allowed us to analyze respiration rate and sniff frequency. Autoradiographs prepared from olfactory bulb sections were analyzed with a computer-based digital image processor which allowed us to make quantitative comparisons between uptake sites.

Both groups of animals showed reliable uptake in a medial group of glomerular complexes 2.5-2.9 mm from the rostral pole of the bulb. In the cyclohexanone-familiar pups, uptake in these areas was significantly higher than in the cyclohexanone-unfamiliar pups. Respiration frequency pattern did not differ between the groups. The enhanced response therefore is odor-specific.

133.5 MORPHOLOGICAL AND METABOLIC CHANGES IN THE OLFACTORY BULB ACCOMPANY THE ENHANCED NEURAL RESPONSE TO FAMILIAR ODORS IN RAT PUPS. C. C. Woo*, R. M. Coopersmith*, and M. Leon. (SPON: D. Aswad) Dept of Pyschobiology, University of California, Irvine, CA 92717.

A familiar odor induces both a behavioral attraction and an increased 14-C-2-deoxyglucose (2-DG) uptake in olfactory bulb glomeruli specific to that odor in Norway rat pups. In this experiment we characterized the glomerular response with other metabolic and morphological techniques. Our strategy was to use the 2-DG technique to localize the glomerular areas of enhanced activity. The areas of enhanced 2-DG uptake were then matched with stained sections. Alternate sections were histochemically processed for cytochrome oxidase (CO), succinic dehydrogenase (SDH), or glycogen phosphorylase (GP). Other pups had alternate sections stained with a silver impregnation technique.

Rat pups received experience with either peppermint odor (peppermint-familiar) or clean air (peppermint-unfamiliar) on postnatal days 1-18. On day 19, all pups were injected with 2-DG (200 uCi/kg) and given a 45-min test exposure to peppermint in an apparatus that recorded their respiration rate and sniff frequency. Autoradiographs and stained sections were analyzed using a computer-based digital image processor and a light microscope.

In the peppermint-familiar pups, the areas of enhanced glomerular response identified in the autoradiographs corresponded to very large glomeruli which protruded into the external plexiform layer. This modification was not seen in the peppermint-responsive areas of the peppermint-unfamiliar pups. Heavy GP staining was also seen in the internal plexiform layer opposite these large glomeruli. In addition, glomeruli within the areas of enhanced 2-DG uptake had heavier CO and SDH staining at their perimeters and lighter staining centers, a pattern not seen in other glomeruli in either group. Early odor experience therefore induces morphological and metabolic changes in the olfactory bulbs of young rats which may underlie the increased neurobehavioral response to familiar odors.

133.6 NEUROPHYSIOLOGICAL CORRELATES OF ENHANCED 2-DG UPTAKE TO FAMILIAR ODORS IN THE OLFACTORY BULB OF NEONATAL RATS. D.A. Wilson, R.M. Sullivan* and M. Leon, Dept. of Psychobiology, Univ. of California, Irvine, CA.

Rat pups have an enhanced olfactory bulb response to familiar odors, as reflected by an increased 14C 2-DG uptake in specific glomeruli (Coopersmith & Leon, Science, 1984, 225:849-851). The present study examined the neurophysiological correlates of this response by recording single units in the mitral cell layer near the areas of enhanced glomerular 2-DG uptake.

Pups were exposed for 10 min/day to either peppermint-scented air or to clean air for the first 18 days of life. All animals received perineal stimulation during the sessions to facilitate odor preference acquisition. On day 19, animals were exposed to peppermint and tested for 1) their 2-DG glomerular uptake, 2) their behavioral attraction, or 3) their mitral cell response.

Single units were recorded in the mitral cell layer in the ventral-lateral area of the olfactory bulb (1.5 - 2.1 mm from rostral pole), corresponding to the area of enhanced 2-DG uptake. Single mitral cells were isolated and identified by depth and/or antidromic activation. Following isolation of a cell, peppermint or orange odor were randomly presented to the external nares for 4 sec, 3 times, with at least 60 sec between presentations. Responses were classified as excitatory when there was an increase in peak frequency above baseline variability, or inhibitory when peak frequencies were less than mean baseline frequency.

Odor-familiar pups preferred the odor and had an enhanced uptake of 2-DG. The mitral cells of such pups demonstrated significantly fewer excitatory and more inhibitory responses to peppermint than controls ($p < 0.001$). There were no differences in excitatory and inhibitory responses to orange odor between groups ($p > 0.10$).

Neonatal exposure to odors selectively alters subsequent mitral cell responsiveness to that odor. Since the enhanced 2-DG uptake to familiar odors appears not to be due to increased mitral cell activity, it may be due to increased activity in either external tufted cells (Macrides, 1984) or inhibitory interneurons.

133.7 GRANULE CELL PLASTICITY IN THE OLFACTORY BULB OF THE MUTANT MOUSE PURKINJE CELL DEGENERATION. Charles A. Greer. Sec. of Neurosurgery and Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

The mutant mouse Purkinje Cell Degeneration (PCD) loses all of its olfactory bulb (OB) mitral cells (MCs) between 12 and 20 weeks postnatal (Greer & Shepherd, 1982). Due to MC loss a subpopulation of the interneurons, granule cells (GCs), are deprived of the reciprocal dendrodendritic synapses normally present and contributing to the network of OB local circuits. Ultrastructural studies have demonstrated that some of the dennervated GCs establish new reciprocal dendrodendritic synapses with another class of OB primary neuron, tufted cells (TCs), which are apparently not affected by the pcd gene (Greer, Halasz & Shepherd, 1983). To further examine the apparent plasticity of GCs and their capacity for reorganizing following dennervation, quantitative Golgi analyses were conducted on dendritic and spine morphology following MC loss.

Affected PCD mice and normal littermate controls were processed for Golgi-Kopsch staining at 16 - 18 weeks postnatal. Granule cells were reconstructed with camera-lucida at 1,000X oil immersion. The distribution and morphological characteristics of dendrites and spine like appendages were quantitatively established.

In littermate control mice the distribution of GCs, their dendritic processes and their spines were comparable to that previously reported for rats. At least 3 catagories were broadly defined: 1) deeply placed somas whose dendrites innervated the deep EPL; 2) superficially placed somas whose dendrites innervated the superficial EPL; and 3) variably placed somas whose dendrites innervated the entire width of the EPL. Mean spine density for these groups was 0.21/1um of dendrite.

In affected PCD mutants the morphology and distribution of GC dendrites was dramatically altered. Dendrites typically extended across the entire width of the EPL in coronal section and did not exhibit the sublaminar distribution found in control mice. Although the mean number of spines/GC decreased by 20%, an accompanying decrease in the length of dendrites resulted in spine density remaining at 0.21/1um. In control mice dendrites were generally oriented radially and somewhat obliquely through the EPL, however, many dendritic branches in the PCD mice assumed a horizontal orientation within the EPL.

The data thus far indicate an extensive reorientation of GC processes in PCD mice following MC loss. Prior EM analyses demonstrated heterologous synaptogenesis following MC loss. The current data reveal a marked reduction in numbers of spines which suggests that not all spines establish new synapses. This may indicate that the availability of synaptic sites on TCs influences, in part, the survival of spines and the extent to which synaptic reorganization of GCs within local circuits may occur.

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133.8 EFFECTS OF EARLY OLFACTORY DEPRIVATION ON THE INTERNAL GRANULAR CELLS OF THE OLFACTORY BULB. E. MEISAMI AND E. NOUSHIN-FAR*. Dept. Physiol.-Anat., Univ. Calif., Berkeley, Calif. 94720, & Inst. Biochem. Biophys., Univ. Tehran, Tehran, Iran.

We have previously shown that early olfactory deprivation by nare closure results in significant reductions in growth and development of the olfactory bulb (OB) as measured by several biochemical and structural parameters. In this study we report on the number, morphometry and spine number in the granule cells(GC)of the internal granular layer (IGL) and mitral cell layer (MCL). Albino rat pups were subjected to olfactory deprivation on one side by unilateral nare occlusion begining in the neonatal period. At day 25 postnatal, both control and anosmic OBs were stained with the Rapid Golgi method and the granule cells were studied quantitatively. It was found that compared to the control OB, the anosmic OB showed significant reductions in several parameters as follows: Number of GC, 19% in IGL, 12% in MCL; total length of GC, 19% in IGL (24% length of main apical branch, 24% soma, 24% diameter of deep branches, 14% diameter of superficial branches). Number of spines per GC in IGL, 25%, in MCL, 19%; number of spines per main apical branch 29%, per deep branches 26%, per superficial branches, 14%. In MCL, number of spines per GC 20%, per main apical branch 31%, density of spines 29%. The stained GC in IGL appeard often as clusters. The number of these clusters in the anosmic OB was reduced by 30% and the number of GC per cluster was reduced by 22%. The entire length of the cluster was reduced by 10% and its width by 3% which were not significant. In the control OB, total spine number per GC of IGL was $111^{\pm}$ 10 of which 42% were on the main apical branch and 26% on the deep branches and 23% on the superficial branches, and 4% on the soma. In the GC of MCL, total spine number per cell was about 81 of which 34% were on the main apical branch, 7% on soma, 28% on deep branches and 29% on superficial branches.

These results indicate early olfactory deprivation reduces the rate of proliferation, growth and spinogenesis of the GC of the OB. Since the GC are not directly connected to the primary olfactory neurons, it follows that the observed effects must be transmitted to the GC either via functional or trophic interactions involving the mitral and tufted cells which make connections with the GC in OB. However, the nature of these interactions remains to be elucidated. In general, these data provide further proof that the development of central neural structures are in part influenced by peripheral factors. (Supported by grants from Univ. of Tehran & Ministry of Science of Iran.)

134.1 ONSET AND DEVELOPMENT OF NEURON-SPECIFIC ENOLASE IMMUNOREACTIVITY IN THE PERIPHERAL VESTIBULAR SYSTEM OF THE MOUSE. C.J. Dechesne*, A. Sans* and A. Keller* (SPON: European Neuroscience Association). Lab. Neurophys. Sens. U.254 USTL Pl. E. Bataillon 34060 MONTPELLIER cedex France.

Immunohistological experiments have suggested that the initial appearance and gradual increase of neuron-specific enolase (NSE) in central nervous system might be correlated with differentiation and/or synaptic activity of cells (Schmechel, D.E. et al, Brain Research, 190 : 195, 1980). Maturation and synaptogenesis in the vestibular system are well documented and display precise sequences of spatio- temporal differentiation. Thus, it was of interest to study the onset and development of NSE immunoreactivity in these structures and compare the observations with the features of morphological maturation.

Antisera against purified mouse NSE were prepared in rabbits and tested as previously described (Keller, A. et al., J. Neurochem., 36 : 1389, 1981). They did not crossreact with non-neuronal enolase. CBA/C57 mice ranging from 14 gestation day (gd) to 15 postnatal day (pd) were fixed with McLean and Nakane's fixative (J. Histochem. Cytochem., 22 : 1077, 1974). Serial cryostat sections of the vestibular receptors and ganglion were processed for immunohistochemical staining using the peroxidase-antiperoxidase method (Sternberger, L.A., Wiley and Sons, NY, 1979). Anti-NSE serum was diluted 1/1000 to 1/5000.

The earliest faint staining of some vestibular ganglion cells was detected at 15 gd. At 17 gd all ganglion cells were stained and the adult intensity of staining was reached between 2 and 4 pd. In the vestibular receptors some NSE-positive sensory cells were detected at 17 gd. Supporting cells were not reactive. NSE immunoreactivity developped in the cristae with two main gradients : (1) a general apex/base gradient and, (2) a center-periphery gradient in each hemi-cristae. At 8 pd all sensory cells displayed an adult intensity of staining.

Staining development, in the vestibular structures studied, paralleled their maturational progression, thus the ganglion cells were positive before the sensory cells. Onset of NSE reactivity in the ganglion cells is concomittant with the formation of contacts without presynaptic differentiations between the afferent fibers and sensory cells, and is observed in hair cells during the period in which the first synaptic structures form. The fact that NSE reactivity develops in the cristae with an apex/base gradient is in accordance with previous findings concerning crista maturation. However this technique revealed the existence of a sequential gradient of maturation along the length of the cristae which had not been observed by other methods. The maturation gradients in the cristae are observed during 5-6 days after the onset of NSE-reactivity and demonstrate that sensory cell development is not simultaneous in the vestibular receptors.

134.2 IN VITRO EFFECTS OF EXCITATORY AMINOACID ANALOGUES ON EMBRYONIC AND NEWBORN MOUSE INNER EAR SENSORY STRUCTURES. J. Raymond* and G. Desmadryl* (SPON : European Neuroscience Association). INSERM U-254, Lab. Neurophysiol. Sensorielle, USTL, 34060 MONTPELLIER Cedex, France.

Excitants related structurally to the transmitter candidates L-Glutamate and L-Asparte are neurotoxic when injected intracerebrally. The most studied of these compounds is kainate (KA) which acts as a potent and selective neurotoxin on various neuron populations in brain or sense organs (for review see Coyle, J.T., J. Neurochem., 41 : 1, 1983). The neurotoxic effects of these excitants were proposed to be associated with overdepolarization following excessive receptor activation and seem to be in relation with the presence of high affinity recognition sites for L-Glutamate (Fagg, G.E. and Foster, A.C., Neuroscience, 9 : 701, 1983). As it has been suggested that glutamate may be involved at the hair cell-afferent fiber synapse in the vestibular and cochlear sensory epithelia (Dechesne, C. et al., Ann. Otol. Rhinol. Laryngol., 93 : 163, 1984 ; Eybalin, M. and Pujol, R., Neuroscience, 9 : 863, 1983), we examined the effects of three of the most selective compounds for the neuronal receptors of this amino-acid on the inner ear structures.

Using an in vitro system for maintaining isolated embryonic and newborn mouse otic vesicles (Desmadryl, G. et al., C.R.Acad. Sci. Paris, 8 : 223, 1984) we exposed the inner ear structures to N-methyl D aspartate (NMDA) quisqualate (QA) and kainate (KA) at different stages of development. Following 1 hour preincubation with fresh medium the isolated organ was exposed to one of these compounds (0,05 to 0,2 mM final concentration) for 5 to 30 minutes in vitro. After processing for ultrastructural observations, the pattern of the acute effects of each amino-acid analog was investigated by light and electron microscopy.

The neurotoxic effects affected the vestibular and cochlear ganglion cells and the NMDA was the less potent of the 3 compounds. In the younger stages, damage was first apparent in the vestibular structures and resulted in a severe swelling of afferent dendrites below the hair cells where are the postsynaptic components of the synapses. The hair cells had a normal ultrastructural appearence. The primary vestibular neurons presented a massive degenerative aspect with vacuolization : the mitochondria and the endoplasmic reticulum were disrupted. In the cochlea, the number of damaged spiral neurons increased with age. The increase in the sensitivity to the neurotoxic effects as a function of age suggests that functional high affinity binding sites to excitatory aminoacid analogues developped in the vestibular and auditory primary neurons in parallel with their synaptic maturation. These neurotoxic effects are consistent with a glutamatergic hypothesis for the neurotransmission in these sensory structures.

134.3 QUANTITATIVE HISTOCHEMICAL MEASUREMENTS OF MALATE DEHYDROGENASE IN NORMAL AND DEAFFERENTED CHICK BRAIN STEM AUDITORY NEURONS. D. Durham, E.W Rubel, and F.M. Matschinsky. Depts. of Otolaryngology and Physiology, U. Virginia Medical Center, Charlottesville, VA 22908 and Dept. Biochemistry, U. of Pennsylvania, Philadelphia, PA 19104.

Cochlea removal in young chickens results in rapid changes in the metabolism of second-order brain stem auditory neurons in n. magnocellularis (NM). Within 8 hours of cochlea removal, histochemical staining for the oxidative enzyme succinate dehydrogenase (SDH) increases in ipsilateral NM neurons, followed after several days by a decrease in reaction product (Durham, D. and Rubel, E.W, J. Comp. Neurol., 231:446, 1985). The surprising initial increase in SDH staining following deafferentation led us to examine the activity of a related metabolic enzyme, malate dehydrogenase (MDH), using a direct fluorometric assay.

The right cochlea was removed in 10 day-old anesthetized chickens. Four hours, 12 hours, or 9 days later birds were anesthetized and their brains quickly removed, blocked and frozen. Cryostat sections were cut, collected, and freeze dried. Tissue samples from NM were dissected from individual dried sections and assayed for MDH activity (oxalacetate formation, Hintz, et al., Am. J. Physiol., 239:C58, 1980).

Mean values (6-14 samples) from individual birds are given below in moles/kg dry wt/min s.e.m. Samples from the left NM contralateral to the cochlea removal and from unoperated animals serve as controls. Asterisks indicate that the mean differs significantly from that in unoperated birds ($p < .01$).

	control	4 hours	12 hours	9 days
right	0.59±.06	0.65±.06 0.64±.07	0.70±.04 0.77±.05*	0.37±.03*
left	0.66±.03 0.63±.03	0.58±.07 0.64±.08	0.51±.02* 0.64±.04	0.57±.04
ratio R/L	0.90	1.12 1.00	1.36 1.21	0.65

The data obtained with this direct assay for MDH are similar to our results with SDH staining. As with SDH, the ratio of MDH activity in ipsilateral NM to activity in contralateral NM was unchanged 4 hours, increased 12 hours and decreased 9 days following cochlea removal. Further study of the short-term increase in metabolism is in progress. (Supported by NIH grants NS 15395 and NS 07466 and the Virginia Lions Club).

134.4 EFFECTS OF EARLY EXPOSURE TO A LOW-NaCl DIET ON RAT CHORDA TYMPANI TASTE RESPONSES. David L. Hill, Dept. of Psychology, Univ. of Toledo, Toledo, OH 43606.

Environmental influences on rat neurophysiological taste responses occur when experimental procedures are imposed during early development. Exposure to a NaCl-free diet from 3 days gestation to 28-45 days postnatal led to flattened NaCl response-concentration functions compared to those in controls.

To learn whether similar alterations occur when rats are fed a low-NaCl diet, multifiber chorda tympani responses were recorded after exposure to a 0.03% NaCl diet for the same period as the earlier study. Additional recordings were made to learn of concomitant changes in receptor membrane components and to determine if exposure to a NaCl-replete diet after deprivation would result in responses similar to controls. As found in rats fed the NaCl-free diet, NaCl response-concentration functions (0.01M-0.5M) in rats fed the 0.03% NaCl diet were flattened compared to controls; NH_4Cl and KCl response-concentration functions were unaffected. Responses to a concentration series of NaCl after lingual application of amiloride (500 μM), suppressed NaCl responses in both deprived and control rats but the magnitude of suppression was disproportionate between groups. The response-concentration functions, expressed relative to the respective 0.1M NaCl response, for both deprived and control rats after amiloride application were similar to those in deprived rats before amiloride. Finally, response-concentration functions for NaCl in rats fed the 0.03% NaCl diet to 28 days postnatal and then fed a sodium replete diet for 10 days or less were similar to rats always fed the 0.03% NaCl diet. Response-concentration functions to NaCl in rats fed the NaCl-replete diet for 15 days, however, were intermediate to those in deprived and control rats.

These results demonstrate that specific neurophysiological taste response alterations occur to NaCl when rats are fed a 0.03% NaCl diet during early development. Response changes were similar to those in rats fed a 0% NaCl diet. A possible mechanism for the changes may relate to increased numbers of functional amiloride-sensitive membrane components. Moreover, the gustatory system is somewhat "plastic" in that responses change with changing environment conditions.

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134.5 CENTRAL REPRESENTATION OF DENTAL STRUCTURES IN THE KITTEN USING TRANSGANGLIONIC HRP TRANSPORT. M.A. Henry*, L.R. Johnson* and L.E. Westrum (SPON: J.D. Loeser). Depts. of Neurological Surgery and Biological Structure, Univ. of Wash., Seattle, WA 98195.

The transganglionic transport of horseradish peroxidase (HRP) following pulpal injections in permanent teeth has been used to demonstrate the central representation of dental structures in the adult cat, but similar detailed studies in kittens with primary teeth are lacking. The present study describes the light microscopic pattern of central labelling following HRP injections in the primary maxillary cuspids of 8-9 week old kittens and compares the results to those of similar studies in adult cats. The periapical area of the injected tooth is also examined to investigate the possible spread of HRP to supporting structures. Kittens had 5 µl of 30% HRP injected into the primary maxillary cuspid on 2 successive days. One day later the animal was transcardially perfused and the brain stem and ganglia processed by the tetramethyl benzidine method of Mesulam. The injected tooth and its supporting tissues were removed as a block and following decalcification were reacted as above. Clusters of HRP cells were located in the ipsilateral ganglion and examination of the trigeminal brain stem nuclear complex (TBSNC) identified an extremely dense projection to each ipsilateral nucleus of the complex. Labelled fibers were found in layers I, II and V of the upper cervical spinal cord. In caudal medullary dorsal horn, the pattern is to the same layers as spinal cord but becomes increasingly more dense (especially in layer V) and broader including a limited projection to layers III and IV near obex. The distribution is in a dorsomedial position in caudal pars interpolaris (PI) with a gradual shift to a ventromedial location in rostral PI. Within pars oralis (PO) there is a gradual shift from a ventromedial to a dorsomedial position as rostral levels are approached. The labelling to main sensory nucleus remains dense especially in the dorsomedial subdivision. Large labelled fibers are seen in the V root entry zone and are seen ascending in the mesencephalic (MES) tract of V, where MES cells are also labelled. Examination of the periapical area of the injected tooth identified HRP within the periodontal ligament of the primary and developing permanent cuspid but not within the permanent tooth itself. This study identifies a more extensive and denser projection to all nuclei of the ipsilateral TBSNC and MES nucleus in kittens as compared to adult cats. This could reflect either 1) a greater central representation of primary teeth and associated structures than permanent ones, that may be modified with exfoliation of primary teeth and eruption of permanent teeth, or 2) a greater ability of the less mature system to transport HRP transganglionically.

Supported by NIH grants DE04942, NS07144, and NS20482. Dr. Westrum is an affiliate of the CDMRC.

134.6 TOPOGRAPHIC REPRESENTATION OF THE FACE WITHIN THE TRIGEMINAL (V) GANGLION OF THE NEONATAL RAT. B.G. Klein, A.M. Szczepanik* and R.W. Rhoades, Dept. of Anatomy, UMDNJ-School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

There is considerable anatomical and physiological evidence for inter- and intra-divisional somatotopy of the V ganglion neurons innervating different parts of the face and head in the adult rat. We have used fluorescent retrograde tracers to examine the representation of the face within the V ganglion of newborn rats. Within 12 hr. of birth, true blue (TB) and diamidino yellow (DY) were injected, in various combinations, into ophthalmic, maxillary and mandibular skin. In addition, tracers were injected into either the A-row and E-row vibrissa follicle regions or into the C1 and C5 regions to examine the intra-divisional representation of the whiskerpad. Animals were perfused transcardially 24-48 hr. later.

In rats which received maxillary (TB) vs. mandibular (DY) injections (N=4) there was no overlap between the TB and DY labelled neurons. TB labelled cells were located medially, throughout the region morphologically analogous to the adult ophthalmic-maxillary division, while the DY labelled neurons were found posterolaterally in the presumptive mandibular division. Similar results were observed for the ophthalmic (TB) vs. mandibular (DY) injections (N=4). In the ophthalmic (DY) vs. maxillary (TB) cases (N=4) all labelled cells were located in the ophthalmic-maxillary zone and there was considerable overlap in the distributions of the cells labelled by the different tracers. However, in 3 of 4 cases, DY labelled cells were concentrated dorsomedial to the TB labelled neurons.

A-row (DY) vs. E-row (TB) whiskerpad injections (N=6) labelled neurons throughout the ophthalmic-maxillary region. DY labelled cells were located medial to TB labelled neurons with little or no overlap. C1 (DY) vs. C5 (TB) (N=5) injections also labelled neurons within the ophthalmic-maxillary division, but provided no clear evidence of an ordered ganglionic representation of the rostrocaudal axis of the whiskerpad.

These results demonstrate inter- and intra-divisional somatotopy of the V ganglion in the neonatal rat. This organization is quite similar to that observed in adult animals.

Supported by EY04170, EY03546, DE06528, the UMDNJ Foundation and the AOA Foundation (RWR) and NRSA NS07240 (BGK).

BGK's current address is Dept. of Neuroscience, New York College of Osteopathic Medicine, Old Westbury, N.Y. 11568.

134.7 RECOVERY FROM EXPERIMENTAL MYOPIA DEPENDS ON THE TYPE OF VISUAL RESTRICTION. Lisa Wentzek*, Michael D. Gottlieb and Josh Wallman. Dept. of Biology, City College, CUNY, New York, NY 10031

Severe myopia can be produced in experimental animals by several different visual restrictions. In monkeys, tree shrews and chicks, total form-deprivation either by neonatal lid-suture or by occluders placed over the eyes results in consistent axial myopia. There are no reports that these experimental myopias are reversible. In chicks, restriction of vision to the frontal visual field also produces severe axial myopia, which can be reversed if the restriction is removed. To explore whether this difference in reversibility is a function of the species used or of the type of visual restriction employed, we now compare the effects of partial and total visual restriction on the course of recovery from experimental myopia in chickens.

Chicks were raised with the visual field of one eye either entirely occluded or restricted to the frontal visual field by means of translucent plastic occluders. The occluders were removed at either 2 or 4 weeks of age, the eyes measured, and remeasured 2 and 4 weeks later to assess ocular refractions, corneal curvatures, and axial dimensions (by A-scan ultrasound).

Development of myopia--As in our previous studies, all treated eyes became myopic after 2 and 4 weeks of treatment. Totally occluded (T) eyes became much more myopic than did eyes restricted to the frontal visual field (F)(median at 2 weeks, T:-32.1 D vs. F:-14.3 D; at 4 weeks, T:-29.9 D vs. F:-12.9 D). These myopias were associated with greatly elongated vitreal chambers. In addition, particularly in the totally occluded eyes, the anterior chambers became deeper and the corneal curvatures steeper.

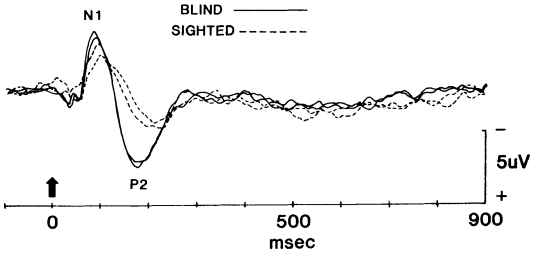
Recovery from myopia--Removal of the occluders caused a dramatic recovery from the myopia in all the eyes restricted to the frontal visual field and in the eyes totally restricted for only 2 weeks. This recovery was associated with a cessation of growth of the vitreal chamber, while the rest of the eye continued normal growth. We interpret this finding as suggesting a functional regulation of axial growth apart from the growth of the eye in general. In contrast to these results, the eyes totally occluded for 4 weeks did not become emmetropic (median at 8 weeks, T:-15.4 D), and the vitreal chambers continued to elongate at approximately the normal rate.

These differences in recovery suggest that only under particular conditions can the growing eye regulate its growth to achieve emmetropia. Perhaps once the eye is beyond a certain degree of refractive error or of vitreal elongation, it loses the normal ability to regulate its growth in the service of its ocular needs. Clarifying the conditions under which this normal growth regulation operates would be of clear importance in our understanding of the etiology of myopia. (Supported by NIH EY-02727.)

134.8 EARLY BLINDNESS REORGANIZES AUDITORY PROCESSING IN HUMANS David L. Woods, C. C. Clayworth* and P. Bach-y-Rita[2] Clinical Neurophysiology Laboratory, Dept. of Neurology, UC Davis, VA Medical Center, Martinez, CA 94553 and [2]Dept. of Rehabilitation Medicine, University of Wisconsin, Madison, WI 53705

We compared auditory evoked potentials (AEPs) in normal subjects and age and sex-matched subjects who had been blind since early infancy. Blind and sighted subjects had comparable brainstem auditory evoked potentials (BAEPs) but blind subjects showed shortened latencies and, in some cases, enhanced amplitudes of middle and long-latency AEPs.

Intergroup differences were first observed at latencies of 50-70 msec in middle latency recordings. The blind also showed amplitude enhancements in tone-evoked N1 and P2 components (below), and shortened latencies of P3s and reaction times in a target detection task. The results suggest that the reorganization of the auditory system following early blindness is primarily the result of changes in auditory structures in the forebrain.



134.9 POSTNATAL DEVELOPMENT OF EVOKED RESPONSE COMPONENTS OF THE COCHLEA AND BRAINSTEM IN RAT. J. Coleman, B. Blatchley*, and W. A. Cooper*. Depts. of Psychology, Physiology and Communicative Disorders, University of South Carolina, Columbia, South Carolina 29208.

Development of auditory function was studied in Sprague-Dawley rats using auditory brainstem recordings. Rat pups were anesthetized with ketamine and acepromazine and pure tone pips presented monaurally. The stimuli consisted of 0.5 msec rise-fall times and 1 or 2 msec at maximum amplitude presented up to 106 dB PeSPL. Repeated vertex-chin recordings were made on postnatal days 9, 10, 12, 14, 15, 16 and 20 from 24 animals. Recordings on postnatal days 9 and 10 showed no response to 3, 8, and 40 kHz stimuli. On postnatal day 12 stimulus dependent responses were reliably recorded. However, response generation was dependent upon the frequency of the stimulus presented. At 3 and 8 kHz stimulation 60% of the animals showed a major deflection at 95 dB. In the same animals 40% showed a response at 40 kHz at 95 dB; four of these animals showed no response at this frequency at 106 dB.

The source of the first response was examined by varying stimulus duration and repetition rate. Doubling stimulus duration correspondingly increased the response envelope by a factor of two. These responses were recorded to random phase presentation suggesting that they were summating potentials which are generated by cochlea. Increasing the repetition rate from 20/sec to 80/sec yielded a response with the same morphology and latency characteristics. The absence of a latency shift implies that this component is not of neural origin.

At 14 days two waves appear to stimulation at 3 and 8 kHz at 95 dB in all animals tested. These waves show neural elements. A third wave now appears in most animals at 3 and 8 kHz and a fourth wave also emerges in some animals. Less than 50% of animals at 14 days show a response to 40 kHz stimulation and later waves are infrequently observed. At 16 days of age four waves appear in all animals at 3 and 8 kHz, although only two routinely appear at 40 kHz. A fifth wave is observed at 20 days to low frequencies, but not to 40 kHz. These results show that for cochlear as well as neural structures the responses to lower frequency stimuli precede those to higher frequencies in the rat. (Supported by the Deafness Research Foundation and BRSG 507 RR07160.)

134.10 DEVELOPMENT OF COCHLEAR AND BRAINSTEM AUDITORY EVOKED POTENTIALS IN THE MONGOLIAN GERBIL. N. K. WOOLF AND A. F. RYAN*, Otolaryngology Research Laboratory, University of California Medical School and Veterans Administration Medical Center, San Diego, CA 92103.

Cochlear N1 compound action potentials (AP) and auditory brainstem evoked responses (ABR) of mongolian gerbils (Meriones unguiculatus) were examined at 10, 12, 14, 16, 18 and 30 days after birth (DAB). This range of ages includes the onset of neonatal hearing through the achievement of mature auditory system characteristics. Neonatal responses were compared to those for adults.

AP was measured utilizing standard round window recording techniques. Click evoked AP was first observable at 12 DAB. AP thresholds at 12 DAB exceeded 120 dB SPL (peak). At that time AP showed significant immaturities with respect to threshold, asymptotic latency and maximum response amplitude. Furthermore, at 12 DAB AP magnitude showed more rapid adaptation with increasing repetition rate than was the case at later ages. Between 12 and 30 DAB AP thresholds decreased approximately 100 dB. Mature asymptotic latency and repetition rate responses for AP were achieved by 16 DAB, while other parameters, including threshold and maximum response amplitude did not reach adult levels until after 18 DAB.

ABRs were first observed at 12 DAB. Only peaks I, II and IV were present in the youngest subjects. The ABR waveforms at 12 DAB had broad peak widths, high thresholds and were very sensitive to repetition rate. The ratio of peak I/peak IV at 12 DAB was greater than one, whereas at later ages it was always less than one. Five positive peaks were apparent for subjects 14 DAB, or older. For all subjects ABR peaks I and II correlated well with the N1 and N2 negative peaks of the AP. With increasing chronological age through 30 DAB, ABR thresholds and latencies decreased, the width of each of the five peaks became more narrow and the relative amplitude of the later peaks increased.

These results indicate that the generators of the peaks in the ABR waveforms mature in a hierarchical fashion, with the more peripheral regions of the auditory system reaching maturity before those for more central regions. For each peak the parameters of latency and rate adaptation reached maturity prior to that of absolute magnitude.

Supported by grants from NIH/NINCDS (NS14945), the Veterans Administration Research Service and the Deafness Research Foundation.

DEVELOPMENT AND PLASTICITY: SENSORY SYSTEM II

135.1 EFFECTS OF ALTERED VISUAL INPUT UPON VISUAL AND SOMATOSENSORY REPRESENTATIONS IN THE HAMSTER'S SUPERIOR COLLICULUS. R.D. Mooney, B.G. Klein and R.W. Rhoades. Dept. of Anatomy, UMDNJ-School of Osteopathic Medicine and Rutgers Medical School, Piscataway, NJ 08854.

It is well known that neonatal removal of one eye and ablation of the ipsilateral superior colliculus (SC) in hamster results in an abnormally extensive ipsilateral retino-SC pathway (eg. So, K-F. and Schneider, G.E., Brain Res., 147:277, 1978) and an abnormal visual map in the superficial laminae of the remaining colliculus (Finlay, B.L. et al., J. Comp. Neur., 183:721, 1979). We used this preparation to determine whether the altered visual map in the superficial SC laminae was associated with a change in the somatosensory representation in the deep layers of this structure.

Hamster pups were subjected to enucleation of the right eye and ablation of the superficial layers of the right SC within 12 hr. of birth and then used in recording experiments no less than 3 mon. later. These experiments showed that the expanded retino-SC projection was roughly mirror symmetic with that observed normally. There were, however, numerous irregularities and discontinuities. The somatosensory representation in the remaining SC was also markedly altered. The changes here included the presence of numerous cells with bilateral or even strictly ipsilateral low threshold receptive fields, cells with split receptive fields and large changes in the magnification factor for different parts of the body. The overall polarity of the somatosensory representation was, however, normal.

Additional anatomical experiments demonstrated only one abnormal somatosensory input to the deep laminae of the left SC: A weak projection from the contralateral somatosensory cortex which innervated the medial portion of the colliculus on this side. Removal of the source of this projection at the time of the recording experiment did not reduce the incidence of abnormalities in the somatosensory representation of the neonatally brain damaged hamsters.

Our results thus show that an altered visual SC map is associated with changes in the somatosensory representation in this structure. The somatosensory changes do not, however, bring this map into alignment with the visual representation.

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135.2 THE EFFECT OF NEONATAL EYE ROTATION ON THE REPRESENTATION OF AUDITORY SPACE IN THE FERRET SUPERIOR COLLICULUS A.J. King*, M.E. Hutchings*, D.R. Moore and C. Blakemore, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, England.

The deep layers of the ferret superior colliculus (SC) contain a two dimensional map of auditory space which is in register with the visual map in the overlying superficial layers (Hutchings, M.E. & King, A.J., 1985, J. Physiol. 358, 31P). In order to determine whether registration between these maps requires sensory experience during postnatal development, we have examined the effect of neonatal eye rotation on the relationship between the representation of visual and auditory space in the SC of the adult ferret.

A 180° intorsion of the left eye was performed in 6 ferrets anaesthetized with halothane and N_2O at or soon after normal eye opening, at 31-36 days of age. The right eye was removed. Animals were between 5 and 7 months old at the time of recording. They were anaesthetized with alphadolone/alphaxalone, surgically prepared for extracellular recording, paralysed with gallamine triethiodide and artificially ventilated. Paralysis was maintained by infusion of gallamine triethiodide and sodium pentobarbitone. White-noise bursts (500Hz-40kHz) were presented under anechoic conditions from a loudspeaker, which was moved in both azimuth and elevation using a hoop system. The visual receptive fields of cells in the superficial and deep layers were mapped with a discrete flashing light source.

The map of the visual world in the contralateral SC was shifted according to the degree of eye rotation. Thus, in animals in which there was no de-rotation, anterior visual space was represented in caudal SC and superior visual space was represented in lateral SC. The responses of 91 auditory units in the deep layers were analysed. The majority of these units responded best to an area of space restricted in both azimuth and elevation. The best areas of most units varied systematically in azimuth from the anterior to the posterior field along the rostro-caudal axis of the SC, and from superior to inferior along the medio-lateral axis. The representation of auditory space in these animals therefore appeared normal and was out of register with that of visual space.

We conclude that the auditory map in the deep layers of the ferret SC does not adapt to alteration of the visual representation, suggesting that the topography of the auditory representation is not regulated during normal postnatal development by the map of the visual world.

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135.3 POST-EMBRYONIC HAIR CELL PRODUCTION IN THE SACCULE OF A TELEOST FISH. A. N. Popper and B. Hoxter*. Dept. of Anatomy, Georgetown Univ. Schools of Med. and Dent., Washington, DC 20007.

In contrast to amniotes, a number of anamniote species continue to add sensory hair cells to the inner ear for at least several years after birth. Post-embryonic proliferation of sensory hair cells is particularly extensive in the teleost Astronotus oscellatus (the oscar). A 2-cm long animal has approximately 5,500 hair cells on the sensory epithelium in one saccule, whereas a 19-cm long animal has over 250,000 sensory hair cells (Popper, A. N. and B. Hoxter, Hearing Res. 15:133, 1984).

Studies of proliferation of sensory hair cells in elasmobranchs and amphibians indicate that the post-embryonically produced sensory hair cells in these animals are added at the edges of the sensory epithelium in these species (Corwin, J. T., Abst. Assn. Res. Otolaryngol. 7:56, 1984).

In order to determine sites of hair cell proliferation in the saccule, specimens of Astronotus were injected with tritiated thymidine for one or more days and sacrificed at various times after injection. Tissue was prepared using standard autoradiographic techniques to locate cells in the saccule that had divided after injection.

Labeled cells of various types were found throughout the saccular sensory epithelium. In animals sacrificed soon after thymidine injection the majority of labeled cells were located just above the basement membrane, at the level of the supporting cells. Animals sacrificed several days or more after injection had labeled cells just above the basement membrane, labeled sensory hair cells, and labeled cells that were located somewhat above the basement membrane but below the level of the hair cells. While some of the labeled cells were located close to the edges of the sensory epithelium, the majority of the labeled cells, including most of the labeled sensory hair cells, were located closer to the center of the epithelium than to the edges.

We found mitosis towards the center of the saccular epithelium. In addition, most labeled cells were found in pairs. These findings lead to the suggestion that there is active production of new cells within the sensory epithelium, rather than such cells migrating into the epithelium from the edges. Thus, our findings demonstrate that post-embryonic hair cell production in Astronotus occurs throughout the saccular epithelium and not just at the edges, unlike the addition of post-embryonic hair cells in other anamniotes or embryonic addition of hair cells in mammals.

Our results also lead to the hypothesis that the newly added sensory hair cells arise from stem cells located just above the basement membrane throughout the saccular epithelium. These stem cells divide and produce cells which grow towards the apical surface of the epithelium and ultimately develop into mature sensory hair cells. (Supported by NIH and NSF.)

135.4 EFFECTS OF UNILATERAL COCHLEA REMOVAL ON THE CYTOARCHITECTURE OF THE THIRD-ORDER AUDITORY NUCLEUS IN A SONGBIRD. J.M. Greenspon*, J. Humpal* and D.H. Reynolds* (SPON: D. Olton) Psychology Dept., Hobart and Wm. Smith Colleges, Geneva, NY 14456; Dept. of Psychol. Johns Hopkins Univ., Baltimore, MD 21218.

The brainstem auditory system (BAS) in avians is considered a model system for investigating the effects of deafferentation on neuronal morphology (Parks, T.N., and Rubel, E.W., J. Comp. Neurol. 164:435-448, 1975). The organization of the chicken BAS is relatively simple. We have examined the neuronal architecture of the third-order auditory nucleus (N. laminaris, NL) in the canary songbird, and the effects of unilateral cochlea removal (UCR) on its neurons. Our investigations reveal that the organization and composition of NL is more complex in a songbird than in the chicken. Concomitantly, the canary's NL exhibits a very different response to UCR than does the chicken's.

In contrast to the chicken's NL, which contains a uniform monolayer of medium-sized, bipolar projection neurons, the canary's NL is composed of three neuronal layers containing several neuronal types: a bipolar projection type; and four types of local circuit neurons. Each neuronal type can be distinguished on the basis of its morphology and position within NL.

Compared to the chicken, the canary's NL exhibits a different response to UCR. In the developing chicken, unilateral damage to the auditory nerve results in severe atrophy of the dendrites of NL neurons which receive bilateral afferents from the second-order nucleus magnocellularis (NM). In contrast, adult chicken NL appears to be unaffected by this damage. In the adult canary, however, NL neurons exhibit a subtle reorganization in response to UCR. There is a decrease in the number and length of higher-order, distal NL dendrite segments in damaged subjects, relative to those contained in intact brains. At the same time, the proximal dendrite segments show a dramatic increase in length. These changes in dendritic morphology observed in birds sustaining UCR were related to patterns of connectivity between NM and NL.

Our studies of the adult canary BAS reveal an order of complexity not observed in the chicken BAS. The complexity in the canary BAS may be related to the songbird's reliance on auditory information for vocal learning, a talent not exhibited by chickens. Our results suggest that the avian BAS is a model for the comparative analysis of structure-function relationships as well as for the study of afferent influences on neuronal morphology.

This research was supported by Hobart and Wm. Smith Colleges and a faculty development grant from the Andrew W. Mellon Foundation through Hobart and Wm. Smith Colleges to J.M.G.

135.5 EARLY DIFFERENTIATION OF THE AFFERENT NERVOUS SYSTEM IN THE OPOSSUM (MONODELPHIS DOMESTICA). Terrell E. Jones* and Bryce L. Munger* (SPON: D.F. Peeler). Department of Anatomy, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033

Early differentiation of afferent fibers innervating the snout skin of Monodelphis domestica was studied by electron microscopy and light microscopic, silver staining techniques. This study was undertaken to investigate the relationship between dermal and epidermal innervation at or following birth. Skin from young opossums was studied at birth (zero day) and postnatal days one, three and five.

Opossum pups were processed by immersion fixation using 10% neutral buffered formalin for paraffin embedding and Karnovsky's fixative for Durcupan ACM plastic embedding. Standard fixation times and procedures were used. Serially sectioned, paraffin embedded tissue was cut at 6-8 microns and processed for silver techniques. Plastic embedded tissue was sectioned at 700-900 Angstroms and processed for normal electron microscopy.

Portions of the afferent nervous system were present in the newborn opossum pup. Neurite bundles were seen within the dermis, epidermis, and penetrating the dermal-epidermal junction. Merkel cells were consistently present in large numbers. Mature Merkel cells had granules polarized toward the associated neurite and were located in the base of the rete pegs. Immature Merkel cells were characterized by a lack of granules and lacked an apposed neurite. Continguous Merkel cells had junctional complexes in zero and one day animals. Schwann cells, identified by their contact with neurite bundles, were present in large numbers, especially in superficial dermal layers. Melanocytes could be identified in the epidermis only in five day pups. No melanocytes were seen in the dermis. Developing rete pegs could be recognized in zero day animals and became prominent in five day pups.

These observations suggest that afferent fibers are present at a much earlier age than previously indicated and that these fibers are anatomically mature. Merkel cells are present early and can maintain themselves without neurite association. Rete pegs form after the time at which nerve fibers can be detected in the epidermis. These findings support the concept of a dependence during differentiation of the dermis, epidermis, and afferent nerve fibers. (Supported in part by USPHS Research Grant NS19462.)

135.6 DEVELOPMENT OF THE MEDIAL LEMNISCUS IN THE POUCH YOUNG OPOSSUM. J.C. Hazlett,[1] T.A. Collins[1], G.F. Martin[2] and T. Cabana[2]. Depts. of Anatomy, Wayne State Univ.[1] and Ohio State Univ.[2]

The development of the medial lemniscus was studied in the neonatal opossum using the axonal transport of WGA-HRP and orthograde degeneration. All animals were anesthetized for the experimental procedures. Specimens from postnatal days 3 and 5 were subjected to WGA-HRP injections of the diencephalon. In the younger group it was difficult to identify individually labeled dorsal medullary neurons. However, a fine dust-like reaction product was observed over cellular nuclei on the dorsal aspect of the contralateral medulla. In the postnatal day 5 animals similar placements resulted in unequivocal neuronal labeling in the contralateral dorsal medulla. The location of these cells was in accord with the positions of the gracile, cuneate and lateral cuneate nuclei in the adult. In an effort to determine the development of the infrathalamic lemniscal connections, lesions of the dorsal column nuclei were performed in litters of 14, 18, 21 and 26 day postnatal animals. Specimens from the 14 and 18 day old litters yielded equivocal results with regard to the course and termination of the medial lemniscus. The axonal debris was quite sparse and difficult to trace. While scattered argyrophilic granules were present in the contralateral inferior olivary nucleus, dorsal aspect of the pontine nuclei, red nucleus and zona incerta, only a few degenerating fibers were observed in the medial lemniscus. Furthermore, there was little evidence for degeneration in the ventrobasal complex. In contrast, material from the 21 and 26 day old litters clearly defined the course of the medial lemniscus. Axonal debris representing the degenerating lemniscus was present in the ventromedial brainstem from inferior olive to mid-pointine levels. From this point the degenerating fillet coursed into the midbrain where it was located ventral and lateal to the red nucleus. At the mesodiencephalic junction degenerating fibers entered the caudal zona incerta and terminated in the overlying ventrobasal complex. All brainstem structures normally innervated by the medial lemniscus in the adult contained axonal debris in both the 21 and 26 day old litters. In summary, on the basis of the HRP studies, we suggest that lemniscal projections to the diencephalon are present as early as the third postnatal day. Lemniscal projections to medullary, pontine and midbrain targets were revealed by degeneration procedures in postnatal day 14/15 animals. We are proceeding with attempts to demonstrate these latter connections at progressively earlier ages. As described here, the early development of lemniscal connections revealed by the HRP technique provides a basis for reevaluating previous studies suggesting a somewhat later appearance for this pathway. (Supported by BNS-8118455 to JCH and BNS-8309245 and NS-10165 to GFM.)

135.7 EVIDENCE FOR A DEVELOPMENTAL SEQUENCE IN THE ORIGIN OF SUPRASPINAL PROJECTIONS TO THE DIENCEPHALON OF THE NORTH AMERICAN OPOSSUM. G.F. Martin, R.H. Ho and T. Cabana, Department of Anatomy, The Ohio State University, Columbus, Ohio 43210.

The origin of supraspinal projections to the diencephalon has been studied in a series of pouch young opossums using the axonal transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). The opossum was chosen because it is born 12 days after conception and is available for experimental manipulation at very early stages of development. All animals were anesthetized for the operative and injection procedures. In the youngest pups subjected to WGA-HRP injections of the diencephalon (postnatal day 3), labeled neurons were difficult to identify. They were never-the-less found in the reticular formation and the dorsal column nuclei. They were probably present in other areas, including the dorsal raphe and superior central nuclei, although masked by spread of the marker and orthograde labeling. Indeed, immunohistochemical processing of brains from animals of the same age revealed evidence for serotonergic axons within the diencephalon and serotonergic neurons within the dorsal raphe and superior central nuclei. The latter nuclei provide most of the serotonergic projections to the diencephalon in the adult opossum. By postnatal day 5, injections in the diencephalon labeled immature neurons in most of the brainstem areas similarly labeled in the adult animal: the dorsal raphe and superior central nuclei, the presumptive coeruleus complex, the reticular formation, the vestibular nuclei as well as the sensory trigeminal and dorsal column nuclei. Where it could be assessed, the pattern and laterality of labeling was comparable to those in the adult. Labeled neurons were also seen in the cerebellar primordium. They were numerous in the nuclear and cortical transitory zones on the side ipsilateral to the injections, but a few were also present contralaterally. Examination of the neurons labeled ipsilaterally in progressively older pups suggested that most of them become incorporated into the parabrachial nuclei. Labeling within the contralateral nuclear transitory zone (presumptive deep nuclei) was well developed by postnatal day 12. At the same age neither orthograde nor retrograde labeling was found in the diencephalon after multiple injections of WGA-HRP into the neocortex. Such labeling was present, however, by postnatal day 18.

Our results suggest that neurons within the brainstem core, including the sensory trigeminal and dorsal column nuclei, innervate the diencephalon very early in development. Crossed cerebellar axons reach the diencephalon somewhat later, followed by those from the cerebral cortex. Such a developmental sequence is best explained by temporal factors such as the birthdates and maturation rates of the neurons in question.

(Supported by BNS-8309245 and NS-10165.)

135.8 ULTRASTRUCTURAL RECONSTRUCTION OF EVENTS RELATED TO THE DEVELOPMENTAL GRADIENTS OF THE COCHLEA. C.D. Fermin and G.M. Cohen. Department of Otorhinolaryngology and Communicative Sciences. Baylor College of Medicine. Houston, Texas 77030, and Department of Biological Sciences. Florida Institute of Technology. Melbourne, Florida 32901.

For this study, chick embryos (Gallus domesticus) ranging between 5 days of incubation and hatching (21 days) were fixed by immersion in 3% cold (4°C) glutaraldehyde in cacodylate buffer, embedded in Araldite 502 and processed for light (LM) and transmission electron microscopy (TEM). Serial sections were cut along the entire cochlear length at 2 and 5 um and examined under the LM. Thin sections (70 to 90 nm) were collected at every 100 um interval and photographed under the TEM.

Reconstructions of the developing chick cochlea (lagena) allowed us to identify ultrastructural events that were finely tuned with time around which the onset of hearing is known to occur (Rebillard and Rubel, Brain Res.,229,15,1981). Our observations were based primarily on synaptogenesis of afferents onto hair cells, cytodifferentiation of hair cells (development of the cuticular plate, stereocilia and of synaptic specializations), modifications of temporary synapses and of the afferent growth cones, and the appearance of the efferent terminals on the bases of the hair cells. We identified a longitudinal and lateral gradient on the chick cochlea (Fermin and Cohen, Acta Otolaryngol.,97:39, 1984) and found a parallel trend on the development of the statoacoustic (VIIIth cochlear) ganglion (Fermin and Cohen, Acta Otolaryngol.,98:42,1984), and the formation of the tectorial membrane (Cohen and Fermin, Hear. Res., In Press, 1985). It seems that these coordinated events taking place in the cochlea around the time of the onset of hearing, are responsible for the gradual improved responses that have been recorded in the past from young and older embryos and from newly hatched chicks. Thus, the contribution of the mentioned events must all be taken into account for explaining the progressive improved hearing of the embryos, and special attributes to individual components of the auditory system should be avoided.

The correspondence between the finely tuned morphological events with electrophysiological data obtained by others in this species (Rubel et al., Ann. Otol. Rhinol. & Laryngol.,93:609,1984) corroborate the usefulness of the chick embryo for this type of study. The importance of these findings will be discussed.

Supported in part by NINCDS grant 10940 and NIH grant RR09032

135.9 THE EFFECT OF NEONATAL INFRAORBITAL NERVE SECTION ON THE PATTERN OF THALAMOCORTICAL PROJECTIONS TO THE SOMATOSENSORY CORTEX OF THE ADULT RAT. K.F. Jensen and H.P. Killackey*. Div. Neurotoxicology, HERL, U.S.E.P.A., Research Triangle Park, N.C. 27711 and Dept. Psychobiology, Univ. of California, Irvine, CA 92717

Each level of the rat trigeminal system between the brainstem and cortex is characterized by a discrete pattern of afferent terminations which is related to the distribution of whiskers on the face of the rat. Previous studies have reported that infraorbital nerve cut at birth abolishes this pattern in the principal sensory nucleus and ventral posterior nucleus. In the present study we have investigated the effect of this same manipulation on the pattern of thalamocortical projections.

The infraorbital nerve of Sprague-Dawley rat pups was sectioned on the day of birth. When the rats reached adulthood, one group was injected with 0.25-0.75 microliters of 10% WGA-HRP into the ventral posterior nucleus. After a survival time of 12 to 24 hours, the rats were perfused, their cerebral cortices removed, flattened, sectioned tangentially, and reacted with TMB. In additional rats, tangential sections of flattened cortex were processed for SDH histochemistry.

Infraorbital nerve section on the day of birth resulted in a marked alteration in the pattern of thalamocortical projections. These projections were distributed in amorphous clusters which were irregularly spaced and of varibale size rather than organized into the rows of discrete clusters characteristic of the normal animal. In addition, the amorphous clusters tended to form bands which extended in a posteromedial to anterolateral orientation and were separated by zones which are relatively free of label. In certain regions, the outside border of this aberrant pattern was as distinct as in normal animals. For example, there was a relatively label free area between the aberrant pattern and the area of the forelimb representation as in the normal animal. The distinctive border of the aberrant pattern suggests the areal extent of the pattern may be the same as in the normal animals. We interpret these results as evidence that thalamocortical projections are maintained and exhibit a limited degree of organization after neonatal nerve cut. This limited organization may reflect the topographic relationship among thalamocortical projections present at birth. Conversely, the aberrant aspects of the pattern may reflect the absence of the normal postnatal peripheral influences which play a role in shaping the terminal arbors of axons.

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135.10 AGE-DEPENDENT REORGANIZATION OF SI CORTEX IN CATS FOLLOWING SPINAL TRANSECTION. P.A. McKinley, W. Jenkins, M.M. Merzenich, S Hamer and J.L. Smith. Dept. Kinesiology, UCLA, Los Angeles, CA 90024 and Coleman Lab, UCSF San Francisco CA 94143

The somatosensory cortex (SI) in mammals has been shown to respond dynamically to limited peripheral changes, such as the amputation of one finger, by reorganization of its somatotopic map (Kaas,J. etal Ann Rev Neurosci., 6:325, 1983). However, the capacity for reorganization after large sensory deficits (such as loss of an entire limb), has not been examined. Also, the extent to which organization is age-dependent has been little explored. In this study we examined the reorganizational responses of SI in chronic spinal cats deprived of sensory input from the hindlimbs due to cord transection at T_{12} at 3 ages: adults, and juveniles of 2 and 6 weeks of age.

Receptive field (RF) characteristics of most of the mediolateral strip of SI were mapped electrophysiologically from multiunit responses to light tactile stimulation in ketamine anesthetized cats. The differences between the cortical maps of the transected animals and the normal cat (Felleman, D. etal.J Neurosci.,3:1648, 1983; McKenna, T. etal. J Neurophysiol., 45:667, 1981) are dramatic and the difference between the age at which transection occurred was striking. Briefly, SI cortex in cats spinalized as adults or at 6 weeks of age was characterized by expansions of the trunk and forelimb representations medialward into the region normally occupied by the hindlimb afferent input. Additionally, the entire trunk representation, including the medially placed part attributed to the lesion, was narrowed along the rostrocaudal axis as compared to normal representation. In contrast, SI cortex in cats spinalized at two weeks of age was characterized by double representation of the the trunk and forelimb, and was organized topologically along the mediolateral axis in two halves that were mirror reflections of each other and occupation of SI cortex along the rostrocaudal axis appeared to be normal for both representations. However, all age groups exhibited some characteristics in common: areas within normally occupied SI cortical zones that were active but unresponsive to light cutaneous stimuli; increased trunk representation, appearance of large receptive fields.

In summary, it is interesting to note that the distances over which reorganization has been observed were greater than those reported in SI cortex of monkeys for more restricted peripheral lesions. Additionally, although the rostrocaudal extent of SI in transected adults was a fraction of that found in normals, it was difficult to distinguish any differences cytoarchitecturally between normal and lesioned animals. Supported by NS 19864

135.11 BEHAVIORAL CONSEQUENCES OF CHRONIC VIBRISSA ACTIVATION OF RAT VIBRISSA-CORTICAL BARREL SYSTEM. K.M. Gallo*, W.J. Carr*, R.L. Craik, P. Hand (SPON: J. Walsh) Beaver College, Glenside, PA 19038 and Dept. of Anim. Bio., Sch. of Vet. Med., Univ. of Penna., Phila, PA 19104

Previous (^{14}C)-deoxyglucose (2DG) studies revealed an enlarged and diffuse pattern of labeling of C3 representation in contralateral SI 90 days after neonatal follicle ablations sparing #3 vibrissa of row C (C3) (Sheu and Hand, Neurosci. Abst., 1982). In a subsequent study, continued stimulation ("enrichment") of one C3 vibrissa on bilaterally follicle ablated rats resulted in an increased local cerebral metabolic rate of glucose (LCMRG) in contralateral SI compared to matched C3 controls (Craik et al, Neurosci. Abst., 1983). These data indicated that (1) a remaining intact receptor organ increased its central representation in SI cortex in response to deafferentation of the surrounding "vibrissal" region of SI and (2) that chronic stimulation of the spared intact receptor organ (C3) produced an increase in LCMRG. The purpose of this study was to determine if there was a correlation between increased metabolic activity and somatosensory related behavior. The present research examined the effect of C3 enrichment on the manner in which rats explore a novel environment, known to evoke wall-hugging (Gallo and Carr, Proc. and Abst., East. Psych. Assoc. 1985). On postnatal days 1-4 (PND 1-4), 20 rats (Sprague-Dawley) had follicles bilaterally ablated, sparing both C3s. Left C3 was mechanically stroked for 10 min per day in 10 animals; the other ten rats had right C3 stroked. "Enrichment" continued from PND 5 to PND 90.

The rats were tested individually in a darkened cylindrical pen (125 cm in diameter) for 2 min per day for 5 consecutive days. It was predicted that prior stroking of the left C3 would tend to evoke clockwise wall-hugging and that prior stroking of the right C3 would evoke counter-clockwise wall-hugging. The findings confirm the hypothesis; i.e., during the ten-minute test, the rats spent significantly more time ($p < 0.05$) and traversed a greater distance ($p < 0.05$) wall-hugging in the predicted direction than in the opposite direction. The experiment reported here suggests that continued mechanical stimulation of spared C3 affects somatosensory related locomotor behavior in rats. The chronic mechanical stimulation of a spared peripheral receptor organ resulted in use of this "enriched" vibrissa to negotiate a novel environment. Such behavioral results appear to correlate with LCMRG in the SI representation of the "enriched" spared C3 vibrissa reported on previously. (Supported in part by BSRG, RR05464, 55-21851)

135.12 MORPHOGENESIS OF DORSAL ROOT GANGLION CELLS WITH PROCESSES IN THEIR SYNAPTIC TARGET ZONE OF EMBRYONIC SPINAL CORD. R.P. Barber* and J.E. Vaughn. Beckman Research Institute of the City of Hope, Duarte, CA 91010.

This study focused upon the morphogenesis of dorsal root ganglion (DRG) cells whose central processes had reached their synaptic target zone in the embryonic mouse (C57BL/6J) spinal cord. On embryonic days 12-15 (E12-15), horseradish peroxidase (HRP; 1-14nl) was pressure injected into the spinal association region through micropipettes. The embryos were then cultured at 35°C for 2-5 hours in an oxygenated medium consisting of heat-inactivated 50% rat serum and 50% Waymouth's solution. Embryos were fixed, cryoprotected, and sectioned on a cryostat. Slide mounted sections were incubated in $CoCl_2$ followed by DAB/H_2O_2, and were then counterstained and coverglassed. Retrogradely labeled DRG cells were measured and classified into four categories representing successive developmental stages: 1) primitive bipolar cells, that were spindle-shaped, symmetrical cells with one process extending from each pole; 2) early transitional bipolar neurons that were asymmetric cells with their two processes approaching one another at an angle exceeding 90°; 3) late transitional bipolar cells that exhibited central and peripheral processes closing toward each other at an angle of 90° or less; and 4) pseudounipolar neurons with a morphology typical of mature DRG cells.

On E12, the mean major and minor somal diameters of labeled DRG cells were 17.06±0.31μm and 8.36± 0.18μm, respectively. Nine percent of the E12 labeled cells were of the primitive bipolar type, whereas 91% were early transitional cells. On E13, the mean major and minor somal diameters were 17.69±0.33μm and 11.04±0.22μm, respectively. Early transitional cells accounted for 77% of the labeled population, while late transitional cells made up 11%. Primitive bipolar cells (10%) and pseudounipolar neurons (2%) accounted for the remainder of the cells labeled at E13. On E14, the mean major and minor somal diameters were 15.39±0.34μm and 12.17±0.28μm, respectively. Early transitional neurons accounted for 60% of the labeled cells, pseudounipolar neurons for 17%, primitive bipolar cells for 13%, and late transitional cells comprised 10% of the labeled population. Finally on E15, the mean major and minor labeled somal diameters were 19.77±0.40μm and 14.60±0.32μm, respectively. Pseudounipolar neurons accounted for 43% of the labeled cells, and late transitional cells made up 36% of the total. Early transitional cells (19%) and primitive bipolar (2%) cells accounted for the remainder. A salient finding of this study was that even the less differentiated forms of DRG neurons had axons within their central synaptic target field during early stages of spinal cord development. Supported by NSF grant BNS-8219831.

135.13 EFFECTS OF DORSAL ROOT GANGLION REVERSALS ON SKIN SENSORY INNERVATION PATTERNS IN CHICK HINDLIMB. S.A. Scott, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

During embryonic development dermatomes and axonal projections of lumbosacral dorsal root ganglia (DRGs) are established in a precise, orderly fashion in the chick, suggesting that sensory neurons may be specified for their axonal pathways or target skin. Alternatively, motor neurons may guide the outgrowth of sensory axons (Landmesser & Honig, Neurosci. Abst. 1982). To investigate these possibilities the rostrocaudal order of DRGs T7-LS3 was reversed to cause sensory axons to enter the limb from a novel location; at St.15-16 either the entire neural tube opposite somites 26-29 was excised, rotated and reimplanted or the dorsal neural tube alone was rotated, leaving the ventral neural tube (including the motor neuron precursors) intact. In some embryos quail neural tissue was transplanted into chicks to aid in later identification of rotated tissue. The resulting dermatomes and axonal projections were studied at St.29-40.

Rotation of the neural tube led to a partial reversal of skin sensory innervation patterns (see also Honig, Neurosci. Abst. 1979), as if some but not all axons from rotated DRGs established "embryologically correct" innervation. For example, in control embryos most skin sensory axons of DRGs T7 and LS1 project in the CFL nerve to serve dermatomes mainly on the thigh; DRG LS2 divides its axons roughly equally between the CFL and CFM nerves, and between the thigh and shank; DRG LS3 projects mostly in the CFM nerve to serve a dermatome mainly on the shank. When neural tube was rotated, the DRGs that developed at T7 and LS1 had a significantly larger percentage of their axons in the CFM and a significantly larger percentage of their dermatomes on the shank, approaching but seldom achieving the innervation patterns of DRGs LS2 and LS3 from which they were derived. In contrast, when only the dorsal neural tube was rotated, leaving motor neuron precursors largely intact, innervation patterns were nearly identical to controls, as if DRG axons established innervation in accord with their position in the embryo. Thus, skin sensory innervation patterns closely follow the motor projection patterns, which are reversed following neural tube rotations (Lance-Jones & Landmesser, J. Physiol. 1980) but are nearly normal following dorsal neural tube rotations. These results suggest that if skin sensory neurons are specified with respect to their peripheral connectivity, the specification is relatively weak, with the innervation patterns being greatly influenced by the corresponding motor neurons. Attempts are now being made to investigate skin sensory innervation patterns in the absence of motor neurons. (Supported by NIH grant NS16067 and an Alfred P. Sloan Foundation Fellowship).

135.14 SELF-MUTILATION AFTER DORSAL RHIZOTOMY: ROLE OF EXTENT AND LOCATION OF LESION. E. Schneider and D. Berman (SPON: A.J. Berman). Psychology Dept., Queens College, CUNY, Flushing NY.

Deafferentation in animals is frequently, but not always, followed by self-mutilation (autotomy) of the affected limb. Rabin et al. (Pain, 21: 117-128, 1985) suggest that, after nerve section in the rat, it is the extent of lesion which is critical, with total denervation of the limb resulting in a markedly different pattern of autotomy than follows partial denervation. The present study is an attempt to elucidate the role of extent and location of dorsal rhizotomy on autotomy in the rat.

Male Long-Evans rats were subjected to section of dorsal roots innervating the left forelimb.

n	Dorsal roots sectioned	
	Location	Number
4	C7-C8	2
5	C6-C8	3
5	C7-T1	3
4	C6-T1	4
5	C5-T2	5

All rats, save two in the C7-C8 group and one in the C6-C8 group, self-mutilated to some degree. There was no significant relationship, however, between the number of roots cut and severity of autotomy. Severity did not differ between rats with lesions of two roots (C7-C8) and those with one of the three-root lesions (C6-C8), nor between rats with the other three-root lesion (C7-T1) and those with four roots cut (C6-T1). Furthermore, rats with section of C7-T1 had as high a level of autotomy as did rats with section of six roots (C5-T2). The particular roots cut, on the other hand, appeared to be critical in determining the course of autotomy. Section of C6 did not affect the phenomenon; autotomy did not differ between rats with lesions of C7-C8 vs C6-C8. Section of T1, however, did affect autotomy; rats with lesions of C7-T1 showed a significantly greater degree of self-mutilation than did rats with section of C7-C8 alone. In fact, all rats with lesions including T1 showed more severe autotomy than did animals with lesions of C7-C8. In addition, rats with lesions including T1 showed deficits in limb placement during ambulation that were not present in rats with lesions excluding T1.

These results are not supportive of the hypothesis that post-dorsal rhizotomy autotomy reflects a pain state (Lombard et al., Pain, 6: 163-174, 1979), unless it is assumed that the addition of T1 to a C7-C8 lesion leads to a greater increment of pain than does the addition of C6. These results do support the view that there is a relationship between functional use of a deafferented limb and relative protection from self-mutilation (Berman et al., Pain, Suppl.1, S105, 1981).

136 SPECIAL LECTURE. MPTP-INDUCED PARKINSONISM: MECHANISMS OF TOXICITY AND SELECTIVE VULNERABILITY. I.J. KOPIN, NINCDS, NIH, Bethesda, MD 20205.

Accidental exposure to 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), either as an impurity in an illicit narcotic or during its synthesis as a pharmaceutical intermediate, produces a parkinsonian syndrome in humans.

In experimental animals the acute pharmacological effects of MPTP differ markedly from the chronic toxic effects. In all species tested the acute effects of MPTP are consistent with evoked amine release, producing in rats a characteristic "serotonin-syndrome" and an autonomic reaction. There is a striking disparity, however, in the susceptibility of various species to the toxic effects. When administered to non-human primates, after dissipation of acute effects, there develops over a period of several days a movement disorder resembling human parkinsonism. There is selective destruction of dopaminergic neurones in the nigrostriatal pathway and biochemical changes in brain and cerebrospinal fluid consistent with loss of these neurones. Dogs are also very sensitive to the toxin, whereas mice require high doses of MPTP to produce limited and largely reversible damage to the brain catecholaminergic neurones. Rats, rabbits and guinea pigs are almost totally resistant to the toxin.

Oxidation of MPTP to its pyridium derivitive, MPP^+, appears to be mediated by monoamine oxidase (MAO). Since drugs such as deprenyl or pargyline which inhibit monoamine oxidase B (MAO-B) and block formation of MPP^+ protect animals from MPTP toxicity, MPP^+ formation is necessary for the toxic effect.

The selectivity of dopaminergic neurones as targets for MPTP appears related to formation of MPP^+ by MAO-B in astrocytes and subsequent uptake of the metabolite into dopaminergic neurones. This does not, however, explain why rodents are relatively insensitive to the toxin. The vulnerability of primates and dogs to MPTP toxicity may be related to the higher concentrations of neuromelanin in the substantia nigra of these species in contrast to its absence in rodent brain and to differences in MPTP metabolism. Neuromelanin exists as a redox polymer which can be a source of free radical generation. The resemblance of MPP^+ to paraquat, a toxin which appears to be mediated by free-radical redox cycling, and the possible role of free radicals in mediating the effects of other toxins such as 6-hydroxy-dopamine, suggest that free radical formation may be involved in MPP^+ toxicity mechanisms. Thus metabolic conversion to MPP^+, accumulation of MPP^+ in specific neurones by the dopamine uptake mechanisms, and involvement of granular neuromelanin in redox cycling of MPP^+-$MPP^{\cdot}$ to yield toxic free radicals may explain the tissue and species specificity of MPTP neurotoxicity.

In addition to providing a useful animal model of Parkinson's Disease, the selective vulnerability of the nigrostriatal neurones of primates and dogs to the toxic effects of MPTP may provide useful insights into the mechanisms involved in the degenerative processes which cause Parkinson's Disease and serve to test therapeutic agents which may retard the degenerative process or replace the missing neurotransmitter systems.

137 SYMPOSIUM. PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF FATIGUE IN THE MOTOR UNITS OF MAMMALS. E. Henneman, Harvard Medical School (Chair); M.J. Kushmerick*, Brigham and Womens Hospital; H.P. Clamann, Medical College of Virginia; D. Kernell*, University of Amsterdam; C.J. DeLuca, Boston University; B. Bigland-Ritchie, J.B. Pierce Foundation.

Fatigue is defined as the inability to produce or sustain an expected level of force. Changes in electrical, mechanical or chemical events precede and accompany fatigue. Any of these could be a cause. The site(s) of fatigue are not the same for all muscles but depend on the structure and function of the muscle and on the pattern of contraction it is called on to produce. M. Kushmerick will discuss intracellular chemical changes in muscle which may cause fatigue. During active force development chemical activity in a muscle produces changes in high-energy phosphate levels and intracellular pH which may be large and quantitatively different for different fiber types. ^{31}P-NMR is a powerful tool which may be used to explore these changes while muscle force is monitored simultaneously. In this way effects of chemical changes on force output may be determined. P. Clamann will examine EMG and force changes of single motor units in response to various stimulus patterns. With all stimulus patterns tested, force of fast fatiguing motor units declines more quickly than EMG. Fatigue resistant motor units fatigue only during high frequency stimulation, and failure seems to be electrical. Rest periods of < 1 s allow recovery from electrical fatigue. When frequency is changed during a stimulus train, force output depends on stimulus history so that there is no unique relation between frequency and force. D. Kernell will discuss central and peripheral aspects of motor unit function during fatigue. Motoneuron discharge rate declines even with constant excitation, resulting in a central fatigue which is most prominent in fast fatiguing motor units. In the periphery, EMG changes precede and accompany mechanical fatigue. The mechanisms have been investigated in vitro. The susceptibility of motor units to EMG and force fatigue is profoundly influenced by chronic stimulation, showing an adaptation to long-term activity. C. DeLuca will interpret changes in the EMG signal recorded during fatigue. The power spectrum of the EMG shifts preceding and during fatigue. This change may be used to predict force loss in a muscle, and may serve as a tool to study the metabolic and electrical events which cause it. B. Bigland-Ritchie will discuss differences in the mechanisms underlying fatigue in human limb and respiratory muscles. In limb muscles, the force of a maximum voluntary contraction (MVC) cannot be increased by stimulating the muscle, showing that fatigue is peripheral. By contrast, the force produced by stimulating the diaphragm declines more slowly than the force of MVC, showing that this fatigue has a central component.

138 SYMPOSIUM. MOLECULAR BASES OF THE IMMUNE RESPONSE TO NEURAL ANTIGENS. L.A. Lampson, Univ. of Pennsylvania Sch. of Med. (Chair); S.I. Rapoport, National Inst. on Aging, NIH; R.T. Johnson, Johns Hopkins Univ. Sch. of Med.; A.A. Zalewski, NINCDS, NIH; R.P. Lisak, Univ. of Pennsylvania Sch. of Med.

The nervous system has long been considered immunologically privileged. Recent work demands a re-evaluation of the extent and nature of this privilege. We will examine factors that permit, or prevent, an immune response to neural antigens. Our goal is to provide a conceptual framework for understanding recent studies of the immune response to infected, damaged, transformed, or transplanted neural tissue, and how that response can be manipulated clinically.

Immune privilege can be understood, first, in terms of a physical barrier. Rapoport will define the physical blood-brain barrier. He will discuss the extent to which it sequesters neural tissue from antigens, and from immunocompetent cells and antibodies. He will illustrate pathological conditions under which the barrier is breached, and discuss how the barrier may be circumvented for therapeutic reasons.

Immune privilege also has a molecular component. Immunocompetent cells must recognize antigen in association with products of the major histocompatibility complex (MHC). Lampson will describe the normal paucity of MHC products in neural tissue. She will discuss circumstances under which greater expression occurs, or can be induced. Illustrations will focus on the immune response to neural tumors.

Viral infections display unusual features in the nervous system. Johnson will discuss the extent to which the latency or progression of viral infections, and the observed pathology, can be understood in terms of the immune response, or its failure.

Transplantation of functional neural tissue holds great clinical promise. In part, its full potential will depend on the extent to which transplants can be made between different individuals or species. Zalewski will discuss factors that influence the survival of neural allografts and xenografts.

Lisak will draw attention to the heterogeneity of immune effector mechanisms. He will review the requirements for stimulating cell-mediated and antibody-mediated responses, and discuss the consequences of the two types of attack against antigens in neural tissue. Examples will emphasize the role of the immune response in demyelinating diseases.

139.1 PET QUANTIFICATION OF DOPAMINE RECEPTORS WITH COMPETITIVE NEUROLEPTIC DRUGS IN THE LIVING HUMAN BRAIN. D.F. Wong*, H.N. Wagner, Jr.*, A. Gjedde*, J. Coyle, R.F. Dannals*, S. Snyder, J. Links*, M.J. Kuhar (SPON: W. Jankel). Dept. Rad. (Nucl. Med.), Neuroscience, Johns Hopkins U., PANUM Inst., Copenhagen, DK., Addiction Res. Ctr., Balto., MD 21205

We have previously shown that C-11 3-N-methylspiperone (NMSP) binds specifically to the human D2 dopamine and, to a lesser extent, to S2 serotonin receptors (Science 222:1264, 1983). After the injection of C-11 NMSP, the caudate/cerebellar ratio (CA/CB), an estimate of D2 receptors, increases linearly with time in both normal and patient subjects (Science 226:1393, 1984). A 3-compartment model was proposed where the binding rate to the receptor, k3, was related to the slope of the CA/CB vs. time.

Two important applications of this PET imaging is comparing potencies of neuroleptics in vivo and in absolute quantification of receptors. We have measured the in vivo competition of neuroleptics with NMSP and compared the relative blockade of dopamine receptors in vivo by the neuroleptics haloperidol (Hal) and molindone (Mol) in normal volunteers, using this approach. Hal (0.05 mg/kg) resulted in a 68% drop in the slope of the CA/CB vs. time. When Mol was given in the clinically equivalent doses that were 4 times that of Hal, in the same people (N=3), a similar degree of blockade was measured, despite the fact that Mol has a 30 times lower in vitro binding affinity at the D2 receptor. A graded response was demonstrated for both Hal (range 0.05-0.082 mg/kg) and Mol (0.16-0.44 mg/kg) (N=3).

We used the blocking studies with Hal to quantify the density of dopamine receptors in normal human volunteers. The most recent model employed the estimate of k1, k2 and k3 from the curve fit of the caudate and cerebellar distribution volumes (tissue/plasma activity conc.) vs. effective time (integral of the plasma activity conc./plasma activity conc., designated PI/P). A correction for metabolites was employed using the cerebellar distribution volume vs. PI/P which agreed well with HPLC metabolite measurements.

We obtained different values of k3, that is, the rate of binding of the ligand to the receptor, with different blocking doses of Hal, and we estimated values for receptor density times koff and the inhibition constant K_i for Hal. Assumption of values of koff and of partition coefficients for Hal allowed an estimate of receptor density B_{max} (5-10 pm/gm) and Ki (1-2 nM) (N=4) which agreed well with values obtained in in vitro binding assays in human brains obtained at autopsy. Hence, it is now possible to compare directly the in vivo blockade of receptors by neuroleptic drugs and to use the blockade to quantify absolute receptor values. (Supported by NS 15080)

139.2 HUMAN BRAIN D-1 DOPAMINE RECEPTORS LABELLED WITH ^{3}H-SCH 23390. J.L.Waddington and K.M.O'Boyle*. Dept. Clin. Pharmacol., Royal College of Surgeons in Ireland, Dublin 2.

SCH 23390 is a potent and selective dopamine D-1 receptor antagonist. Recently, the binding of ^{3}H-SCH 23390 to rat striatal membranes has been described and may be superior to ^{3}H-piflutixol as a selective D-1 receptor ligand. We have investigated ^{3}H-SCH 23390 binding in human putamen.

A crude membrane preparation was prepared from human post-mortem putamen tissue. Radioligand binding assays were carried out essentially as described previously for ^{3}H-piflutixol. Specific binding was defined as that displaced by 100 nM piflutixol. The results of saturation studies were consistent with binding to a single population of sites with a B_{max} of 14.2 pmol/g wet weight and a K_d of 1.3 nM (mean of 3 estimations on the same tissue pool). The number of D-2 receptors was measured twice on this same tissue pool using ^{3}H-spiperone and amounted to 8.1 pmol/gm wet weight. Relative potencies of dopamine and reference dopaminergic antagonists to displace the binding of 0.8 nM ^{3}H-SCH 23390 are shown in the Table. Non-dopaminergic antagonists such as prazosin (α_1) and ketanserin (5-HT_2) had IC_{50} values >25μM.

Displacing Agent	IC_{50} (nM)
SCH 23390	2.1
R-SK&F 83566	4.2
S-SK&F 83566	>1,000
Fluphenazine	106
Domperidone	4,910
Sulpiride	>100,000
Dopamine	2,216

means of 2-5 estimations

In this study, ^{3}H-SCH 23390 showed a typical D-1 receptor profile, being displaced potently by SCH 23390 and stereospecifically by the R- but not the S- enantiomer of the selective antagonist SK&F 83566. The non-selective agents fluphenazine and dopamine itself were also active. Among the selective D-2 antagonists domperidone was 2000-fold less active than SCH 23390 and sulpiride was inactive. The ratio of D-1:D-2 sites was approximately 2:1. The binding dissociation constant and drug displacement potencies are similar to those reported in rat striatum, consistent with selective labelling of D-1 receptors in human brain.

We thank Schering for ^{3}H-SCH 23390, Smith Kline & French for enantiomers of SK&F 83566, and Dr. J.Dinn for human brain tissue. This work was supported by the Medical Research Council of Ireland and the Royal College of Surgeons in Ireland.

139.3 QUANTITATIVE AUTORADIOGRAPHIC EVALUATION OF [^{3}H]-SCH 23390 AS A DOPAMINE D-1 RECEPTOR ANTAGONIST IN RAT AND HUMAN BRAIN. T.M. Dawson, D.R. Gehlert*, S.S. Stensaas, H.I. Yamamura, A. Barnett and J.K. Wamsley. Depts. Psych., Pharm. and Path., Univ. Utah Sch. Med., SLC, UT 84132, Dept. Pharm., Univ. Arizona, Tucson, AZ 85724, Schering, Bloomfield, NJ 07003

Agonist-stimulated adenylate cyclase indicates the existence of dopamine (DA) D-1 receptors in the prefrontal cortex of rats. Localization of D-1 receptors by binding techniques would be the next logical step in further characterization of DA receptors in the central nervous system.

Classic saturation and competition experiments were combined with quantitative autoradiography to determine the suitability of [^{3}H]-SCH 23390 as a D-1 receptor antagonist in several discrete microscopic regions of brain. Ten micron, slide-mounted, serial tissue sections from rat brain or human postmortem cortex were incubated with 1 nM [^{3}H]-SCH 23390 with varying concentrations of cold SCH 23390, fluphenazine, SKF 38393, cis-(Z)-piflutixol, trans-(E)-piflutixol, sulpiride, methysergide and ketanserin. Saturation isotherms were generated by incubating the slide-mounted tissue sections in varying concentrations of [^{3}H]-SCH 23390. Autoradiograms were produced by apposition of the labeled tissue sections along with tritium standards to LKB Ultrofilm. Data was analyzed using computerized microdensitometry.

Binding of [^{3}H]-SCH 23390 to several discrete microscopic regions of rat brain (substantia nigra reticulata, accumbens nucleus, caudate-putamen, olfactory tubercle, prefrontal cortex) and human brain (striatum, superior frontal gyrus, hippocampus, substantia nigra) was saturabe, of high affinity and stereospecific. Displacement data indicate that [^{3}H]-SCH 23390 binding to human and rat brain structures, under the conditions employed for autoradiography, is selective for the D-1 receptor.

The anatomic distribution and density of D-1 receptors was then determined. The highest concentrations of D-1 receptors were noted in the substantia nigra and striatum of both human and rat brain. D-1 receptors were hetereogenously distributed in the amygdaloid and hippocampal structures. Low to very low concentrations of D-1 receptors were identified in the cortices of both human and rat brain with the deeper laminae containing approximately a two-fold greater concentration than the superficial laminae.

The localization of D-1 receptors in the frontal cortex provides strong evidence for a role of these receptors in the mediation of behavior and thought. This suggests the possibility of new therapeutic modalities, free from the adverse side-effects associated with most neuroleptics, in the treatment of schizophrenia.

139.4 COMPARISON OF THE DISTRIBUTION OF D-1 AND D-2 DOPAMINE RECEPTOR SUBTYPES IN THE RAT CNS BY QUANTITATIVE AUTORADIOGRAPHY. J.K. Wamsley, T.M. Dawson and D.R. Gehlert*. Depts. Psychiatry and Pharmacology, Univ. Utah Sch. of Med., Salt Lake City, UT 84132.

Two subtypes of dopamine receptors have been reported to exist in the CNS. The stimulation of adenylate cyclase is believed to be mediated by agonist interaction with the D-1 receptor subtype while the D-2 receptor appears to be negatively linked or not coupled to this enzyme. These subtypes can be identified in ligand binding studies on the basis of distinct pharmalogical properties. Recently, a radiolabeled form of the highly selective D-1 antagonist SCH 23390 has been reported to bind with high affinity and specificity to slide-mounted sections of rat brain (Dawson et al., Eur. J. Pharmacol., 108:323, 1985; this meeting). In order to compare the distribution of D-1 and D-2 receptors in the rat brain, we have labeled serial sections with either [^{3}H]-SCH 23390 or the selective D-2 antagonist [^{3}H]-sulpiride for the detection of receptors by quantitative autoradiography. We have also localized the high and low affinity conformations of these receptor subtypes and investigated their guanine nucleotide sensitivity.

The D-1 receptor was labeled with [^{3}H]-SCH 23390 (1 nM) while the D-2 receptor was labeled with [^{3}H]-sulpiride (20 nM) (Gehlert et al., Eur. J. Pharmacol., 98:311, 1984; Neurochem. Int., in press). Additional sets of sections were incubated with various concentrations of SKF 38393 (D-1) or ADTN (D-2) in the absence or presence of 10 micromolar Gpp(NH)p. Autoradiograms were produced by apposition to tritium-sensitive film in x-ray cassettes for appropriate exposure periods. Autoradiograms were quantitated using computerized densitometry with radioactive standards.

A highly discrete distribution was observed for each receptor subtype. Structures such as the caudate-putamen, nucleus accumbens and olfactory tubercle contained a high density of both D-1 and D-2 receptors. Relatively high D-1, but low D-2 receptor binding was detected in the substantia nigra, cerebral cortex, endopiriform nucleus, claustrum and the suprachiasmatic nucleus. ^{3}H-SCH 23390 binding was very low in the pituitary and olfactory bulb areas which exhibited a high density of ^{3}H-sulpiride binding sites. Sparse, but differential labeling was noted for both ligands in areas of the hypothalamus, thalamus, brainstem and cerebellum. A distinct regional distribution for the guanine nucleotide-sensitive high affinity agonist conformation of each receptor subtype was also noted. These results not only provide additional evidence for the existence of D-1 and D-2 receptor subtypes; but indicate distinct brain regions, for further study of the functional role that these subtypes may play in neurotransmission.

139.5 CHRONIC BLOCKADE OF D1 DOPAMINE RECEPTORS INCREASES ^{3}H-SCH 23390 BINDING SITES IN THE RAT STRIATUM. M.L. Porceddu*, E. Ongini and G. Biggio, Institute of Biology, Chair of Pharmacology, University of Cagliari, Italy.

Chronic administration of SCH 23390 (0.03 mg/kg s.c., three times daily), a selective D1 receptor blocker, increased markedly the ^{3}H-SCH 23390 binding in the rat striatum. As revealed by the Scatchard plot analysis of saturation data from striatal homogenates, SCH 23390 increased the total number of binding sites by 40% when compared to tissue from solvent-treated rats, but failed to change the apparent affinity of ^{3}H-SCH 23390 for its binding sites. Moreover, chronic treatment with haloperidol (0.5 mg/kg s.c., three times daily), a dopamine receptor antagonist with mixed selectivity for D1 and D2 receptor sites, produced a slight but significant increase of D1 dopamine receptor subtypes. In fact, this treatment increased the Bmax of ^{3}H-SCH 23390 binding sites by 15% and failed to change the apparent affinity of ^{3}H-SCH 23390 for D1 receptor sites. In contrast with the effect of these drugs, chronic administration of (-)sulpiride, a selective blocker of D2 receptor site, failed to change ^{3}H-SCH 23390 binding. The Scatchard plot analysis of saturation data carried out on (-)sulpiride treated and solvent-treated rats, revealed that this treatment failed to change the number as well as the apparent affinity of ^{3}H-SCH 23390 binding sites in striatal membrane preparation. The results indicate that a) chronic blockade of D1 receptor sites by a selective antagonist (SCH 23390) can lead to an increase in their total number; b) ^{3}H-SCH 23390 is a selective ligand for D1 dopamine receptor subtype.

139.6 DIFFERENTIAL RECOVERY RATES OF D-1 AND D-2 DOPAMINE RECEPTORS IN RAT STRIATUM AS A FUNCTION OF AGING AND CHRONIC DRUG TREATMENTS. Andrew B. Norman*, George Battaglia and Ian Creese. Dept. of Neuroscience, M-008, U.C.S.D. School of Medicine, La Jolla, CA 92093.

Changes in the Bmax of a number of neurotransmitter receptors have been reported following chronic pharmacologic blockade or as a function of aging. Since receptors are not stable components of cell membranes but rather are subject to constant degradation and synthesis, we investigated whether the processes regulating striatal dopamine receptor numbers might be altered in response to aging and chronic reserpine treatment. We found a reduction in the Bmax of both striatal D-1 and D-2 receptors of 21% and 26% respectively in senescent (28 months) compared to mature (4 months) Fischer 344 rats. We investigated the time-dependent recovery of D-1 and D-2 receptors by following increases in the Bmax of [^{3}H]flupentixol (in the presence of 30nM spiperone) and [^{3}H]spiperone (in the presence of 40nM ketanserin) binding after irreversible blockade of these receptors by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (10mg/kg i.p.) In senescent rats the rate of recovery was markedly slower than in mature rats for D-1 (3.8 vs 6.7pmol/g tissue/day) and D-2 (1.4 vs 2.5pmol/g tissue/day) receptors. However, whilst the Bmax of both D-1 and D-2 receptors returned to control values 16 days following EEDQ treatment in senescent rats, the maximal recovery (up to 32 days) of both D-1 and D-2 receptors in mature rats achieved only 80% of control values. Interestingly, the Bmax in mature rats at 16 and 32 days was similar to the control Bmax observed in senescent rats. These data indicate that the turnover of the majority of D-1 and D-2 receptors may be markedly slower in senescent compared to mature rats and furthermore, that a subpopulation of D-1 and D-2 dopamine receptors may be lost as a function of aging.

21 day reserpine treatment (0.5mg/kg/day) produced a 31% increase in the Bmax of D-2 receptors in mature rats but no significant change in senescent rats. Similarly, chronic reserpine treatment produced a small (10%) but significant increase in the number of D-1 receptors in mature rats but no change in senescent rats. Although the rate of recovery of D-2 receptors was faster in reserpinized mature rats (4pmol/g tissue/day), there was no significant effect of reserpine on the rate of recovery of D-2 receptors in senescent rats. However, no significant increase in the apparent rate of recovery of D-1 receptors was observed following chronic reserpine treatment in either mature or senescent rats. These data demonstrate that recovery rates of distinct receptors can be differentially regulated by chronic drug treatments and that this regulation may differ as a function of aging.

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139.7 SPIPERONE AND SCH-23390 DISCRIMINATE (^{3}H)FLUPHENAZINE LABELED D-1 AND D-2 DOPAMINE RECEPTORS IN MOUSE STRIATUM. D.G. Morgan and C.E. Finch. Andrus Gerontology Center, and Dept. of Biol. Sciences, Univ. Southern Cal., Los Angeles, 90089-0191.

D-1 and D-2 dopamine receptors in mouse striatal membranes were labeled in vitro with (^{3}H)fluphenazine. Assays were conducted in a physiological ions buffer for 30 minutes at 25°C, and filtered with a cell harvester. Nonspecific binding was reduced considerably by prewetting the GF/C filters with 0.1% polyethyleneimine, and including 0.15 M NaCl in the wash buffer. Apomorphine (100 µM) was used to estimate specific binding, and, typically, displaced less (^{3}H)fluphenazine than the more hydrophobic dopamine antagonists. Some of these dopamine antagonists also displaced (^{3}H)fluphenazine from heat-denatured membranes; apomorphine did not.

The D-1 and D-2 specific binding of (^{3}H)fluphenazine was discriminated by the selective dopamine antagonists SCH-23390 (D-1 selective) and spiperone (D-2 selective). Saturation analyses of these two sites indicated that the density of D-1 sites in mouse striatum was 1400 fmol/mg protein, and the D-2 density was 700 fmol/mg protein, irrespective of the competitor used to discriminate the D-1 and D-2 components. The affinity of (^{3}H)fluphenazine for the D-2 site was slightly greater than for the D-1 site (equilibrium dissociation constant, K_d = 0.7 and 3.2 nM, respectively).

Dopamine had a greater affinity than other neurotransmitters for both sites labeled by (^{3}H)fluphenazine. Moreover, displacement curves from a series of dopaminergic and nondopaminergic antagonists indicated that the pharmacological specificity of (^{3}H)fluphenazine binding in the striatum was dopaminergic. Only small amounts of dopamine specific (apomorphine sensitive) (^{3}H)fluphenazine binding were found in other brain regions. However, chlorpromazine displaced (^{3}H)fluphenazine binding from all brain regions, particularly in cerebellum, suggesting the existence of a nonmonoaminergic (^{3}H)fluphenazine binding site with, perhaps, a phenothiazine specificity.

Replacement of the physiological ions buffer with one containing 4 mM EDTA, increased specific (^{3}H)fluphenazine binding at a concentration of 1.5 nM, and truncated the plateau region of the spiperone (but not SCH-23390) displacement curves. Preliminary experiments suggest this increase is due to a greater affinity of dopamine antagonists for D-1 binding sites in the EDTA buffer.

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139.8 DOPAMINE RECEPTORS IN HIPPOCAMPUS: IN VIVO AND IN VITRO CHARACTERIZATION, AND THEIR PHARMACOLOGICAL RELEVANCE. S. Bischoff*, A. Bruinink* and A. Vassout* (SPON: A. Matus). Res. Dept., Pharma Div., Ciba-Geigy, Basel, Switzerland.

A large body of evidence supports our hypothesis of the existence of a dopaminergic system in the hippocampus (Bischoff,S., in Isaacson and Pribram (Eds), The Hippocampus, Plenum Press, 1985). In particular, dopamine (DA) receptors have been identified using the in vivo (^{3}H)spiperone radioreceptor assay. The present study was aimed to further characterize these receptors.

In rat hippocampus, under in vivo conditions, spiperone bound specifically (80%) and stereoselectively to DA receptors. No significant binding was found either to spirodecanone acceptor sites or to serotonin or alpha-adrenergic receptors. The effects of neuroleptics in the hippocampus correlated (r: .963) well with those on spiperone binding in striatum. However, the atypical neuroleptics (eg. clozapine and benzamides) inhibited spiperone binding at lower doses in hippocampus than in striatum. They also exerted an almost complete inhibition in hippocampus whereas in striatum some of them displayed only partial effect.

Previously, DA receptors have not been identified in the hippocampus in vitro. Now, we found that in vitro, in hippocampal homogenates, (^{3}H)spiperone (25pM) bound to several high affinity sites. One of them appeared to be DA D_2 receptors as defined by the displacement of (^{3}H)spiperone binding by 1 uM l(-)sulpiride (IC_{50}: 18 nM). The density of the sites was about 70times lower than in striatum. As concluded from pH dependency, the other sites may predominently be spirodecanone sites.

To check the pharmacological relevance of these DA receptors in hippocampus, we compared the potency of neuroleptics to inhibit in vivo (^{3}H)spiperone binding in hippocampus and to antagonize apomorphine-induced climbing in mice. A close correlation was found between both parameters even with atypical neuroleptics suggesting that the hippocampal DA receptors are of the same type than those responsible for the apomorphine-induced climbing, and therefore, that their inhibition may reflect the antipsychotic efficacy of a drug.

139.9 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF PUTATIVE A13 DOPAMINE NEURONS. M.K. Sanghera, Depts. of Psychiat. and Physiol., Univ. of Texas Halth Science Center, Dallas, TX. 75235.

The rat hypothalamus contains four distinct groups of dopamine (DA)-containing nuclei designated A11-A14 by Dahlstrom & Fuxe, (Acta physiol. scand., 62:1-52, 1964). Of these, perhaps the tuberoinfundibular system (A12), located in the arcuate nucleus, has been most thoroughly studied and shown to be intimately involved in the regulation of prolactin from the adenohypphysis. The A11 group, located in the caudal hypothalamus, has been demonstrated to project caudally to the spinal cord region. On the other hand, comparatively little is known about the A13 and A14 DA neurons.

The purpose of the present study was to determine whether the A13 neurons have similar electrophysiological properties as the midbrain DA neurons. Anatomically, DA neurons were labelled with antibodies raised to tyrosine-hydroxylase (TH) using the immunoperoxidase staining method. These neurons are located medial to the zona incerta at the infundibular level of the hypothalamus. Electrophysiologically, extracellular single cell recordings were made from cells in the A13 in the chloral hydrate anesthetized male rat. The center barrel of a 5-barrel micropipette was used for recording neuronal activity and to mark the recording site at the end of the experiment with Fast Green dye. One barrel was filled with 3M NaCl and was used as the current balance and the other barrels were filled with DA hydrochloride (0.1M), sodium glutamate (GLU, 0.05M), and GABA (0.01M). The cells in the A13 were found to have action potentials greater than 2 msec in duration, similar to midbrain DA neurons. Firing rates ranged from silent to 2 impulses/sec. The silent cells could be made to fire by the iontophoretic application of GLU. Application of DA onto these cells inhibited the GLU-induced activation of these cells. This inhibition was selectively attenuated by the intravenous administration of haloperidol (0.1 mg/kg) while GABA continued to inhibit the firing rates of the cells. Histological verification of the recording sites indicated that these putative DA-containing cells were located within the A13-TH rich region of the hypothalamus. These data indicate that A13 DA neurons have action potential and autoreceptor characteristics similar to midbrain DA neurons. However, these A13 neurons are normally silent in the chloral hydrate anesthetized preparation. Research supported by grants MH-30546 and MH-39699.

139.10 BEHAVIORAL EVIDENCE FOR A D_2-LIKE DOPAMINE RECEPTOR IN THE CNS OF RHESUS MONKEYS. W.L. WOOLVERTON AND J.B. KAMIEN.* DRUG ABUSE RESEARCH CENTER, UNIVERSITY OF CHICAGO, CHICAGO, IL 60637.

Rhesus monkeys (N=4) were trained in a two-lever, food-reinforced drug discrimination paradigm to discriminate apomorphine (APO; 0.12 or 0.25 umol/kg, IV, 5 minutes presession) from saline. When the discrimination was acquired, various doses of APO, as well as other dopamine (DA) agonists, were injected before test sessions. In addition, DA antagonists were tested in combination with several doses of APO. APO (0.06-1.0 umol/kg, IV, 5 minutes presession) produced a dose-related increase in the percent of responses that occurred on the drug lever during test sessions. In substitution tests, the selective D_2 agonist piribedil (0.12-4.0 umol/kg, IV, 5 minutes presession) produced a dose-related increase in drug lever responding that was comparable to that seen with APO, while the selective D_1 agonist SKF38393 (0.12-64 umol/kg, IV, 5 minutes presession) resulted in principally saline lever responding, even at doses which substantially reduced response rate. Administration of DA (1.0-2.0 umol/kg, IV, 5 minutes presession), which does not readily cross the blood-brain barrier, also resulted in principally saline lever responding. Pimozide, (0.007-0.125 umol/kg, IV, 120 minutes presession), a selective D_2 antagonist, blocked the ability of APO to occasion drug-appropriate responding and this blockade could be overcome with a higher dose of APO. Domperidone (0.07 umol/kg, IV, 120 minutes presession), a potent D_2 antagonist, but one that does not cross the blood-brain barrier, did not block the APO discriminative stimulus. SCH23390 (0.007-0.25 umol/kg, IV, 30 minutes presession), a D_1 antagonist, reduced drug lever responding following APO in one monkey but not in another. This blockade could also be overcome with a higher dose of APO. The results from substitition tests with DA agonists and the blockade of the APO cue with pimozide suggest that the discriminative stimulus properties of APO are based on its action at a receptor that is similar to the D_2 receptor. The failure of DA to substitute for APO as well as the failure of domperidone to block the APO discriminative stimulus indicate that this receptor is located in the central nervous system (CNS). The results with SCH23390 raise the possibility that D_1 and D_2 receptors are not independent in the CNS. This experiment provides in vivo evidence for the existence of a functional D_2 receptor in the CNS of rhesus monkeys. (Research supported by N.I.D.A. Grant DA-00250).

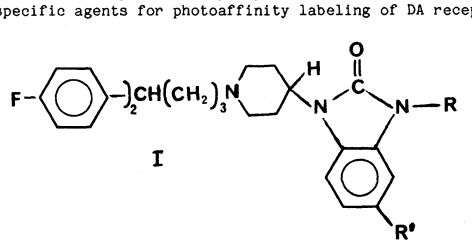
139.11 SOLUBILIZATION AND ISOLATION OF ^{3}H-SPD BINDING SITES FROM THE CAT BRAIN CORPUS STRIATUM. L. L. Hsu, K. Moroi* and J. R. Yu*. Dept. of Psychiatry and Behav. Sci., Univ. of Texas Med.Branch, Galveston, TX 77550.

We have previously characterized one population of ^{3}H-spiperone (SPD) binding sites in the crude mitochondrial membranes of the cat brain corpus striatum. The apparent K_d value for this popualtion of SPD binding sites was 1.00 nM and the appatent B_{max} value was 395 fmol/mg protein. We have solubilized these SPD binding sites and further purified them to apparent homogeneity by gel filtration and DA-affinity chromatography as previously described (Moroi & Hsu, J. Neurosci. Res. 12:113, 1984). Twenty-five adult cats were used for this study. Cats were anesthetized with Nembutal, decapitated & brains were rapidly removed. Corpus striata were carefully dissected over ice, frozen on dry-ice and stored at -60 °C until used. 25 pairs of striata were thawed, homogenized in 9 vol. of 0.32 M sucrose in 10 mM Tris-HCl, pH 7.4, containing 2 mM EDTA, 0.1 mM phenylmethylsulfonylfluoride (PMSF), and 0.2% NaN_3. The homogenate was subjected to differential centrifugation to yield P_{2M} membranes according to the method of de Robertis et al (J.B.C. 242:3487,1967). The SPD binding sites were solubilized from P_{2M} by 1% digitonin in 0.05 mM Tris-HCl,pH 7.4,containing 1 mM EDTA, 0.1 mM PMSF, and 1 mM DTT. The solubilized SPD binding sites in the digitonin extract were further purified by gel filtration, DA-affinity chromatography and disc gel electrophoresis. Results indicated that the ^{3}H-SPD binding to the digitonin extract was also saturable with an apparent K_d value of 3.43 nmol and an apparent B_{max} value of 310 fmol/mg protein. Bio-Gel A-5m gel filtration resulted in several protein peaks with fractions 30-50 having the highest ^{3}H-SPD binding. Subsequent DA-affinity chromatography of these fractions resulted in a single protein band on the native polyacrylamide gel after electrophoresis. This apparently pure SPD-binding protein from cat striatum showed specific ^{3}H-SPD binding of 15,986 fmol/mg protein, indicating a 127 fold purification over the homogenate. Furthermore, this purified SPD-binding protein also exhibited a single type of subunit as from the rat brain cortex, with an apparent molecular weight of 72 K on the SDS gel. The identity of this purified SPD-binding site as a subtype of DA receptors remains to be determined.

(This work was supported in part by a Robert A. Welch grant H-827.)

139.12 SYNTHESIS AND PHARMACOLOGICAL CHARACTERIZATION OF PIMOZIDE DERIVATIVES: A NEW CLASS OF POTENTIAL PHOTOAFFINITY LABELS FOR DOPAMINE RECEPTORS. Randall B. Murphy, Miroslav Trampota* Frank R. Arnold and David I. Schuster. Department of Chemistry, New York University, New York, NY 10003.

Pimozide (structure I, R = R' = H) is a highly active neuroleptic drug with a high affinity toward dopamine (DA) receptor sites as measured by binding in competition with radiolabeled spiperone, haloperidol and apomorphine (Seeman, P. Pharmacol. Rev. (1980) 32, 229). As part of a program to characterize dopamine receptors using the technique of photoaffinity labeling, we have synthesized some derivatives of pimozide which might serve as effective photolabels for such sites. The affinity of these compounds for D_2 sites in rat striatal homogenates has been determined in each case by a standard binding assay vs. [^{3}H]-spiperone. The phenethyl derivative of clopimozide (R = $PhCH_2CH_2$, R' = Cl) was prepared by reaction of the parent compound with phenethyl bromide and sodium hydride in dry THF for three weeks; the IC_{50} for this compound was 6.3 nM. The p-nitrobenzyl derivative of pimozide (R = p-NO_2-Ph-CH_2, R' = H) was readily prepared by treatment of pimozide with the corresponding bromide, KH and [18]-crown-6 in dry THF under nitrogen. This compound was reduced to the corresponding p-aminophenyl derivative which was converted to the p-azidophenyl analogue. The IC_{50}'s for these compounds in the binding assay were 25, 28 and 25 nM. Products of ring iodination of the amino- and azidophenyl systems are currently being purified and characterized. The relatively high dopaminergic activity of this series of compounds has prompted us to explore their utility as specific agents for photoaffinity labeling of DA receptor sites.



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140.1 RATES OF FORGETTING IN H.M.: SIX-MONTH RECOGNITION.
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Using a picture-recognition paradigm that provided amnesic subjects with additional study time in order to make their initial yes/no recognition comparable to that of control subjects, Huppert and Piercy (1979) examined rate of forgetting in two etiologies of amnesia. These authors concluded that H.M., with bilateral medial temporal-lobe pathology, demonstrated abnormally rapid forgetting over the course of a week, whereas patients with diencephalic pathology due to Korsakoff's Syndrome did not. Freed, et al. (1984) reported that H.M. did not display rapid forgetting over the course of a week in either yes/no or forced-choice recognition when the mean of 4 experiments was examined. This study emphasized the variability of H.M.'s performance and how this factor could account for Huppert and Piercy's results. It was noted that there were differences in memory performance as assessed by yes/no and forced-choice recognition techniques at the 24-hour retention interval. In the present study H.M.'s recognition memory was tested 6 months after initial recognition testing that employed a week delay. H.M.'s 6-month recognition was evaluated on each of 3 different forms of the rate of forgetting test over the course of a year. He performed at chance levels when tested using a yes/no recognition technique, but his delayed-match-to-sample (DMS) and delayed-non-match-to-sample (DNMS) performance were comparable to that of control subjects 6 months after initial testing. In addition, H.M.'s recall of photographs is impoverished at a time when his forced-choice recognition memory is comparable to that of control subjects. These results indicate that there are important differences in the memory performance of amnesic subjects as assessed by different recognition techniques. Despite being severely amnesic, with appropriate techniques H.M. can demonstrate intact recognition memory over extended periods of time.
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140.2 RECOGNITION OF SPATIAL LOCATION IN THE AMNESIC PATIENT H.M.
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Spatial location, recency, and frequency are often encoded equally well under incidental and intentional conditions; in contrast, memory for specific items can be improved by instruction to remember (e.g., Mandler, Seegmiller, & Day, 1977; Hasher & Zacks, 1979). Despite the encoding commonality among temporospatial contextual attributes, the ability to recall the location of an item and the item itself appear to be subserved by medial temporal-lobe structures (Smith & Milner, 1981), whereas memory for when and how often an event occurred is subserved by frontal-lobe structures (Corsi, 1972; Milner, 1977; Smith & Milner, 1983). Smith and Milner, studying recall of location in an incidental learning situation, found that the globally amnesic patient H.M. was severely impaired in the immediate and delayed recall of spatial location, although immediately after presentation (but not 24 hours later) he did show appreciation of the random spatial array of items. The present experiment used an intentional learning paradigm and a forced-choice recognition procedure in order to measure the course and nature of forgetting of spatial location in H.M. and in age-matched control subjects. Each of 352 words was presented in 1 of 20 locations for 2 seconds on a computer screen. At various times throughout the test, subjects had to decide which of 2 words had been seen or in which of 20 locations a word had appeared. Content and location questions occurred 1, 3, 6, 10, 15, 25, 50, 100, and 150 items following the initial presentation of the stimulus; thus, the rate of forgetting for content and for location could be computed separately and compared directly. Distances between inaccurate location responses and the correct locations were also calculated. H.M.'s recognition of content fell to chance by the stimulus-test interval of 15; his recognition of exact location was at chance by the interval of 6. When scoring for location reflected the distance of errors from target locations, his performance was better than chance at intervals where his recognition of items was at chance. H.M. apparently maintained an estimate of target locations over most of the intervals tested. In contrast to Smith and Milner's finding of grossly impaired incidental recall of spatial location in H.M., our study, which used intentional recognition testing, found relative preservation of information about spatial location. The forced-choice recognition format may provide contextual cues that can be taken advantage of by amnesic patients, and that are not available in free recall. We conclude that recognition of spatial location, like that of recency and frequency, is relatively spared in global amnesia resulting from temporal-lobe pathology.
Supported by grants MH 24433, RR 00088, and 2 T32 GM07484.

140.3 CONSTRAINTS UPON THE ACQUISITION OF COGNITIVE SKILLS IN AMNESIA: STUDIES WITH PATIENT H.M. AND PATIENTS WITH ALZHEIMER'S DISEASE.
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The progression of experiments searching for preserved learning in amnesia has revealed, successively, the lasting acquisition of motor, perceptual, and cognitive (or problem-solving) skills in the face of impaired fact learning. In one such study, patients with global amnesia showed improvement in the application of a mathematical rule 24 hours and 17 weeks after initial exposure to problems (variants of the Fibonacci series) that had to be solved by the discovery and use of that rule (Wood et al., 1982). The present studies investigated the range and limits of the acquisition of cognitive skills in the patient H.M., whose severe and pervasive amnesia is the consequence of a bilateral resection of medial temporal-lobe structures, and in patients with memory disorders due to Alzheimer's disease. H.M. had previously learned the solution to another cognitive task, the Tower-of-Hanoi puzzle (Cohen & Corkin, 1981); there are no reports of such cognitive skill learning in the latter group.

H.M. demonstrated considerable improvement in the application of the Fibonacci rule 24 hours, though not 1 hour, after initial exposure to 15 problem-sets. However, H.M. showed similar improvement 24 hours after testing when asked to supply the three correct numbers that completed an arbitrary five-number string presented 15 times. This result indicates that his 24-hour improvement on the Fibonacci problems was due to a combination of minimal declarative learning and task constraints, and does not reflect normal procedural learning of any rule. In a second experiment, H.M. and two patients with Alzheimer's disease took part in a problem-solving task derived from Barry and Broadbent (1984). Control subjects (N = 40) showed considerable improvement on this task despite poor conscious awareness of the nature of the solution as indicated by questionnaire responses. The two patients with Alzheimer's disease also demonstrated acquisition of the cognitive skill and retained the skill for at least 24 hours. In contrast, H.M. failed to show any learning within sessions or across days. These results suggest that only certain cognitive skills, perhaps under certain testing conditions, may be acquired and retained normally in the face of amnesia, and that different kinds of cognitive skills may be mastered by patients whose amnesias differ both in etiology and in severity.
Supported by grants MH 24433, MH 32724, 2 T32 GM07484, and RR 00088.

140.4 THE SUBICULAR CORTICES IN ALZHEIMER'S DISEASE: NEUROANATOMICAL RELATIONSHIPS AND THE MEMORY IMPAIRMENT, B.T. Hyman, G.W. Van Hoesen, L.J. Kromer and A.R. Damasio. Departments of Neurology and Anatomy, University of Iowa, Iowa City, IA 52242.

The subicular cortices constitute a grey matter passageway interposed between the hippocampus and the temporal cortex. Anatomical studies in the primate have shown that the subicular cortices give rise to the final common pathway for hippocampal efferents to the cerebral cortex. The subicular cortices also have powerful connections with the thalamus, basal forebrain and amygdala. The subiculum proper is one of the principal areas of pathological involvement in Alzheimer's disease, but the full extent of subicular pathology and the degree of involvement of the parasubiculum and presubiculum has not been addressed. For these reasons we examined the entire antero-posterior extent of all subicular cortices in 10 cases of Alzheimer's disease and in 8 age-compatible controls using Nissl, Congo red, thioflavin S and silver staining methods. We observed that the pathologic changes of Alzheimer's disease occur specifically in only certain subdivisions of the subicular cortices, leaving adjacent divisions spared. The Alzheimer brains showed neurofibrillary tangles in the subicular-CA1 zone, including the transitional area of the prosubiculum, while the dentate gyrus, and CA2, CA3 and CA4 hippocampal subfields were largely spared. In all cases the subicular-CA1 zone was more heavily involved than the more medial aspect of the subiculum proper, or the adjacent presubiculum. In contrast, the parasubiculum contained many neurofibrillary tangles. At the level of the uncal and genual parts of the hippocampal formation, many cells contained prominent tangle involvement. Caudally, at the level of the lateral geniculate nucleus, the entire subiculum proper was heavily involved, and remained so throughout the posterior extent of the structure. Neuritic plaques appeared throughout the subiculum. They formed a prominent line in the deepest part of the molecular layer of the subiculum, a zone that in lower mammals, receives serotinergic projections from the raphe complex. Five age-matched control brains, and cases of multi-infarct dementia, pseudodementia, and Huntington's chorea, did not show subicular changes. From a neural systems viewpoint, the subicular pathology in Alzheimer's disease deprives the hippocampal formation of its major output, including the critical link to the cortex. Such changes are likely to contribute to aspects of the memory impairment in much the same way that anoxic damage to Sommer's sector or bilateral hippocampal destruction do. (Supported by NIH grants NS14944, IF32EY05720, and PO NS 19632).

140.5 ALZHEIMER'S DISEASE SPARES MOTOR LEARNING. P.J. Eslinger and A.R. Damasio. Dept. of Neurology, Univ. of Iowa, Coll. of Med., Iowa City, IA 52242.

A progressive impairment of recent memory is an early and characteristic sign of senile dementia of the Alzheimer type (SDAT). Patients with surgical ablation of the medial temporal lobes, herpes simplex encephalitis, and alcoholic Korsakoff's syndrome also have pervasive memory impairments, but their ability to learn motor and perceptual skills are not affected. Our study ascertained if SDAT patients would exhibit a similar dissociation between motor learning and learning of specific sensory-based information.

Eight patients diagnosed with probable SDAT ($\bar{X}$ age = 71) and eight healthy older persons ($\bar{X}$ = 71) were examined in 3 experiments of learning and memory, each comprising 5 consecutive learning trials and one 20-min delayed trial, assessing retention. In the first experiment, total time on target was recorded in a rotary pursuit task. In the second experiment, subjects learned a 10-item grocery list, and in the third experiment, subjects learned to recognize 8 unfamiliar faces. Analysis of time on target revealed a significant motor learning effect ($p<.001$), but no group difference. The SDAT group showed an improvement of 161%, with every patient improving. On the delay trial, neither group exhibited significant decline in performance level. In contrast, controls exhibited significant verbal learning and facial learning while the SDAT group did not. On the delayed memory trial, SDAT performance fell to almost zero for the verbal recall task (73% decline vs 11% decline for controls) and to chance level for the facial recognition task (29% decline vs 2% decline for controls).

The striking dissociation between the acquisition and retention of specific sensory-based information (i.e., declarative knowledge such as words and faces) and a motor skill (i.e., rotary pursuit performance), in SDAT patients suggests there is a selective impairment of declarative memory systems, causing an overt amnesic syndrome. But the memory systems responsible for learning and retaining a new motor skill remained untouched. Declarative memory mechanisms have been associated with the limbic system, especially the hippocampal region, while perceptual-motor learning is likely to be mediated by primary sensory cortices and motor system structures of the frontal lobe, basal ganglia, thalamus, cerebellum and related pathways, all of which operate apart from limbic system control and are relatively resistant to degenerative changes of Alzheimer's disease. These behavioral findings may be correlates of the recently reported specific hippocampal system damage in SDAT (Hyman et al. 1984). Supported by NIH Grant PO1S19632-02.

140.6 COVERT RECOGNITION OF FAMILIAR FACES BY PROSOPAGNOSICS: EVIDENCE FOR INTRAMODAL REPRESENTATION OF LEARNED STIMULI. D. Tranel and A.R. Damasio (SPON: K. Johnson). Department of Neurology (Division of Behavioral Neurology), University of Iowa College of Medicine, Iowa City, IA 52242.

Prosopagnosia is a defect of visually triggered contextual memories related to a specific face. The ability of two prosopagnosic subjects to recognize familiar faces was tested with a newly developed technique, using the electrodermal skin conductance response (SCR). It has been shown previously that the SCR is sensitive to stimuli that have special significance, or "signal value," for the perceiver. Furthermore, we have established that, in normal subjects, highly familiar faces have "signal value," and tend to elicit more frequent and larger amplitude SCRs than unfamiliar faces. Against this background, we hypothesized that prosopagnosics, despite their complete inability to recognize familiar faces, might nonetheless produce discriminatory covert responses to these faces via an autonomic route which might be indexed with the SCR.

We studied two prosopagnosic subjects with stable bilateral lesions to the occipito-temporal region. They were shown several series of faces while skin conductance responses to these faces were recorded and measured. Each series comprised several familiar faces (of family members and other persons well-known to the patient) randomly embedded amongst unfamiliar faces (belonging to persons definitely not known to the patient). We found that both patients generated more consistent and significantly larger amplitude SCRs to the familiar faces than to the unfamiliar ones.

The accurate electrodermal discrimination of familiar faces shown by our subjects indicates that an early step of the physiological process of recognition is still taking place, in spite of the inability to allow the process to unfold to a conscious level. The results of this early step are not made available to awareness, yet can be indexed via the electrodermal skin conductance response.

We believe this covert recognition depends on the availability of intramodal memories for specific faces, organized in the form of a dynamic representation system which is unconsciously accessed and "matched" by incoming percepts of previously learned stimuli.

140.7 STRENGTH AND DURATION OF PRIMING EFFECTS IN AMNESIC PATIENTS AND NORMAL SUBJECTS. A. P. Shimamura*, L. R. Squire and P. Graf*. Dept. of Psychiatry,, Univ. of CA, La Jolla, CA 92093, Vet. Admin. Med. Ctr., San Diego, CA 92161, and Dept. of Psychology, Univ. of Toronto, Canada.

In a series of experiments, we assessed priming and recognition performance in amnesic patients, alcoholic subjects, and healthy normal subjects. Priming was assessed by showing word fragments of previously presented words (e.g. DEF for DEFEND) and asking subjects to complete each fragment with the first word that came to mind (word completion test). Tests of word completion and recognition memory were given after an immediate, 2-hour, and 4-day delay. In Experiment 1, word completion was assessed with cues that could be completed to form several common words (e.g., MOT for MOTHER, MOTEL, MOTOR) one of which had been presented previously. Priming effects were equivalent in amnesic patients and control subjects and disappeared within 2 hours. Recognition memory was impaired in amnesic patients at all test delays. In Experiments 2 and 3, the duration of word completion was tested with cues that uniquely specified the study words (e.g., JUI for JUICE; or A__A__IN for ASSASSIN). In these cases, amnesic patients exhibited a smaller word completion effect than control subjects at immediate testing, and their word completion effect disappeared within 2 hours. By contrast, control subjects exhibited completion effects that lasted at least 4 days. One exception to this pattern was that other amnesic patients, patients prescribed bilateral electroconvulsive therapy (ECT), exhibited word completion effects that persisted for at least 1 day (Experiment 3). In Experiments 2 and 3 we also varied the degree of semantic encoding during study (semantic vs nonsemantic orienting conditions). We found that the semantic condition increased the level of word completion performance of control subjects but had a negligible effect on the performance of amnesic patients. Moreover, in the nonsemantic condition control subjects and amnesic patients performed similarly. We suggest that the weaker and more transient word completion effects found in amnesic patients and in control subjects under nonsemantic conditions involve processes (i.e., priming) which do not depend on the integrity of the brain structures damaged in amnesia. The longer-lasting word completion effects by control subjects in the semantic conditions appear to reflect declarative processes that are impaired in amnesic patients.

140.8 HUMAN AMNESIA AND THE MEDIAL TEMPORAL REGION: MEMORY IMPAIRMENT FOLLOWING A BILATERAL LESION LIMITED TO THE CA1 FIELD OF THE HIPPOCAMPUS. S. Zola-Morgan, L.R. Squire and D.G. Amaral. V.A. Medical Center, San Diego, CA 92161, Dept. Psychiatry, UCSD, San Diego, CA 92093 and Salk Institute, San Diego, CA 92138.

Bilateral medial temporal lobe damage has been known for a quarter of a century to cause profound amnesia in humans. Much of the relevant information comes from surgical cases in which portions of the temporal lobes have been removed in an effort to relieve severe epilepsy. These cases have suggested that medial temporal amnesia depends at least in part on damage to the hippocampal formation.

Despite this evidence converging on the hippocampus, the status of memory functions in these cases often is based on anecdotal reports or on incomplete histological information, and there are few if any well-studied cases of amnesia where the damage is limited to the hippocampus. Recently, we have obtained extensive clinico-pathological information from a deceased 57 year old male, who developed memory impairment as a result of a hypoxic-ischemic episode that followed cardiac bypass surgery. This patient survived for five years following the episode, during which time he participated in our long-term studies of the neuropsychology of amnesia. We present here a well-documented amnesia with neuropathological evidence of bilateral damage limited to the hippocampus.

Neuropsychological Data: Following surgery patient R.B. showed no signs of dementia or significant cognitive impairment other than memory (WAIS = 111; WMS=91). Compared to control patients he was markedly impaired on tests of anterograde memory (e.g. Paired Associates: 2.2 vs 9.4 for Control Group; Delayed Story Recall: 1.0 vs 5.0 for Control Group; Delayed Diagram Recall: 5.3 vs 24.3 for Control Group. His retrograde amnesia was mild, extending back for approximately 1-2 years prior to the onset of his amnesia. Despite his memory impairment, he exhibited an intact capacity for priming.

Neuropathological Findings: Histological analysis of Nissl and fiber stained serial sections throughout the brain revealed a bilateral lesion limited to the CA1 field of the hippocampus and involving its entire anterior-posterior extent. In addition, there were two small focal unilateral lesions, one in the left somatosensory cortex and one in the right striatum. All other brain regions, in particular the mammillary bodies, the dorsomedial nucleus of the thalamus and the amygdala appeared normal.

This case shows that a circumscribed lesion limited to a subfield of the hippocampus can produce a clinically meaningful memory impairment. The possible reasons for the apparent selective vulnerability of CA1 pyramidal cells will be discussed.

140.9 RETROGRADE AMNESIA FOLLOWING COMBINED HIPPOCAMPUS-AMYGDALA LESIONS IN MONKEYS. D.P. Salmon*, S. Zola-Morgan, and L.R. Squire (Spon: D. Amaral). Dept. of Psychiatry, Univ. of California Med. Sch. (San Diego) and San Diego VA Med. Ctr.

It is well established in both humans and non-human primates that bilateral combined lesions of the hippocampus and amygdala (H-A) result in a deficit in recognition memory (i.e. declarative knowledge) while the ability to learn and retain motor skills (i.e. procedural knowledge) is preserved. Here, we present a first attempt to examine the status of premorbid memory (i.e. retrograde amnesia) following H-A lesions in monkeys by assessing the retention of information acquired at various time periods prior to surgery.

Eight cynomolgus monkeys were trained on five sets of 20 two-choice object discrimination problems. A different set was administered beginning 32, 16, 8, 4, and 2 weeks prior to surgery. Retention of each set of discrimination problems was assessed 24 hrs after acquisition by presenting a single trial with each of the 20 stimulus pairs. Additionally, six weeks prior to surgery, all monkeys were trained on a motor skill task. Monkeys learned to retrieve a candy lifesaver that was threaded onto a metal wire by moving the lifesaver around a right angle bend to the end of the wire.

Surgery took place after acquisition of the fifth and final discrimination set. The monkeys were divided into an operated group (bilateral hippocampus-amygdala lesion, N 4) and a control group (N 4) such that the average 24-hr retention test scores were approximately equal for the two groups on each of the five problem sets, and their rate of acquisition of the motor skill task was comparable.

Three weeks after surgery remote memory for the object discriminations was assessed by presenting a single trial with each of the 100 stimulus pairs. The control group evidenced little forgetting for discriminations in any of the five preoperative time periods. The H-A group, in contrast, demonstrated a profound loss of memory for the discriminations from all time periods. Retention of the motor skill task was relatively well preserved in both groups.

To examine fully the status of retrograde amnesia in experimental animals, additional studies will be needed. Some of the methodological issues that need to be addressed in such studies will be discussed.

140.10 ENHANCEMENT OF TRISYNAPTIC PATHWAY IN THE HIPPOCAMPUS DURING PERFORMANCE OF SPATIAL WORKING MEMORY TASKS: A 2-DG BEHAVIORAL STUDY IN RHESUS MONKEYS. H.R. Friedman and P.S. Goldman-Rakic, Sec. Neuroanat., Yale Sch. Med., New Haven, CT. 06510.

Knowledge of the role of the hippocampal formation (HPC) in learning and memory is largely based on examination of behavioral deficits that follow surgical ablation. We used Sokoloff's ^{14}C-2DG technique to directly examine HPC metabolism in intact monkeys while they performed tasks with different mnemonic demands. We report that functional activity in subsectors of the HPC is augmented during performance of spatial working memory tasks relative to performance on a reference memory task or a task with no memory requirement.

Male rhesus monkeys were trained to criterion on 1 of 4 behavioral paradigms. Two of the tasks, spatial delayed response and spatial delayed alternation, were working memory tests which require that memory of reward placement be updated on every trial. The two control tasks were (1) a visual discrimination (VD) test which presents the same problem on every trial and therefore taps only reference memory processes, and (2) a sensorimotor task with no memory component. Monkeys were given a single intravenous dose (100uCi/kg) of ^{14}C-2DG and tested for 45 minutes. Local cerebral glucose utilization (LCGU) values for discrete brain regions on X-ray autoradiographs were obtained using a digital image processing system. Quantitative densitometry of separate laminae in the dentate gyrus (DG) and Ammons horn (CA fields) was accomplished by superimposing the X-ray autoradiograph on nissl-stained tissue sections. The LCGU values obtained were subjected to statistical analysis.

LCGU in the DG, CA1 and CA3 fields was augmented in monkeys performing working memory tasks relative to monkeys performing the VD and sensorimotor control tasks. This elevation of functional metabolism was most pronounced in the granule and molecular layers of DG and the molecular and pyramidal layers of CA1. To a lesser extent metabolism in the molecular and pyramidal cell layers of CA3 also was increased. In contrast, LCGU in the oriens layer of CA1 and CA3 was similar for all groups. It is significant that the zones of raised metabolic activity represent synaptic links in the perforant pathway - a major source of cortical afferents to the HPC. These results are the first in vivo visualization of functional activation of this trisynaptic pathway during performance of spatial mnemonic tasks in the monkey and provide evidence of a special role for this pathway in spatial working memory.

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140.11 EFFECTS OF MAMMILLARY BODY VS. DORSOMEDIAL THALAMIC LESIONS ON SPATIAL MEMORY IN MONKEYS. E.J. Holmes and C.E. Tenke*. Depts. of Psychiatry and Neuroscience, Kennedy Center, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Neuropathological reports of the consistent and prominent degeneration of the dorsomedial nuclei of the thalamus (DMT) and the mammillary bodies (MB) of the hypothalamus in alcoholic Korsakoff patients has led to the belief that these nuclei may be of critical significance in the mediation of short-term memory. Earlier findings have shown that lesions of the mammillary nuclei in monkeys do indeed affect the short-term retention of spatial location in a 30-sec. delayed alternation task (Holmes et al., Neurosci. Abstr., 8:23, 1982). As an extension of these findings, monkeys with MB lesions were directly compared to monkeys with DMT lesions on another task of short-term memory, i.e., delayed response.

The subjects were 17 M. fascicularis monkeys. Four monkeys received bilateral MB lesions, 4 monkeys received bilateral DMT lesions, 6 monkeys served as operated controls for the MB and DMT groups, and 3 monkeys remained unoperated. Training and testing was conducted in a Wisconsin General Test Apparatus. During pre-operative training, the subjects were allowed to watch the experimenter bait one of two foodwells in a test tray and subsequently cover both foodwells with identical Plexiglas plaques. Initially, the subjects were allowed to displace one of the plaques and retrieve the reward immediately after the foodwells were covered until they performed at a criterion of 27 correct responses in 30 consecutive trials. After attaining criterion with the 0 sec. delay, progressive response delays of 1 sec., 2 secs., etc., up to 5 secs. were inserted while obscuring the subject's view from the test tray. The subjects were required to attain the criterion of 27/30 correct at a particular delay before proceeding to the next delay. After surgery, the animals were retrained on the 5 sec. delay problem until they reattained the pre-operative criterion. Following retraining, successive delays of 10, 20, and 30 secs. were systematically inserted to determine the effect of timed intervals on retention.

The results show that all of the control animals were at 90% performance at all delays during testing. The results also show that although the monkeys in the MB and DMT groups were at 90% performance in the retention of the 5 sec. delay task, the subjects in both groups were impaired in varying degrees on the 10, 20, and 30 sec. intervals with some of the animals performing consistently at or near chance even before the 30 sec. delay. The severity of the impairment for both the MB and DMT groups seemed to coincide well with the extent of the neuronal damage. Thus, it appears that both the mammillary and dorsomedial nuclei share responsibility for the retention and retrieval of spatial information. It remains to be determined, however, if the quality or character of the information to be retained is the same since the efferents and afferents of these two nuclei are quite different.

140.12 THE EFFECT OF SELECTIVE LESIONS TO THE PERIARCUATE CORTEX ON THE PERFORMANCE OF TWO NONSPATIAL CONDITIONAL TASKS. M. Petrides. Dept. of Psychology and the Montreal Neurological Institute, McGill Univ., Montreal, Quebec, Canada, H3A 1B1

It has been demonstrated that damage to the periarcuate region (areas 8 and 6) of the dorsolateral frontal cortex in the monkey is critically involved in the performance of conditional associative tasks. In these tasks, the animal has to learn and perform according to the conditional rule: if stimulus A is presented, select response X, and if stimulus B is presented, select response Y (see Petrides, Behav. Brain Res., 5: 407, 1982).

It must, however, be emphasized that although the periarcuate cortex can be distinguished from other frontal cortical regions on the basis of certain anatomical characteristics, such as direct input from first-order, modality-specific "association" cortical areas, there are also differences in the connections of various parts of this region. These anatomical results suggest that different parts of the periarcuate cortex may be predominantly involved in the control of different kinds of conditional tasks.

In the present experiments, performance on two nonspatial conditional tasks after lesions restricted to the anterior (area 8) or posterior (area 6) arcuate cortex was investigated. The two tasks differed only in the responses that were required, the type of stimuli used and their mode of presentation being the same. In one experiment, the monkey was faced with two white perspex boxes placed close to each other. On any given trial one of the boxes, chosen according to a random but balanced sequence, was lit and the other remained unlit. The monkey was trained to select the lit box when object A was shown and the unlit box in the presence of object B. After mastery of this task, monkeys (Macaca nemestrina) received bilateral lesions of the anterior or the posterior arcuate cortex. Monkeys with damage to the anterior arcuate cortex were severely impaired in comparison with normal controls, but those with damage to area 6 were not. In contrast to this, the animals with posterior arcuate lesions (but not those with anterior lesions) were impaired in acquiring a go, no-go task with symmetrical reinforcement. In this conditional task, the subject was rewarded for responding when one stimulus was presented, and for withholding responding for a given period of time when the other stimulus was shown.

The results are consistent with the known anatomical connections of these parts of the periarcuate cortex. The anterior arcuate cortex, damage of which impaired performance on a conditional task requiring visual responses, is preferentially connected with the visual system; in contrast, the posterior arcuate cortex, damage of which impaired performance on a task involving two kinaesthetically distinct responses (go or no-go), is preferentially linked to the motor cortex and the somatosensory system.

140.13 RHINAL CORTEX: A THIRD TEMPORAL-LOBE COMPONENT OF THE LIMBIC MEMORY SYSTEM. E.A. Murray, J. Bachevalier, and M. Mishkin. Laboratory of Neuropsychology, N.I.M.H., Bethesda, MD 20205

Monkeys trained on a visual recognition task, delayed nonmatching-to-sample (DNMS) with trial-unique objects, were given a six-week long performance test in which they were required to remember, first, single objects for increasingly longer periods of time (30, 60, or 120s) and, then, increasingly longer lists of objects (3, 5, or 10). Previous experiments indicated that whereas normal monkeys learn the basic task in 100 trials and score over 95% correct responses on the performance test, monkeys with combined ablation of the amygdaloid complex and hippocampal formation, including a large portion of the ventromedially adjacent rhinal (i.e. ento-, pro-, and peri-rhinal) cortex, relearn in about 1000 trials and obtain an average performance score of about 60%. An equally severe deficit followed combined ablation of the amygdaloid complex plus rhinal cortex (relearning, 2000 trials; performance, 60%), but only a mild deficit followed ablation of the hippocampal formation plus rhinal cortex (relearning, 340 trials; performance, 84%). These findings in monkeys with one limbic structure spared appeared to support the view that the medial temporal lobe contained just two structures critical for memory, the amygdaloid complex and the hippocampal formation, and that ablating or disconnecting both was necessary and sufficient to insure a severe memory loss. According to this view, the rhinal cortex ablation was effective when combined with amygdalectomy simply because it deafferented the hippocampus from its neocortical inputs.

This view was brought into question, however, by results from monkeys in which the pathways connecting the two limbic structures with the basal forebrain/diencephalon were transected while the rhinal cortex itself was largely spared. For example, monkeys with combined transection of the amygdalofugal pathways and fornix and those with combined amygdaloid ablation plus fornical transection relearned DNMS in an average of 280 to 350 trials and obtained average performance test scores of 69 to 74%.

To test whether the rhinal cortex participates in memory processes independently of serving as a sensory gateway to the hippocampus, we prepared monkeys with combined ablations of the amygdaloid complex and hippocampal formation that spared the rhinal cortex. These animals relearned DNMS in an average of 350 trials, and preliminary results on the performance test indicate scores well above those found earlier for monkeys with combined ablation of the amygdaloid complex and hippocampal formation that included most of the rhinal cortex. The results suggest that the rhinal cortex, perhaps via its diencephalic projections, can sustain a substantial level of visual recognition memory in the absence of both the amygdaloid complex and hippocampal formation.

140.14 INFEROTEMPORAL OUTFLOW TO AMYGDALA AND FRONTAL LOBE IN DISCRIMINATION AND REINFORCEMENT IN THE MONKEY. D. Gaffan* and S. Harrison* (SPON: B. H. Turner). Dept. of Experimental Psychology, University of Oxford, Oxford OX1 3UD, England.

Bilateral amygdalectomy is known to impair monkeys' spontaneous emotional reactions to exteroceptive stimuli. Our first question was whether it impairs also their response to exteroceptive stimuli with an acquired reinforcing significance under experimental control. Monkeys learned visual simultaneous discriminations with purely auditory reinforcement during acquisition, as follows. In each daily session 30 new discrimination problems were presented serially. A correct visual choice produced white noise and an incorrect choice produced clicks. A food reward was delivered only if there were 4 correct choices in succession. If there were 4 wrong choices in succession there was 10 sec of darkness. After either of these events the current problem was terminated and the next began. Thus non-auditory reinforcement was delivered only after solution of a problem had succeeded or failed.

Bilateral amygdalectomy reduced the rate of successful solution from 92 percent to 66 percent. Solution rate was unimpaired by crossed unilateral lesions of visual association cortex and amygdala. In a similar but simpler task, learning was profoundly impaired by crossed unilateral lesions of visual association cortex and of the area of termination of the uncinate fascicle in the inferior convexity of the frontal lobe. These results show that the direct input to amygdala from sensory association cortex is not essential for the modality of the retrieval cue in learning and memory, and imply that the importance of the amygdala is rather in securing the effectiveness of reinforcers. Further they suggest that the direct input to frontal lobe from temporal association cortex in the modality of the retrieval cue is crucial for the effectiveness of the cue. The uncinate fascicle may have been unduly neglected in hypotheses of the neuropathology of amnesia.

DEVELOPMENT AND PLASTICITY: VISUAL PATHWAYS I

141.1 ADAPTATION EFFECTS FROM CONDITIONING AREA 17 CORTICAL UNITS IN KITTENS DURING PHYSIOLOGICAL RECORDING. A.B. Saul* and J.D. Daniels (SPON: J.A. Anderson). Center for Neural Science and Division of Engineering, Brown University, Providence RI 02912.

The most typical demonstration of plastic changes in visual cortex involves abnormal rearing of kittens in a restricted visual environment, after which most cells respond poorly to deprived stimuli but respond well to experienced stimuli. We have been studying the changes which occur in the activity of a single neuron by recording immediately before and after conditioning, as well as monitoring the responses during the conditioning session.

These experiments were performed on eight fully anaesthetized and paralyzed kittens, aged 4-8 weeks. We first derived detailed receptive field (RF) plots under computer control. One eye was then occluded during one hour of conditioning with drifting spots, bars, gratings, or complex stimuli. Conditioning was unidirectional and repetitive, although stimulus sweeps were alternated with blank trials of approximately equal duration. At the end of each hour of conditioning the RFs were replotted. Often, this sequence was repeated while units were held for up to 14 hours.

We observed changes, albeit less dramatic than those seen in awake kittens. While some cells gain or enhance a preference for the experienced stimulus, the most striking result is that most changes involve loss of responsiveness to certain patterns which share features of the conditioning stimulus. The effects include shrinkage of RFs, weakened response to the conditioned direction, movement of the orientation tuning curve away from the conditioned orientation, and in general adaptation to the conditioning stimulus. Response properties were selectively altered, as opposed to a general weakening of response as in fatigue. RFs remained in their adapted state for at least 4 hours in some cases.

Ocular dominance and orientation selectivity do not change consistently. Direction selectivity changes most dramatically, typically decreasing in cells conditioned in their preferred direction. Directional preference reverses in some cells.

Responses consistently fall during the hour of conditioning. The level of evoked activity at the beginning of the session is rarely matched at any later point. Responsiveness oscillates as it decays, with both sides of its envelope falling.

Although these results are obtained in anaesthetized kittens, the behavior of single cells appears similar to psychophysical data. Presumably, adaptation is an integral part of the plastic phenomena addressed by longer-term experiments, despite the fact that the induced changes are in the opposite sense.

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141.2 ORIGINS OF CORTICAL PLASTICITY IN VISUAL CORTEX AND CLAIRE-BISHOP AREA IN THE CAT. D.N. Spinelli. Center for System Neuroscience, Univ. of Masschusetts at Amherst, MA 01003.

We have demonstrated that unique visual experiences during development result in unique functional properties of single cells in visual cortex and in the Claire-Bishop area of cats (Spinelli&Jensen,Science, 203, 75-78, 1979.) A unique visual experience, for animals with frontally located eyes, is one that requires associating vertical bars viewed by one eye, with horizontal bars viewed by the other. Signature cells, that is cells which are binocular, but respond best to vertical bars for one eye and horizontal bars for the other, can then be found in visual cortex, Claire-Bishop and other cortical areas. We refer to these cells as signature cells because they have incorporated the signature of the experience. Cells with these properties are not found in cats that did not have these experiences. To define the parameters that lead to this adaptation and to study the anatomical circuitry that is involved we have exposed three groups of normally reared kittens to simple alternations of vertical bars for one eye and horizontal bars for the other. The stimuli were switched every 300 msecs. in one group and every 400 and 500 msecs. respectively in the other two groups. No behavior was required and no reinforcement delivered. These stimulus sequences were presented for about 8 minutes a day over a period of three months.

Visual receptive fields of single cell in visual cortex, area 17, and Claire-Bishop were mapped. At the end of the recording session Horseradish Peroxidase (50% solution, 1 microliter) was injected in the area of the microelectrode penetration to trace connectivity. The results show that significant numbers of signature cells are present both in area 17 and Claire-Bishop indicating that simple stimulus alternation is sufficient to produce unusual functional properties in visual neurons even in normally reared kittens. The optimum duration for each stimulus proved to be 400 msecs. The anatomical study shows that even in Claire-Bishop the principal inputs are from the lateral geniculate nucleus and that there is very little intracortical connectivity. Local connectivity and time relationships between the stimuli seem to account for the observed results.

141.3 EFFECTS OF UNEQUAL ALTERNATING MONOCULAR OCCLUSION AND DIFFUSION ON CAT VISUAL SYSTEM. W.G. CHRISTEN*and G.D. MOWER, Dept. of Neurology, Children's Hospital, Boston, Mass. 02115.

Strabismic animals that alternately fixate do not develop visual deficits while animals that preferentially fixate do. In preferential fixation the lesser used eye is placed at a disadvantage not only because it receives less normal visual input than the more used eye, but also because it receives more abnormal stimulation.

We have explored, separately, the effects of these imbalances on cortical and LGN physiology using various ratios of unequal alternating monocular occlusion and diffusion. Four ratios of eye usage were used (4/4,7/1,50/1 and 100/0) and each eye received visual exposure daily. A contact lens was used to restrict vision to one eye during visual exposure sessions and rearing was continued until the time of physiological investigation (5 months of age or older). Some cats were reared with opaque contact lens occluders (OCCLUSION) to investigate the effects of different amounts of normal visual input between the two eyes. These occluders eliminated the VEP to both form and light stimulation so that a particular eye experienced either normal visual stimulation or no stimulation at all. Other cats were reared with translucent diffusers (DIFFUSION) which abolished the VEP to form but permitted diffuse light stimulation. These animals were used to investigate the effects of an imbalance in normal and abnormal input between the eyes.

In visual cortex no differences between OCCLUSION and DIFFUSION cats were observed. Binocularity was reduced in all cats but for both groups a ratio of 50/1 was needed before takeover by the more experienced eye was seen (65-75% of cells dominated by the more experienced eye). Takeover was greater in the 100/0 cats (90-96% domination).

In the LGN only DIFFUSION animals developed an X-cell acuity deficit in the less experienced eye. This deficit occurred in 50/1 and 100/0 cats but not in 4/4 and 7/1 cats. All OCCLUSION cats developed normal X-cell acuities in both eyes in spite of the reduced usage of one eye. This result shows that the X-cell acuity deficit occurs only with abnormal stimulation and that a relatively small amount of normal input is sufficient to negate this deleterious effect.

In summary, these results indicate that occlusion and diffusion have different effects on the developing visual system and that surprisingly little normal vision is needed to prevent the development of eye specific deficits.

141.4 OCULAR DOMINANCE, SELECTIVITY, AND RESPONSIVENESS IN KITTEN AREA 17 NEURONS, AFTER DARK REARING PLUS BRIEF MONOCULAR EXPERIENCE. J. D. Daniels & A. B. Saul*. Center for Neural Sciences and Division of Engineering, Brown University, Providence, Rhode Island, 02912.

If a young kitten lives in total darkness for a few weeks, the responses of its visual cortical neurons become sluggish and lose (or fail to develop) selectivity. Binocularity, however, is retained. If such a kitten is then allowed monocular experience (ME), after several days visual cortical neurons will become *responsive, monocular, and selective* for direction and orientation. How does ocular dominance (OD) plasticity correlate with the improvement in responsiveness and selectivity?

We have given dark-reared 4-9 week old kittens 0-24 hours of ME, then have recorded from area 17 neurons, documenting OD, selectivity, and responsiveness.

In order to assess correlations quantitatively, we record averaged orientation tuning curves for each eye's receptive field. We use a selectivity rating $SEL = 1 - \frac{mean}{max}$ $(0 \leq SEL \leq 1)$ for the responses to stimulus sweeps within $\pm 90°$ of the optimal direction. Picking the best response for each eye's field, we compute an ocular dominance rating $OD = \frac{open}{open + closed}$ $(0 \leq OD \leq 1)$. To quantify responsiveness, we rely on *variability* of the unit over several sweeps.

From our data, the following seems probable:

(1) Within hours of the beginning of ME, some cells improve their *responsiveness*, producing more spikes per stimulus presentation and showing *less variability* over a series of stimulus sweeps than do sluggish dark-reared neurons.

(2) Along with the improvement in responsiveness, some neurons begin to shift ocular dominance (average OD = .71). This shift occurs without substantial improvement in selectivity (average SEL = .46).

(3) Other cells shift OD more slowly, and a number of these cells show a differential (open vs closed) change in selectivity before the shift is complete.

Over the time we have studied, 0-24 hours of ME, the improvement in responsiveness is the most apparent feature, with ocular dominance and selectivity changing at slower rates.

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141.5 OCULAR DOMINANCE AND VISUAL ACUITY CHANGES FOLLOWING TERMINATION OF REVERSE OCCLUSION IN THE KITTEN. K.M. Murphy* and D.E. Mitchell, (SPON: D.P. Phillips). Dept. of Psychology, Dalhousie University, Halifax NS. Canada.

Early visual experience exerts a strong influence upon the functional development of the visual system. Monocular deprivation early in the critical period shifts cortical ocular dominance and visual acuity in favor of the nondeprived eye. These effects may be reversed if vision is restored to the deprived eye sufficiently early in the critical period and at the same time the other eye is occluded (reverse occlusion).

Previous work has shown that following 5 or 6 weeks of monocular deprivation only 18 days of reverse occlusion will promote the maximum shift in cortical ocular dominance. We have examined the effect on visual acuity and ocular dominance in area 17, of a period of binocular visual experience provided subsequent to reverse occlusion. Control kittens recorded from immediately upon termination of 18 days of reverse occlusion, after either 5 or 6 weeks of monocular deprivation, were virtually identical to those reported by Movshon (1976). Following a subsequent period of binocular vision (4-8 weeks) the percentage of exclusively monocular cells (groups 1 & 7, < 20%) and binocular cells (groups 2-6, > 80%) was similar for both conditions. However, the distribution of binocular cells differed in the two conditions, with a tendency for the initially nondeprived eye to dominate when monocular deprivation was extended until 6 weeks of age. Measurement of visual acuity made using the jumping stand technique demonstrated extremely poor acuity for both eyes following either deprivation regimen. Visual acuity recovered to only 1-3 cycles/deg following 5 weeks of initially monocular deprivation and was marginally better (2-4 cycles/deg) after 6 weeks initial monocular deprivation. Although binocularity was restored in area 17 following these deprivation regimens, visual acuity was very poor and never recovered.

Movshon, J. A. (1976) J. Physiology (London), 261, 125-174.

141.6 SPLIT CHIASM AND MONOCULAR DEPRIVATION DURING DEVELOPMENT: A MODEL OF NONCOMPETITIVE MECHANISM IN VISUAL CORTEX NEURONS. U. Yinon*, A. Hammer* and Z.D. Weiser*. (SPON: M.S. Myslobodsky). Physiol. Lab., M & G Goldschleger Eye Res. Inst., Tel Aviv Univ., Sackler Faculty of Med., C. Sheba Med. Ctr., Tel Hashomer 52621, Israel.

The effects of the absence of the conventional binocular convergence on cortical cells following optic chiasm split and of monocular deprivation were studied during development.

In five kittens absence of binocularity was induced at six weeks of age by a sagittal section of the optic chiasm and monocular deprivation was induced by lid suturing (OCX-MD). In this way binocular competition between the "experienced" and "deprived" visual inputs on cortical cells may either be cancelled or take place via the indirect callosal pathway. Unit recording (438 cells) was made in these cats during adulthood from area 17-18 boundary, the callosal projection zone of the visual cortex. For comparison, recordings were similarly made from thirteen normal adult cats(544 cells) six adult cats monocularly deprived as kittens (MD; 283 cells) and twelve cats with chiasm split made during adulthood(OCX;729 cells).

The majority of the responsive cells in each hemisphere of the OCX-MD cats reacted monocularly to the ipsilateral eye,as in the OCX cats. A minor proportion of contralaterally driven cells were found reacting to the experienced (3.6%) and to the deprived(0.4%) eye. Only very few cells with binocular input were found in the OCX-MD cats as in the MD cats, in contrast to the result in our normal cats. While 67.2% of the cells in the hemisphere ipsilaterally to the experienced eye were responsive and 32.4% of the cells were unresponsive, the opposite result, namely 31.4% responsive cells and 64.9% unresponsive cells, were found in the hemisphere ipsilaterally to the deprived eye.

We conclude that except for a small interference due to an active callosal transfer, a neuronal effect of a noncompetitive process has been found, as reflected by the fact that each hemisphere is almost independently dominated by its own eye.

Research supported by the Israel Institute for Psychobiology, Charles E. Smith Family Foundation grant No. 1/84 (to U. Yinon).

141.7 EFFECTS OF CORTICAL ACTIVITY BLOCKADE ON OCULAR DOMINANCE PLASTICITY IN KITTEN VISUAL CORTEX. H.O. Reiter, D.M. Waitzman and M.P. Stryker. Div. of Neuroscience and Dept. of Physiology, School of Medicine, UCSF, San Francisco, CA 94143.

Monocular visual deprivation during early life has long been known to reduce the response of visual cortex neurons to stimulation through the deprived eye. Many lines of evidence suggest that this effect occurs principally in the cortex and is due to a modification of the electrical activity of cortical neurons. Our experiments tested the hypothesis that a blockade of electrical activity in the visual cortex during the period of monocular deprivation would prevent the shift in ocular dominance.

A 33 ga. cannula attached to an osmotic minipump (Alza 2002) was implanted into area 17 of 4-5 week-old kittens. The pump was filled with a mixture of 10^{-5}M tetrodotoxin (TTX) and ^{3}H-proline (1μCi/ml) for experimental animals or saline alone for control animals. Two days after implantation of the pump and cannula, the lid of the contralateral eye was sutured closed. Five days later, acute extracellular single-unit recordings were made to assess the extent of cortical activity blockade anterior to the cannula. Both spontaneous and evoked discharges of cortical neurons were absent or severely suppressed over a distance of at least 6-8mm anterior to the cannula and to a depth of several millimeters. After disconnecting the minipump and closing the ipsilateral eyelid as well, we found that the blockade wore off over the following 20-36 hours. At this point a series of single-unit recordings from cortical neurons was made in the previously blocked area anterior to the cannula. Receptive field characteristics, including ocular dominance, were determined for each cell.

The principal result was that cortical activity blockade largely prevented the ocular dominance shift found in control animals. This provides further evidence for the idea that a binocular imbalance in cortical activity is necessary for monocular deprivation to exert its effect during the critical period.

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141.8 EVIDENCE FOR AN ENHANCED ROLE OF GABA INHIBITION IN AMBLYOPIC CATS. G.D. Mower, W.G. Christen*, F.H. Duffy. Laboratory of Developmental Neurophysiology, The Children's Hospital, Boston, MA.

Application of the GABA antagonist bicuculline reveals binocular responses in visual cortical neurons of monocularly deprived (MD) and strabismic cats. A similar effect occurs in monocular cells of normal cats. The question arises, therefore, as to whether abnormal visual rearing results in an enhanced role of GABA inhibition. The present study addressed this question by comparing the effects of iontophoretic bicuculline on the ocular dominance of cortical cells in normal, MD, and strabismic cats. To date, 117 cells have been studied in the three preparations.

In all preparations, GABA disinhibition produced changes in ocular dominance in a higher proportion of originally monocular (51%) than originally binocular cells (15%). Thus, GABA inhibition affects a higher proportion of the total population of cells in MD (44%) and strabismic (51%) cats than in normal cats (20%). This result suggests that the role of inhibition is physiologically enhanced as a result of abnormal visual experience during early life.

Comparison of the pre-drug and during-drug ocular dominance distributions provides insights into the role of inhibition in producing amblyopic deficits in visual cortex. The pattern of ocular dominance that is"revealed" by disinhibition appears to reflect underlying changes in afferent connectivity. During disinhibition, a high proportion of cells are binocularly driven in MD (48%) and strabismic cats (67%). The shape of the during-drug distribution, however, is not normal in either preparation. In MD cats, most cells remain more responsive to the non-deprived eye. In strabismic cats, most cells remain dominated by the originally effective eye. These patterns are consistent with the changes in afferent connectivity from the two eyes in MD (loss of input from one eye) and strabismic cats (exaggerated segregation of the two eyes).

We conclude that the role of inhibition on cortical ocular dominance is enhanced as a result of abnormal visual rearing. GABA inhibition appears to sharpen underlying biases in afferent input to determine overall cortical ocular dominance in amblyopic preparations.

141.9 DARK-REARED KITTENS: EFFECTS OF A GABA ANTAGONIST ON RESPONSIVITY OF VISUAL CORTICAL CELLS. T. Tsumoto and R. D. Freeman. Group in Neurobiology, School of Optometry, University of California, Berkeley, CA, 94720 and Dept. of Neurophysiol., Osaka University Medical School, Kitaku, Osaka, 530 JAPAN

In dark-reared kittens neurons in the visual cortex are unresponsive or only weakly responsive to visual stimulation. By ionophoretic application of an excitatory amino acid (DL-homocysteate) these cells can be activated visually (Ramoa et al., Soc. Neurosci. Abstr. 10, 1077, 1984). One possible explanation of this finding is that intracortical inhibition, mediated mainly by γ-aminobutyric acid (GABA) (Sillito, J. Physiol. 250, 305, 1975), masks excitatory inputs to cortical neurons in dark-reared kittens. To test this possibility we studied the effects of ionophoretic application of bicuculline methiodide (BIC), a GABA antagonist, on visual responsivity of neurons in 14-19 week-old dark-reared kittens. Standard physiological techniques were used along with a four-barreled glass microelectrode with a sharpened tungsten wire projecting from one of the barrels to enable simultaneous recording of single units and ionophoresis of BIC.

Of 110 cells recorded from 4 cats, 59 were unresponsive or very weakly responsive to visual stimuli. Of the 51 visually responsive cells, only 24 were selective for stimulus orientation. These results are in accord with findings of previous studies. About 80 % of the originally unresponsive or weakly responsive cells became clearly responsive during BIC application, although the cells remained largely unselective to stimulus orientation. In addition, for cells which had been responsive initially BIC generally increased levels of responsivity. However, for most of these cells, BIC did not alter selectivity. Thus, the effect of BIC in dark-reared kittens is somewhat different from that found for normal adult cats. In the latter case, selectivity for stimulus orientation is often lost during BIC application but tuning characteristics do not usually seem to be affected in dark-reared animals. This suggests that there may be two types of cortical inhibition. The first kind is responsible for orientation tuning and the second is a type of tonic inhibition which exerts an influence over general levels of cortical responsivity (Tsumoto et al., Exp. Brain Res. 34. 351, 1979). The present results suggest that inhibitory synapses related to selectivity are either not well developed in the visual cortex of dark-reared kittens or become dysfunctional during lack of visual stimulation, and those related to general or tonic inhibition are fully functional.

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141.10 ACETYLCHOLINE, NOREPINEPHRINE AND THE EXTRATHALAMIC CONTROL OF VISUAL CORTICAL PLASTICITY. Mark F. Bear and Wolf Singer.* Max Planck Institute for Brain Research, 6000 Frankfurt 71, F.R.G.

It is now quite clear that cortical NE depletion, by itself, is not a sufficient condition to prevent ocular dominance (OD) plasticity in kitten striate cortex. We therefore turned our attention to a possible cholinergic contribution to experience-dependent cortical development. This possibility was examined by destroying the cortically-projecting cholinergic neurons of the basal telencephalon and then testing for a deficit in the ocular dominance shift that normally results from brief periods of monocular deprivation (MD).

Unilateral NMDA lesions of the diagonal bands of Broca and the substantia innominata were made in 4- to 5-week-old kittens. The effectiveness of the lesions was confirmed in all cases by using AChE histochemistry. Despite a drastic reduction in the density of AChE+ axons in striate cortex, 7-10 days of MD caused an apparently normal OD shift to the open eye. However, when cortical ACh and NE were both unilaterally depleted, the OD shift was significantly retarded on that side. The combined depletion was accomplished either by making chemical lesions of both the basal forebrain and the dorsal noradrenergic bundle, or by surgical lesions of the cingulum bundle. In either case, HPLC analysis confirmed that cortical NE was reduced by more than 50% in the hemispheres with the lesions.

These results indicate that while removal of either system alone is ineffective, the combined depletion of ACh and NE is sufficient to block plasticity. This suggests that these two extrathalamic projections are able to functionally compensate for one another, and raises the possibility that they exert their effects on synaptic plasticity via a common molecular mechanism.

141.11 6-HYDROXYDOPAMINE INTERFERES WITH CHOLINERGIC TRANSMISSION IN STRIATE CORTEX. Wolf Singer* and Mark Bear (SPON: L.N Cooper). Max Planck Institute for Brain Research, 6000 Frankfurt 71, F.R.G.

Continuous intracortical infusion of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) will disrupt the normal plastic response to monocular deprivation (MD) in kitten striate cortex. However, this effect probably is not attributable solely to the depletion of cortical norepinephrine (NE) since other methods of NE depletion leave cortical plasticity intact. Because the concurrent removal of both cholinergic and noradrenergic projections does appear to disrupt the normal ocular dominance shift after MD (Bear and Singer, this session), we examined the possibility that 6-OHDA also inferferes with cholinergic transmission in striate cortex. This was accomplished by testing the effects of iontophoretic ACh (2.0 M, +15- +60 nA) on cortical neurons before, during and after 6-OHDA iontophoresis (0.2 M, +40- +80 nA) from a piggyback microelectrode assembly. While the 6-OHDA had variable effects on neuronal discharge by itself, it consistently attenuated the cellular response to ACh. The ACh-dependent enhancement of visual responsiveness was usually reduced by about 50%, but in some instances the application of 6-OHDA completely abolished the ACh effect. These results suggest that intracortical 6-OHDA may disrupt plasticity in striate cortex by a combined action on noradrenergic and cholinergic neurotransmission.

141.12 TWO METHODS OF CATECHOLAMINE DEPLETION THAT YIELD DIFFERENT EFFECTS ON PLASTICITY BOTH DEPRESS NE*STIMULATION OF ADENYLATE CYCLASE (ACase) ACTIVITY. C. Aoki & P. Siekevitz, Rockefeller Univ., NY,NY 10021 & J.D.Daniels, Brown Univ., Providence, R.I. 02912.

Upon release, NE can bind to postsynaptic beta-adrenergic receptors and stimulate ACase, an enzyme that converts intracellular ATP to cAMP. Kasamatsu et.al. (J.Comp.Neurol.185:163) have hypothesized that this cascade of events is involved in maintaining ocular dominance plasticity. One evidence supporting this hypothesis is that when visual cortical catecholamines are depleted by intracortical perfusion with a neurotoxin, 6-OHDA, at 6-wk of age (acute lesion), the procedure prevents the loss of binocular cells following monocular deprivation. Bear et.al. (Nature 302:245), however, found that a cortical depletion achieved by IP injection at birth of 6-OHDA (chronic lesion) does not alter plasticity. Could the difference in the state of plasticity be explained by the difference in the stimulation of ACase by the residual NE? To answer this idea, the basal, the maximal catalytic and the NE-stimulated activities of ACase were compared between the tissues lesioned by the two methods versus control tissues. The assay was done blind, without the knowledge of whether the tissues were controls or experimentals.

Three cats were acutely lesioned. From each brain, one block within the 5 mm radius of the site of 6-OHDA injection (lesioned site), another within the 5 mm radius of the site of the vehicle injection in the contralateral hemisphere (control site), and another 10 mm anterior to the 6-OHDA injection site (anterior site) were collected. The blocks were then homogenized and stored frozen at -70°C until the time of the assay. Basal and maximal catalytic activities were the same in all three cases at every site. In contrast, in two animals, sites of lesion exhibited significant depressions in NE-stimulation when compared to the control sites (-35%, -38%) consistently over the range of NE concentrations of 5 up to 250 uM. In another animal, there was no consistent decrease of NE stimulation at the lesioned site.

Five cats wer e prepared for the chronic lesion study, two of which were injected with 6-OHDA and three that were injected with the vehicle solution IP at birth. Tissues were prepared for the assay exactly as described above. Basal and maximal catalytic activities were similar, but the NE-stimulations were significantly depressed, compared to controls (-67%, -33%).

Thus, NE-stimulation of ACase decreased following both methods of lesion. The results corroborate Kasamatsu's earlier finding that after acute lesions, an exogenous source of NE reinstates plasticity, since as is shown here, ACase remains stimulatable by NE. However, since both methods of lesion depress NE-stimulation of ACase activity, the reason for the difference in the state of plasticity appears to reside elsewhere. *NE=norepinephrine

141.13 RECOVERY OF NEURONAL PLASTICITY IN THE KITTEN VISUAL CORTEX PERFUSED WITH A β ADRENORECEPTOR ANTAGONIST. T. Shirokawa and T. Kasamatsu. Smith-Kettlewell Institute of Visual Sciences, 2232 Webster St., San Francisco, CA 94115.

In the visual cortex of developing kittens, the number of β adrenoreceptor binding sites rapidly increases with age, showing a prominent peak larger than the adult value between the 5th and 11th week after birth (Jonsson and Kasamatsu, Exp. Brain Res. 50: 449, 1983). Recently, we showed that direct perfusion of β adrenoreceptor antagonists into kitten visual cortex suppressed, in a dose-dependent manner, the shift in ocular dominance following brief monocular lid suture. When the kitten visual cortex was directly perfused with 10^{-2} M propranolol (in an osmotic pump) for a week, in conjunction with monocular lid suture, the expected shift in ocular dominance was maximally blocked. The resulting binocularity of 67% was compared to that of ~15% in control (Shirokawa and Kasamatsu, ARVO abst., 25. 1984). Taken together, these findings strongly suggest that normally functioning β adrenoreceptors may be critically involved in the regulation of neuronal plasticity in the immature visual cortex (Kasamatsu and Shirokawa Exp. Brain Res., 1985, in press). In the present study, we further examined whether visuocortical plasticity which had been lost to a blockade of β adrenoreceptors by propranolol could be restored some time after the end of its cortical perfusion.

First, the visual cortex of kittens (5-6 weeks old) was directly perfused with 10^{-2} M propranolol for a week. About 60% of visually responsive cells remained binocular, when tested immediately after the end of monocular deprivation which had begun upon the end of propranolol perfusion. Reemergence of the usual extent of the shift in ocular dominance was clearly observed, however, when the lid suture was started one week after the end of the blocker perfusion (binocularity, 20-30%). These results were interpreted as suggesting that β adrenoreceptors blocked by propranolol became functional again, at least partly, within two weeks after the end of the blocker perfusion, causing the obvious shift in ocular dominance following brief monocular lid suture.

Next, noradrenaline (NA, 5×10^{-5} M) was perfused directly into the visual cortex, immediately after the end of the propranolol treatment, over a period of one week concurrently with monocular deprivation. Exogenous NA apparently accelerated the recovery of visuocortical plasticity; about 60% of cells in the NA-treated cortex exclusively responded to stimulation of the nondeprived eye, reducing binocularity to ~20%. This level of binocularity was significantly lower than that (~60%) obtained in the comparable condition but without exogenous NA.

Thus, we concluded that reactivation of β adrenoreceptors by endogenous NA gave rise to reemergence of the visuocortical plasticity and that exogenous NA seemed to further accelerate the recovery.

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142.1 DEMONSTRATING THE NONLINEAR INTERACTION BETWEEN EXCITATION AND INHIBITION IN DENDRITIC TREES USING COMPUTER-GENERATED COLOR GRAPHICS: A FILM. P. O'Donnell*, C. Koch, and T. Poggio. Artificial Intelligence Laboratory and Center for Biological Information Processing, M.I.T., Cambridge, MA 02139.

Computer simulations of the action of synaptic conductance changes on the somatic potential in anatomical reconstructed cat retinal ganglion cells have shown that the specific interaction between synaptic excitation and inhibition with a reversal potential close to the resting potential of the cell (silent inhibition) implements a synaptic veto-operation, inhibition shunting excitation only if it is on the direct path between excitation and inhibition (Koch, Poggio, and Torre, 1982). We have examined the effect of two different types of inhibition, silent inhibition (as for instance activated through the $GABA_A$ receptor) and inhibition with a reversal potential negative to the resting potential (hyperpolarizing inhibition; $GABA_B$ receptor), onto the somatic potential evoked by excitatory synaptic input. If the inhibitory conductance change is above a minimal value, the major difference between these two inhibitions is their specificity: while silent inhibition needs to be localized exactly on-the-path between excitation and the soma in order to effectively reduce the EPSP, hyperpolarizing inhibition is far less demanding, usually reducing the potential in the dendritic tree within a given neighborhood of the synapse. Moreover, we have examined the action of a passive membrane, of the branching geometry, and of the density of active channels on the ability of the inhibition to veto the spike. Our major tool is a set of high-level computer programs enabling us to automatically construct the appropriate circuits describing active and passive electrical properties in complex neuronal structures from the measured morphological data. A graphic front-end processor allows us to directly inject current or place synaptic input onto the graphic version of the reconstructed cell and its dendritic tree. The computations are performed using an electrical circuit simulation program, SPICE. Subsequently, the voltage throughout the whole dendritic tree is displayed on a color monitor as a function of time, different colors coding for different voltages. We will present a color film demonstrating the above effects.

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142.2 K^+ CONDUCTANCE ACTIVATED BY GANGLIONIC α_2 RECEPTORS MAY BE Ca^{2+} INSENSITIVE. P.E. Rafuse, J.A. Zidichouski* & P.A. Smith. Department of Pharmacology, University of Alberta, Edmonton, Canada, T6G 2H7.

The adrenaline induced hyperpolarization (Ad_H) in bullfrog sympathetic ganglia was investigated using the sucrose gap technique. Experiments were performed in the presence of 0.5 μM desipramine. Previous work suggested that the Ad_H was generated via an α_2 adrenoceptor and subsequent activation of potassium conductance (g_K, Smith, J. Physiol, 347, 377, 1984). In agreement with this, we found that the Ad_H was blocked by the K^+ channel blocker, Ba^{2+} (2 mM) yet was not altered when isethionate was substituted for extracellular Cl^-. The response was antagonized by phentolamine (IC_{50}=0.53 μM), idazoxan (IC_{50}=0.59 μM) and yohimbine (IC_{50}=6.2 nM) but not by β blockers or by prazosin (1 μM). Since mobilization of intracellular Ca^{2+} has been associated with α_1 receptor activation (Boulton, Physiol. Rev. 59, 606, 1979, Exton, pp 117 in Adrenoceptors and Catecholamine Action I ed. Kunos G. Wiley, NY, 1981), we investigated whether this mechanism could also be involved in the generation of an α_2 adrenergic response such as the Ad_H. If this were so, the Ad_H might result from activation of Ca^{2+} sensitive g_K following mobilization of intracellular Ca^{2+}. To test this, we compared the effects of various K^+ channel blockers on the Ad_H with their effects on spontaneous, rhythmic, caffeine induced hyperpolarizations. (R. $Caff_H$, Kuba, J. Phyiosl., 298, 251, 1980). These responses, which are attributed to activation of Ca^{2+} sensitive g_K were recorded intracellularly during continuous superfusion of 5 mM caffeine. R. $Caff_H$ responses were antagonized by 10 mM tetraethylammonium (TEA) or by 50 μM quinidine but not by 1 mM 4 aminopyridine (4-AP). On the other hand, the Ad_H was antagonized by both 1 mM 4-AP and 50 μM quinidine but not by 10 mM TEA or 0.5 μM apamin. The differential sensitivity of the two responses to K^+ channel blockers suggest that different K^+ channels may be involved in each case. Furthermore, the Ad_H was not affected by dantrolene (60 μM), a substance thought to interfere with intracellular Ca^{2+} movements. These results imply that a Ca^{2+} sensitive g_K may not be activated during the Ad_H and that the effects of α_2 receptor stimulation may be independent of the mobilization of intracellular Ca^{2+}.

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142.3 **CHARACTERIZATION OF SYNAPTIC TRANSMISSION IN THE INFERIOR MESENTERIC GANGLION OF THE RABBIT.** M. A. Schumann* and D. L. Kreulen (SPON: S. Hameroff). Department of Pharmacology, University of Arizona, College of Med., Tucson, AZ 85724.

The properties of the inferior mesenteric ganglion of the rabbit were studied with intracellular microelectrodes. Isolated ganglia with attached nerve fibers were pinned in a recording chamber and nerve fibers were stimulated with bipolar electrodes. The resting membrane potential averaged -54 mV (± S.E. 1.2 mV) and the cell input resistance averaged 24 MΩ (± S.E. 8 M). Eight per cent of the cells studied (23 of 280) had continuous excitatory postsynaptic potentials (EPSPs) which were blocked by hexamethonium (10^{-4}M). Most cells received convergent nicotinic cholinergic synaptic input from all of the nerve fibers attached to the ganglion. Following repetitive stimulation (30 Hz) of any of the nerve fibers, slow changes in the membrane potential were observed in 84% of cells tested. In 71% of the cells a slow hyperpolarization was followed by a slow depolarization; in 13% of the cells repetitive stimulation produced a slow depolarization only. Both potentials were abolished in low Ca^{+2} high Mg^{+2}. The slow hyperpolarization was associated with a decrease in input resistance, its amplitude ranged from 2 to 15 mV (mean: 7 mV, ±S.E.4 mV) and its duration ranged from 2 to 20 sec (mean: 8 sec, ±S.E. 5 sec). Atropine depressed the slow hyperpolarization by 60% and hexamethonium (10^{-4}M) abolished it. Adenosine (10^{-6}M) and dipyridamole ($3x10^{-6}$M) either together or separately increased both the amplitude and the duration of the hyperpolarization. The slow depolarization that followed the hyperpolarization was associated with a 20% increase in input resistance, its amplitude ranged from 2 to 16 mV (mean: 7 mV, ±S.E. 4 mV) and its duration ranged from 1 to 4 min. The depolarization was not depressed by either atropine or hexamethonium. Adenosine and/or dipyridamole decreased both the amplitude and duration of the depolarization. These studies demonstrate some unique characteristics of the rabbit inferior mesenteric ganglion compared to this ganglion in other species: continuous activity and both hyperpolarizing and depolarizing noncholinergic synaptic potentials. (Support:HL27781)

142.4 VOLTAGE DEPENDENT SYNAPTIC AND NON-SYNAPTIC CONDUCTANCES IN THE MAUTHNER CELL. D.S. Faber and H. Korn, Div. Neurobiol., Dept. Physiol., SUNY, Buffalo NY 14214, and Lab. de Neurobiologie Cellulaire, INSERM U261, Institut Pasteur, Paris, France.

Current clamp analyses of the Mauthner (M-) cell have indicated that its soma-dendritic membranes are electrically passive. We have used single and double electrode voltage clamp techniques, and report that the M-cell's leakage conductance and a synaptically activated inhibitory conductance are both decreased during hyperpolarization. The M-cell was penetrated with one or two KCl microelectrodes, injected with sufficient Cl^- ions to permit detection of IPSPs evoked by stimulation of either the recurrent inhibitory network or individual presynaptic interneurons, and voltage clamped at resting potential (-70 to -85 mV). Leakage conductance was measured during command pulses of 15 to 30 msec duration and -50 to +30 mV magnitude; at the pulse onset the M-cell membrane was quite linear, and its input conductance averaged 3.85 μS (s.d.=1.91 μS, n=21). However, during hyperpolarizations of 10 mV or more, the leakage current often decayed up to 50% by pulse offset. This current sag presumably reflects voltage dependent properties of the M-cell rather than the charging characteristics of its distributed soma-dendritic membranes since: 1) the responses to hyper- and depolarizing command pulses were asymmetrical, and ii) there was no overshoot at the termination of the hyperpolarizing command pulses. Since there was Cl^- loading, these results suggest the M-cell has a voltage dependent potassium conductance which is normally maximal near resting potential.

Inward IPSCs were also recorded at resting potential and during the hyper- and depolarizing command pulses. In some cases, the IPSC current-voltage relation was non-linear, with the synaptic conductance appearing to decrease during hyperpolarizations of 15 to 20 mV or more. Although this voltage dependence was somewhat labile, its occurrence was correlated with neither the level of Cl^--loading nor the magnitude of the synaptic current. In addition, it was not due to a shift in the Cl^- equilibrium potential during the clamp pulses as it was apparent within a few msec after the onset of hyperpolarization. Two possible mechanisms could explain these observations: i) inadequate voltage control of the synaptic membrane, with an increased non-synaptic resistance in that region underlying the decreased synaptic current, and ii) a voltage dependent shift in the kinetics of the activated synaptic channels. The latter is more likely since hyperpolarization consistently decreased the decay time of the synaptic currents in a voltage dependent manner. Thus, hyperpolarization may decrease the mean lifetime of transmitter activated Cl^- channels. Supported in part by NIH Grant #NS-15335.

142.5 INHIBITORY SYNAPTIC CONDUCTANCES AND DRIVING FORCE IN THE MAUTHNER CELL: COMPARISON OF VOLTAGE AND CURRENT CLAMP DATA. H. Korn and D.S. Faber, Lab. de Neurobiologie Cellulaire, INSERM U261, Institut Pasteur, Paris, and Div. Neurobiology, Dept. Physiology, SUNY at Buffalo, Buffalo, NY 14214.

In order to directly measure the driving force (ΔV) and the peak conductances of its Cl^--dependent IPSPs, the Mauthner (M-) cell was penetrated with one or two KCl microelectrodes for single (SEVC) or double (TEVC) electrode voltage clamping, iontophoretically loaded with Cl^- ions, and clamped at resting potential. Unitary inhibitory postsynaptic currents (IPSCs; measured with SEVC) were evoked by intracellular stimulation of single presynaptic neurons, while activation of the M-cell's recurrent inhibitory network produced the full-sized collateral IPSC (measured with SEVC or TEVC). SEVC chopping frequency was 18-33 kHz (Axoclamp, Axon Instr.). IPSCs were measured at the holding potential and during brief (~20 msec) depolarizing and hyperpolarizing command potentials. The driving force was determined either by extrapolation from plots of IPSC amplitude vs. membrane potential, when linear, or directly with depolarizations to the reversal potential. Analysis of the results obtained with these two techniques indicated that: i) g_{coll}, the peak conductance during the collateral IPSC, averaged 5.93 μS (n=10, s.d.=4.75 μS), which is comparable with the previous estimate of 6.08 μS obtained with current clamp techniques (Faber, D.S. and Korn, H. J. Neurophysiol. 48:654, 1982), and ii) the average unitary inhibitory conductance, g_{ipsc}, was 144 nS (n=5, range: 80-179 nS).

Previous indirect estimates of the unitary conductances (mean=172 nS) involved normalizing unitary IPSP amplitudes (V_{ipsp}) relative to the magnitude of the collateral IPSP (V_{coll}), since the latter was found to be a constant fraction (k=0.5) of the driving force. That is, although ΔV varied from one experiment to the next, it could be taken as $2xV_{coll}$ in the formula $g_{ipsp} = [V_{ipsp}/(\Delta V - V_{ipsp})]g_M$, where g_M is the M-cell's input conductance. We have now confirmed that conclusion during voltage clamping: in 16 experiments where V_{coll} ranged from 6 to 22 mV, ΔV was between 10 and 48 mV, and k averaged 0.54 (s.d=0.09). In addition, even when strychnine, which acts postsynaptically to reduce or block IPSPs in the M-cell, decreased g_{coll} by greater than 50%, there were no significant changes in ΔV. In summary, these experiments strengthen our earlier conclusions and derivations of both the size of the binomial quantal conductance and the relative synaptic strengths of individual presynaptic neurons (Korn, H., Mallet, A., Triller, A. and Faber, D.S. J. Neurophysiol. 48:679, 1982). Supported in part by NIH Grant #NS-15335.

142.6 A SEROTONIN-INDUCED INCREASE OF CALCIUM CURRENT IN IDENTIFIED MOLLUSCAN NEURONS MIMICKED BY cGMP BUT NOT BY cAMP. D. Paupardin-Tritsch*, C. Hammond*, and H.M. Gerschenfeld* (SPON: Y. Ben Ari), Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75230 Paris 05, France.

In cardiac muscle cells, beta-adrenergic agonists evoke an increase of the duration of the Ca-action potential by inducing a cAMP-dependent increase of the Ca-current (see Reuter, Nature 1983,301,569; Tsien, Ann.Rev.Physiol. 1983,45,381). In contrast, neurotransmitters like serotonin (5HT) and dopamine have been reported to induce an increase of the duration of the Ca-spike of molluscan neurons by decreasing the S-current (Klein et al., PNAS 1982,77,543), a cAMP-dependent K-current (Klein & Kandel, PNAS 1978,75,3512; PNAS 1980,77,3512; Paupardin-Tritsch et al., Brain Res. 1981,217,201; J.Neurosci. , in press; Deterre et al., PNAS 1982,79,7934; Siegelbaum et al., Nature 1982,299,413).

We have now observed that in two identified neurons located on the ventral face of the left parietal ganglion of the snail Helix aspersa 5HT increases the duration of the Ca-spike and that this effect is not mimicked by cAMP. Voltage-clamp studies revealed that in these neurons 5HT did not affect the outward current recorded in a Ca-free extracellular medium containing 30 mM TEA and did not evoke any current when the potential was held at +10 mV. In contrast, 5HT markedly increased the inward current recorded in a TTX-TEA-BA medium. The I-V curves showed that 5HT increased the Ca-current.

This effect of 5HT on the Ca-current was mimicked neither by intracellular injections of cAMP nor by forskolin, whereas the intracellular injection of cGMP did increase the Ca-current. When 5HT evoked a maximal increase of the Ca-current, the intracellular injection of cGMP became ineffective and conversely, when cGMP evoked a maximal increase of the Ca-current, 5HT application became ineffective. IBMX and RO 20-1724 had no effect on the Ca-current. Another phosphodiesterase inhibitor, zaprinast (M&B 22-948) 100 uM also evoked an increase of the Ca-current but did not potentiate the effects of 5HT. Phorbol-ester TPA, which also evoked an increase in Ca-current, did not interfere with 5HT effects. Intracellular injection of EGTA did not affect the 5HT-induced increase of Ca-current.

These results show that 5HT can also increase the duration of the Ca-spike of molluscan neurones by a second mechanism involving an increase in Ca-current. The possible involvement of cGMP as a second messenger will be discussed.

142.7 PHORBOL ESTERS INDUCE THE TRANSLOCATION OF SOLUBLE Ca^{2+}/PHOSPHOLIPID-DEPENDENT PROTEIN KINASE IN ADRENAL CHROMAFFIN CELLS. B. C. Wise and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

We have shown (Wise and Costa, J. Neurochem., in press) that the bovine adrenal medulla and its constituent chromaffin cells contain significant quantities of the Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C) and endogenous substrate proteins for the enzyme in both cytosol and membrane fractions. With the finding that tumor-promoting phorbol esters activate this enzyme (Castagna et al., J. Biol. Chem. 257: 7847, 1982) by mimicking the action of diacylglycerol, an important tool became available to study the in vivo activation and function of the enzyme. In the present studies, we have investigated the effects of phorbol esters on the subcellular distribution of protein kinase C activity and on catecholamine secretion in bovine adrenal chromaffin cells maintained in primary culture. Pretreatment of chromaffin cells with 12-O-tetradecanoylphorbol 13-acetate (TPA) causes a dose-dependent (EC_{50}= 20 ng/ml) decrease in Ca^{2+}-dependent enzyme activity in the soluble fraction with a maximal effect (90% decrease) seen at between 0.1-1 μg/ml TPA. There is a concomittant increase in the levels of enzyme activity in the membrane fraction by TPA pretreatment with an EC_{50} value of 10 ng/ml and a maximal effect (93% increase) seen at 100 ng/ml. With doses of TPA up to 100 ng/ml, there is no change in the total cell level of enzyme activity and, therefore, the results indicate an apparent translocation of enzyme activity induced by TPA from a soluble fraction (containing about 50% of total enzyme activity under resting conditions) to a membrane fraction. The translocation is rapid with near maximal effects seen after 5 min of TPA treatment. Other phorbol esters (such as 4β-phorbol 12,13-dibenzoate, 4β-phorbol 12,13-dibutyrate, and 4β-phorbol 12,13-didecanoate) which stimulate protein kinase C in vitro are also effective in causing the enzyme translocation. Inactive phorbol esters (i.e. ineffective enzyme activators in vitro) are ineffective in situ in chromaffin cells. We also find that TPA treatment of cells results in a dose-dependent release of both norepinephrine (NE) and epinephrine (E) with an EC_{50} value of about 10 ng/ml and a maximal effect (10-13% stimulated release) seen at 100 ng/ml for both NE and E release. The TPA-induced release of catecholamines is lower than the nicotine-induced release which suggests that other components may be involved in the normal physiological release of catecholamines. The results suggest that the translocation of protein kinase C from a soluble to a membrane-bound form may be an important step in the activation and functioning of the enzyme in such processes as exocytosis.

142.8 NOVEL EFFECT OF PHORBOL ESTERS ON REPETITIVE FIRING IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. J. M. Baraban, S. H. Snyder and B. E. Alger, Dept. of Neurosci,. Johns Hopkins Univ. Sch. Med., Batimore, MD 21205 and Dept of Physiol., Univ. of MD. Sch. Med., 21201, Baltimore, MD.

Phorbol esters stimulate protein kinase C, a calcium- and phospholipid-dependent phosphorylating enzyme present in high concentrations in mammalian brain. In order to examine the role of protein kinase C in neuronal function, we studied the electrophysiological effects of phorbol esters on hippocampal CA1 pyramidal neurons in the slice preparation with intracellular recording techniques. We found that phorbol esters block the afterhyperpolarization (AHP) initiated by a brief train of action potentials (Proc. Natl. Acad. Sci. USA., 82:2538, 1985). The calcium-dependent potassium conductance responsible for this AHP also contributes to the marked slowing of firing ("accomodation") that is observed during prolonged depolarizing current pulses in many cell types. Accordingly, during administration of 1 uM phorbol 12, 13 diacetate, an active phorbol ester, blockade of accomodation develops in parallel with inhibition of the AHP. However, blockade of accomodation is transient. After approximately twenty minutes, the number of spikes elicited during a two-second depolarizing pulse decreases dramatically, even though the AHP following a 100 msec pulse remains blocked. We refer to this phase as "paradoxical accomodation".

To investigate the mechanism of this effect, we have studied repetitive firing elicited in CA1 pyramidal cells by both carbachol and 2 sec depolarizing current pulses. In addition to decreasing the number of spikes produced by depolarizing current pulses, phorbol 12,13 diacetate, 1 uM, also markedly reduces the repetitive firing elicited by application of carbachol, 1-20 uM. Nevertheless, individual spikes induced either synaptically or by current injection are unaffected, indicating that the suppression of repetitive firing is not due to a local anesthetic effect of phorbol esters. In the presence of 100-200 uM $CdCl_2$, which blocks synaptic transmission and Ca entry into the cell, phorbol esters still initially increase the number of action potentials evoked by a 2 sec depolarizing pulse and then produce a paradoxical accomodation pattern. Small, discrete, voltage-dependent depolarizing potentials appear to trigger individual action potentials during prolonged membrane depolarizations. Phorbol esters block these small depolarizations, a phenomenon which may account in part for paradoxical accomodation.

These findings support the conclusion that phorbol esters inhibit repetitive firing via a postsynaptic mechanism distinct from a Ca-dependent K conductance.

142.9 VOLTAGE CLAMP ANALYSIS OF SLOW CHOLINERGIC SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. D.V. Madison, B. Lancaster†, R.A. Nicoll and P.R. Adams†. Depts. of Pharmacology and Physiology, University of California, San Francisco, CA. 94143; and †Dept. of Neurobiology, State University of New York, Stony Brook, NY. 11794.

A slow muscarinic excitatory postsynaptic potential (slow EPSP), accompanied by an increase in membrane input resistance and by a blockade of calcium-activated potassium afterhyperpolarizations (AHPs), can be elicited in CA1 pyramidal neurons in vitro. This is achieved by electrical stimulation of cholinergic afferents within the hippocampal slice (Cole and Nicoll, J. Physiol. 352, 173; 1984). In the present study we have used a single-electrode voltage clamp to examine the membrane current underlying the slow EPSP. Of particular interest was the possible role of two muscarine-sensitive K^+ currents, I_{AHP}, which produces the afterhyperpolarization, and a voltage-sensitive current, I_M (Halliwell and Adams, Brain Res. 250, 79; 1982). I_M was seen as a time-dependent inward relaxation in membrane current during hyperpolarizing voltage commands (1 sec.; 10-20mV) from holding potentials of -30 to -40 mV. I_{AHP} was elicited by depolarizing commands (1 sec.; 20-40mV) from holding potentials of approximately -60mV.

Under voltage clamp the slow EPSP is a slow inward current (slow EPSC) which decreases in amplitude as the membrane potential approaches E_{K^+}. During the slow EPSC, membrane conductance was decreased and I_{AHP} was reduced dramatically, while I_M was unaffected (n=20). Only in two cells, which were bathed in eserine and stimulated with several times the strength needed to produce a slow EPSC, was I_M reduced noticibly. This finding differs from that described in cultured CA3 neurons reinnervated by cholinergic fibers (Gahwiler and Brown, Nature 313, 577, 1985).

To assess the relative sensitivity of I_M and I_{AHP} to cholinergic agonists, bath applications of carbachol were made. Carbachol concentrations which produced an inward current comparible to the slow EPSC (ie. 1uM), decreased membrane conductance and I_{AHP} with little effect on I_M. Only with higher carbachol concentrations (eg. 10uM) was I_M clearly decreased. All actions of carbachol were sensitive to 0.1uM atropine.

We conclude that in CA1 pyramidal cells I_{AHP} is more sensitive than I_M to muscarinic receptor activation, and that selective reductions in I_{AHP} are observed during most slow EPSCs. Although I_M can be reduced by high agonist concentrations or very strong synaptic activation, blockade of I_M is not a prerequisite for the generation of the slow EPSC. We are currently investigating whether the block of I_{AHP} can account in full or in part for the production of the slow EPSC.

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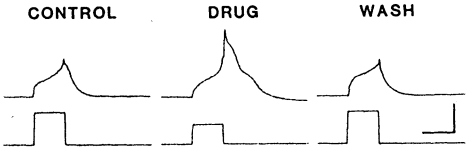
142.10 EPILEPTIFORM ACTIVITY INDUCED BY INHIBITORS OF THE Na/K PUMP IN RAT HIPPOCAMPAL SLICES: FACILITATION OF Ca^{++} SPIKES. M. McCarren and B.E. Alger. Dept. Physiol., Univ. MD, Sch. Med., Balto., MD 21201.

Block of the Na/K ATPase (Na/K pump) can lead to epileptiform activity in mammalian brain, but the mechanism is unknown. Using intracellular recordings from the stratum pyramidale region of CA1 of the rat hippocampal slice preparation, we have investigated the effects of Na/K pump inhibition by cardiac glycosides (CGs) on pyramidal cell membrane properties and synaptic potentials.

We have assessed Na/K pump antagonism by measuring $[K^+]_o$ with K^+-sensitive electrodes and glial cell membrane potentials. As anticipated, perfusion with CGs (strophanthidin or dihydroouabain) increased resting $[K^+]_o$ from a baseline of 5.4 mM and decreased the rate of K^+ clearance following its elevation by stimulus trains.

Field potential recordings indicated that CGs caused repetitive firing (bursts) in response to single stimuli. Intracellular recordings showed that neuronal resting potentials became depolarized by 2-8 mV. Ipsps were reduced, although not as markedly as reported previously for ouabain, a more potent CG. Furthermore, even with ipsps blocked by picrotoxin, CGs facilitated bursting and depolarizing afterpotentials, but interpretation of these data is complicated by possible effects on synaptic transmission and Na^+ gradients. To test whether CGs might directly enhance neuronal burst generating properties, we studied Ca^{++} action potentials (in 1 uM TTX and 5 mM TEA) in the presence and absence of CGs. In the cell illustrated below, a Ca^{++} spike was elicited by less current in 10 uM strophanthidin than was necessary in either control or wash solutions. (Calibration: 40 mV, 0.5 nA and 100 ms). The facilitation of Ca^{++} spikes by CGs was not due simply to depolarization, since it persisted when the membrane was repolarized to the control membrane potential and was not mimicked by perfusion with 6-9 mM K^+_o. Input resistance either did not change or increased slightly (20%) in CG, yet the facilitation was not blocked by simultaneous perfusion with a variety of K^+-conductance antagonists (Ba^{++}, 4-AP, TEA, carbachol), suggesting an action of the CGs distinct from blockade of a K^+ current.

It appears that interference with the Na/K pump can lead to burst firing due not only to a depression of ipsps but also to an increase in the intrinsic excitability of the pyramidal cells.



142.11 THE TEMPERATURE DEPENDENCE OF HIPPOCAMPAL CA1 POSTSYNAPTIC FIELD POTENTIALS - EVIDENCE FOR ENHANCEMENT SUBSEQUENT TO COOLING. R.M. Lebovitz. Dept. Physiology, University of Texas Health Science Center, Dallas, Texas 75235.

Hippocampal slices (400 micron) taken from 150-250 g Sprague-Dawley rats were equilibrated at 35.5°C for 3 hours in an interface-type chamber. Temperature was monitored by a thermistor located just below the slice and, in separate calibration experiments, by a bead thermistor embedded in the slice from above. Stable temperature shifts were imposed by a Peltier device placed below the chamber. Orthodromic evoked potentials were elicited by a bipolar electrode placed on the Schaffer collaterals in stratum radiatum of CA2-3. The CA1 population spike and excitatory postsynaptic potential (EPSP) fields were recorded via glass microelectrodes located in stratum pyramidale and stratum radiatum, respectively. Field data were obtained as a function of stimulus intensity (I/O curves) at the base temperature, at several stable temperatures as the slice was cooled to 24-26°C, then again after rewarming. The slice was stimulated at a constant rate (0.1 Hz) at 50% of maximal intensity (population spike amplitude) continuously throughout each experiment.

The CA1 population spike showed evident saturation at high stimulus intensities so that stimulus-dependent and stimulus-independent (plateau) segments of the I/O relation could be defined. Both the slope of the intensity-dependent and the magnitude of the plateau segments were reduced by cooling, with little or no change in intercept response threshold. The immediate effect of cooling on population spike discharge was not, therefore, well characterized as a "shift" in the I/O curve but rather as a flattening. The EPSP field potential slope was proportional to stimulus intensity over a broad range (5-80 V); saturation generally was not observed. EPSP slope was reduced by cooling with little or no change in intercept threshold, so that the slope of the I/O curve for this response component declined with cooling. Upon rewarming to the base temperature in a substantial number of instances (70%) the population spike I/O curves were observed to be shifted to the left, relative to baseline. In these instances, the I/O curves recovered to baseline form 60-120 minutes after the return to the base temperature. Comparable enhancement and shift of the I/O curves could be seen after moderate warming of the slice, but not after repeated I/O determinations at a fixed baseline temperature. The data suggest the appearance of a reversible potentiation of the response to low-rate stimuli that is made evident by temperature cycling of the preparation.

143.1 NALTREXONE INDUCES DOSE-DEPENDENT DECREASES IN SELF-INJURIOUS BEHAVIOR. B.H. Herman, M.K. Hammock*, J. Egan*, C. Feinstein*, I. Chatoor*, R. Boeckx*, N. Zelnick*, R. Jack*, and J. Rosenquist*. Brain Res. Cen. and Depts. of Psychiat., Neurosurg., Lab. Med. and Child Health & Develop. of Children's Hospital Nat. Med. Cen. and George Washington Univ. Sch. of Med., Washington, D.C. 20010.

Opioid systems in the brain have been implicated in the regulation of antinociception in animals and humans. Over-activity in some brain opioid pathway may underlie the self-injurious behavior (SIB) exhibited by some severely mentally retarded individuals. This is the first report demonstrating that the potent orally administered opiate antagonist - Naltrexone HCl (NALTRX) - is effective in reducing the frequency of SIB.

The subject (S) was a 17 year old male with severe SIB since the age of 7, phenylketonuria, and severe mental retardation. S was in good health: heart rate, blood pressure, body weight, EEG, CAT scan, and clinical chemistry tests (e.g., aspartate transaminase, serum alanine aminotransferase) were within normal range. No side effects of NALTRX were observed.

The frequency of the following SIB types were recorded: hand-to-ear hits, chin-to-shoulder hits, and self-biting. Each test session was 10 min long with five one min test trials surrounded by one min rest intervals. S was tested 1h and 4h after oral administration of NALTRX (DuPont Pharmaceuticals). Behavior was rated by three individuals (two blind to drug and one not blind to drug) (inter-rater reliabilities $\geq +0.96$, p's $< .001$). Sessions were videotaped for blind behavioral analyses. S was tested on drug or placebo for two days followed by two no drug days.

Table. Effect of Naltrexone on Total SIB Frequency

Condition	Day	SIB Frequency
Placebo (Before)	1	45.7 ± 1.7*
Placebo (Before)	2	39.7 ± 0.7
0.5 mg/kg NALTRX	1	35.7 ± 0.9
0.5 mg/kg NALTRX	2	32.0 ± 1.8
1.0 mg/kg NALTRX	1	13.0 ± 0.6
1.0 mg/kg NALTRX	2	6.7 ± 0.3
1.5 mg/kg NALTRX	1	9.3 ± 0.7
1.5 mg/kg NALTRX	2	0.0 ± 0.0

*Mean ± S.E.M. of three raters.

The table shows the effects of NALTRX, 1h after drug, on total SIB frequency as rated by three individuals. There is a dramatic dose-dependent decrease in SIB frequency with increasing doses of NALTRX. Indeed, the second day of 1.5 mg/kg NALTRX reduced SIB frequency to zero. The long half-life of NALTRX may explain the enhanced Day 2 drug effects.

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143.2 FURTHER CHARACTERIZATION OF AN ENDOGENOUS OPIOID ANTICONVULSANT: MEASUREMENT OF OPIOID PEPTIDE IMMUNOREACTIVITY IN RAT CEREBROSPINAL FLUID FOLLOWING A GENERALIZED SEIZURE. J.B. Long, C.J. Molineaux[1] and F.C. Tortella. Neuropharm. Br., Dept. of Med. Neurosci., Div. of NP, Walter Reed Army Inst. of Res., Washington, D.C. 20307 and [1]Dept. of Pharmacol., Unif. Ser. Univ. Hlth. Sci., Bethesda, MD 20814.

Seizure-activated endogenous opioid systems appear to inhibit subsequent seizure activity. Repeated maximal electroshock (MES) in rats produces a progressive decrease in seizure severity which is abolished by both naloxone and morphine tolerance (Tortella, et al., Brain Res. 332:174, 1984). Similarly, MES produces a naloxone-reversible increase in the threshold to a subsequent flurothyl-induced seizure (Tortella and Cowan, Life Sci. 31:2225, 1982). We recently observed that cerebrospinal fluid (CSF) collected from rats following an MES-induced seizure causes an increase in flurothyl seizure threshold when administered intracerebroventricularly (icv) to naive rats (Tortella and Long, Science, In Press). This anticonvulsant action was blocked by opioid antagonists and enhanced by peptidase inhibitors. Additional results from these and other experiments (Tortella and Long, INRC, 1985) indicate that the endogenous anticonvulsant substance in CSF is a peptide with a MW less than 10,000 daltons, and that the anticonvulsant effects of this substance involve an interaction with δ opioid receptors. Therefore, in the present experiments we sought to identify convulsion-induced alterations in CSF concentrations of known opioid peptides in order to distinguish which, if any, of these opioids serves as an endogenous anticonvulsant.

Ten min following a single MES seizure or sham treatment, CSF was collected from the cisternal space of donor rats into tubes on ice containing aprotinin and bacitracin (756 kallikrein units and 1mg per ml of CSF, respectively). CSF was acidified with acetic acid and heated at 90°C for 10 min prior to radioimmunoassay for β-endorphin, dynorphin, met-enkephalin, and leu-enkephalin -like immunoreactivity. MES caused a generalized tonic-clonic convulsion which was associated with a selective 3.5 fold increase in β-endorphin immunoreactivity relative to sham treated rats (265 ± 15 vs 62 ± 10 pg/ml). In contrast, CSF levels of dynorphin, met-enkephalin, and leu-enkephalin (101 ± 6, 196 ± 11, and 147 ± 13, pg/ml, respectively) were unaltered following MES.

From these results it appears that if the seizure-activated anticonvulsant activity in rat CSF is attributable to a known endogenous opioid, it is most likely associated with a molecule immunologically recognized as β-endorphin.

143.3 β-FUNALTREXAMINE (β-FNA) AND THE RESPONSE OF NEUROBLASTOMA IN MICE. I.S. Zagon, P.J. McLaughlin, A.E. Takemori* and P.S. Portoghese*. Department of Anatomy, The M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033 and Departments of Pharmacology and Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55455.

Previous studies with a variety of opioid agonists and antagonists have shown that endogenous opioids and opioid receptors control neuro-oncogenic events (Zagon, I.S. and McLaughlin, P.J., Life Sci., 28:1095, 1981; Science, 221:671, 1983; Life Sci., 35:2057, 1984). Unfortunately, all of the compounds utilized to date have a cross-reactivity with various opioid receptor types, limiting our knowledge as to which specific receptor(s) is (are) involved in neural cancer. Recently developed selective antagonists provide a unique opportunity to elucidate the importance of each receptor. In the present experiments, the effects of β-FNA, a highly selective and irreversible μ opioid receptor antagonist, on tumor response in mice inoculated with neuroblastoma was determined.

S20Y neuroblastoma, cloned from the A/Jax mouse C1300 neuroblastoma, were injected subcutaneously (10^6 cells/animal) into A/Jax mice. Tumors were measured in 2 dimensions with vernier calipers every 3rd day beginning on the first day a measurable (5 mm or larger) tumor was observed. Incidence of tumor appearance, total survival time, as well as time between tumor cell inoculation and tumor appearance, were analyzed. Beginning 2 days after tumor cell inoculation, mice were injected with either 2 mg/kg or 10 mg/kg β-FNA, or sterile water. Injections were given every 48 hr and continued until all tumor-bearing mice were dead.

Inoculation of neuroblastoma cells in control subjects resulted in 100% tumor incidence within 16 days. Mean and median survival times of 36 and 35 days, respectively, following tumor inoculation were recorded in control animals. Tumor incidence and survival times were comparable to controls for mice given chronic injections of 2 mg/kg and 10 mg/kg β-FNA. Tumor size on the day of death did not differ between control mice and those given 2 mg/kg β-FNA, but tumor size of mice in the 10 mg/kg β-FNA group was 21% smaller than control levels. Both dosages of β-FNA were found to block morphine-induced analgesia for 48 hr. These results suggest that, in and by themselves, μ receptors selectively antagonized by β-FNA do not play an important role in neuro-oncogenic events.

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143.4 CSF FROM MORPHINE DEPENDENT RATS PRECIPITATES OPIATE ABSTINENCE SYNDROME. D.H. Malin, L. Dussack*, P. Jenkins*, J. Zografos, P.D. Bruce*, L. Harter* and R. Monfort*. Univ. of Houston-Clear Lake, Houston, Texas, 77058.

There have been several reports that injection of CSF or brain extract from morphine dependent rats could induce tolerance to morphine analgesia. In addition, there were earlier reports that brain extract from dependent rats interferes with morphine effects on the vas deferens assay. These findings raise the possibility of an endogenous opiate antagonist in opiate dependent organisms. It was therefore of interest to determine whether CSF from dependent rats could, like the artificial antagonist naloxone, precipitate withdrawal in morphine treated rats. The third ventricle was chosen as a target for CSF injection, based on preliminary studies showing that i.c.v. naloxone at that site precipitated the most rapid onset of abstinence signs.

Twelve recipient rats were cannulated in the third ventricle and implanted subcutaneously with 2 Alzet 2001 osmotic minipumps, infusing them with 0.35 mg/kg/hr morphine sulfate, or with saline alone. Twelve donor rats were implanted with one larger Alzet 2ML1 minipump, infusing them with 3.5 mg/kg/hr morphine sulfate, or with saline alone. After 7 days of infusion, the donors' pumps were removed and 6 hours was allowed for clearance of morphine from their CSF. CSF (75 μl) was then withdrawn, under ether anestesia, from the cisterna magna of each donor and was gradually injected by motorized syringe (10 μl/min) into the third ventricle of a conscious recipient rat. Recipients were observed for a standard checklist of abstinence signs for 30 min following the onset of CSF injection.

In the 4 cases where the donor and the recipient had both been morphine infused, 37.75 ± 9.66 abstinence signs (M ± SEM) were observed, beginning shortly after the onset of CSF injection. The predominant signs were abdominal writhes, wet-dog shakes and ptosis, with teeth chattering, scratching, head shakes and seminal ejaculation also observed.

In the 4 control cases where the donor had been saline infused and the recipient had been morphine infused, there were 3.25 ± 2.85 abstinence signs. In the 4 control cases where the donor had been morphine infused and the recipient had been saline infused, there were 3.25 ± 2.32 abstinence signs. According to Dunnett's multiple comparison test, there were significantly more abstinence signs, $p < .01$, in the group where both donors and recipients had been morphine infused than in either of the control conditions. The results are thus consistent with the hypothesis that prolonged opiate exposure induces in the CSF a substance with certain opiate antagonist properties. (University of Houston-Clear Lake Organized Research Grant).

143.5 STEREOSPECIFIC MODULATION OF NEURO-ONCOGENESIS BY OPIOID ANTAGONISTS. P.J. McLaughlin and I.S. Zagon. Department of Anatomy, The M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033

Growth of murine neuroblastoma has been shown to be modulated by opioid agonists and antagonists (Zagon, I.S. and McLaughlin, P.J., Life Sci. 28:1095, 1981; Science 221:671, 1983; Life Sci. 35:2057, 1984). The mechanism underlying this regulation of oncogenesis is suggested to involve endogenous opioid systems. If tumor response is regulated at the level of the opioid receptor, this interaction should be stereospecific. To address this question, the response of neuroblastoma in mice following administration of (-) and (+) isomers of naloxone was studied.

S20Y neuroblastoma, cloned from the A/Jax mouse C1300 neuroblastoma, were injected subcutaneously (10^6 cells/animal) into A/Jax mice. Tumors were measured in 2 dimensions with vernier calipers every 3rd day beginning on the first day that a measurable (5 mm or larger) tumor was observed. Incidence of tumor appearance, total survival time, as well as time between tumor cell inoculation and tumor appearance, were analyzed. Beginning 2 days after tumor cell inoculation, mice were injected with either 15 mg/kg (-) naloxone, 15 mg/kg (+) naloxone, or sterile water. Injections were continued daily until all tumor-bearing mice were dead.

By 13 days after tumor cell inoculation, 100% of the mice in the control and (+) naloxone groups had measurable tumors but only 47% of the mice in the (-) naloxone group had tumors. Five of the 17 mice ($p<0.01$; chi-square test) in the (-) naloxone group did not develop a tumor by day 56 when the experiment was terminated. The control and (+) naloxone mice did not differ from each other in regard to mean and median total survival time, survival time after tumor appearance, or time between tumor cell inoculation and tumor appearance. However, the (-) naloxone mice had median and mean survival times that were 43% and 28%, respectively, longer than control mice. Latency time between tumor cell inoculation and appearance of a measurable tumor was significantly ($p<0.01$) delayed by 33% for mice in the (-) naloxone group in comparison to control and (+) naloxone mice. No difference in the pattern of tumor growth was observed between naloxone-treated and control animals. In opioid agonist challenge experiments, (-) naloxone blocked morphine-induced analgesia for 4-6 hr; analgesic responses of (+) naloxone and control animals to morphine were similar.

These results demonstrate that opioid antagonists can modulate the course of neural neoplasia, and that these effects are stereospecific. Data from this investigation provide further evidence that endogenous opioid systems play a role as regulators of neural cancer.

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143.6 BEHAVIORAL AND BIOCHEMICAL PROFILES OF AGONISTS AND ANTAGONISTS AT THE SIGMA (SKF 10,047) RECEPTOR. G.F. Steinfels, S.W. Tam, and L. Cook, E.I. DuPont de Nemours & Co., Biomedical Products Dept., Wilmington, DE 19898, U.S.A.

Over the past several years there has been a growth in the use of drug discrimination (DD) procedures as a tool for investigating the interoceptive cues produced by opiates. Much interest has been focused on the sigma receptor because it is believed to be responsible for the production of opiate-produced psychotomimetic behaviors in humans. Although there have been studies in rats indicating that (±)-N-allynormetazocine (NANM/SKF 10,047) can be used as a discriminative stimulus (DS), there are recent studies now indicating that the two stereoisomers of NANM have different pharmacologic profiles. For example, using guinea pig brains we can demonstrate that the (-)-but not (+)-isomer of NANM has high affinity binding to the kappa receptor. An isolated guinea pig ileum preparation indicates that the kappa receptor binding is agonistic. However, despite the high affinity kappa receptor agonist binding, analgesic studies in rats demonstrate that (-)-NANM does not possess the same degree of intrinsic analgesic activity as do other kappa agonist analgesics (e.g. U50488H). Rats were trained to discriminate between 3.0 mg/kg (+)-NANM and saline in a two-choice discrete trial avoidance task. To correlate DD behavior with receptor affinity (IC50), the drugs were tested in a brain sigma receptor binding assay using (+)-[^{3}H]NANM. At the highest dose tested (18 mg/kg), (-)-NANM (IC50=496nM) only partially generalized (65%) to (+)-NANM (IC50=68nM) and (-)-NANM caused sedation and ataxia. However, pretreatment with naloxone (1 mg/kg), which presumably blocks the mu, kappa, and delta receptor effects of (-)-NANM, results in complete generalization of (-)-NANM with (+)-NANM. (-)-NANM was 1/3 as potent as (+)-NANM. Cyclazocine (IC50=79nM), completely generalized to and was 1.1 times as potent as (+)-NANM. In contrast, PCP (IC50=777nM), despite its relatively low affinity for the sigma receptor, completely generalized to and was 1.7 times more potent than (+)-NANM. Thus although there is previous biochemical data suggesting that PCP and (+)-NANM occupy different receptor sites, these drugs share similar DS properties. Although it has been inferred that the pharmacologic properties of sigma agonists may be mediated through the dopamine receptor, the dopamine receptor agonist apomorphine did not generalize to (+)-NANM. Haloperidol, which has high affinity binding to the dopamine and sigma receptor, antagonized the DS properties of (+)-NANM. Since (+)-NANM apparently lacks apomorphine generated cues, the antagonism of (+)-NANM produced by haloperidol was probably mediated at the sigma receptor site. The alpha-2 antagonist yohimbine, has no measurable affinity for the sigma receptor, yet it could antagonize the DS properties of (+)-NANM. These data may indicate a link between the sigma receptor and noradrenergic system, the latter of which may have an important role in psychotomimetic behavior.

143.7 EFFECTS OF A SUSTAINED DECREASE IN OPIOID TONE ON LH SECRETION IN INTACT AND LONG-TERM CASTRATED RATS. Nicole Fournet*, L.G. Allen*, P.S. Kalra* and S.P. Kalra (SPON. W. Luttge) Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610

Considerable evidence suggests that endogenous opioid peptides (EOP) may be involved in the regulation of LH release in male rats. The observations that a bolus injection of an opiate receptor antagonist, naloxone (NAL), stimulated LH release in intact but not in long-term castrated rats, led to the hypothesis that EOP may exert a tonic inhibitory influence on LH release only in intact rats. We tested this hypothesis by continuous NAL infusion to achieve a sustained decrease in opioid tone in intact and long-term castrated rats. Two side-by-side cannulae were placed intracardially in intact and castrated rats (18-20 days post-castration). NAL (2 mg/h/1.2 ml) or saline alone was infused continuously through one cannula, and 100 µl blood samples were withdrawn through the other with the aid of a peristaltic pump at 5 min intervals for 2-1/2 - 3 h for estimation of plasma LH by radioimmunoassay. In intact rats, saline infusion did not change basal LH secretion. However, NAL infusion evoked a significant 2-fold increase in LH release ($p < 0.05$) after 20 min and elevated levels were sustained for an additional 10 min. LH secretion gradually returned to pre-NAL levels and the basal LH secretion pattern was seen as in control saline-treated rats for the remainder of the NAL infusion period. In long-term castrated rats, saline infusion did not influence episodic LH secretion in any fashion through the 3 h infusion period. Quite unexpectedly, NAL infusion produced a bimodal LH response - increase followed by sustained decrease. Within 15 min after NAL infusion, LH rose significantly ($p < 0.05$), elevated levels were observed for another 15 min. LH secretion gradually returned to pre-NAL infusion levels and thereafter, despite continuous NAL infusion, it slowly decreased and stayed below the control castrate levels ($p < 0.02$) during the remaining 105 min infusion period. These studies show that (1) a sustained curtailment of opioid influence induced an immediate excitation of LH release, the magnitude of LH response was quite similar in intact and long-term castrated rats, (2) in addition, in castrated rats continuous NAL infusion decreased LH release. Since our previous studies showed a marked decrease in the hypothalamic LHRH content of castrated rats, it is likely that the attenuation of NAL-induced LH release observed in these rats may be due to exhaustion of releasable stores of LHRH after initial hypersecretion, and (3) contrary to previous evidence based on bolus NAL injection, these studies show that a tonic EOP inhibitory influence may exist in intact as well as castrated rats. (Supported by NIH HD 11362).

143.8 MORPHINE EFFECTS ON [^{3}H] THYMIDINE INCORPORATION INTO NEONATAL RAT BRAIN DNA. H.I. Kornblum*, S. Loughlin and F.M. Leslie. Department of Pharmacology, California College of Medicine, Irvine, CA 92717.

The early appearance of opioid peptides and receptors in the developing brain has led to the hypothesis that these systems may play a role in neuronal development. One possible function is control of brain growth. Chronic administration of the opioid antagonist, naltrexone to neonatal rats causes an increase in brain size (Zagon,I. and McLaughlin,P.; Science, Vol 221, 1983, 1179-80.) Furthermore, opioid peptide immunoreactivity has been detected in the germinal zone of the lateral ventricle of rats 1 to 5 days of age (Loughlin,S., Massimiri,T., Kornblum,H. and Leslie,F., Neuropeptides, Vol 5, 1985, 469-472), and in the proliferative zone of the cerebellum of 10 day old rats (Zagon,I., Rhodes,R. and McLaughlin,P., Science, Vol. 227, 1985, 1049-1051.) In order to test the hypothesis that opioids control cellular proliferation within the brain, we have examined the effects of morphine on [^{3}H] thymidine incorporation into brain DNA of one day old rats. Pups were injected with morphine (5mg/kg) followed 30 minutes to four hours later by [^{3}H] thymidine (1µCi/gm). Animals were sacrificed 30 minutes following the last injection. Brains and spleens were removed and homogenized in 1 M NaOH. DNA was extracted by acid precipitation and hydrolysis in 10% $HClO_4$ (PCA). A sample of homogenate was taken prior to DNA extraction in order to determine [^{3}H] thymidine availability to the target tissue. Morphine administration depressed [^{3}H] thymidine incorporation in brain by approximately 40 %. This effect was observed at the shortest (30 min) and longest (4 hr) time periods of treatment examined and was not due to decreased availability of [^{3}H] thymidine. Inhibition of brain DNA synthesis by morphine was antagonized by pretreatment with 5 mg/kg naloxone. Naloxone alone had no effect on DNA synthesis. Morphine did not depress incorporation into spleen DNA, although it did decrease the availability of [^{3}H] thymidine to spleen. In order to test the hypothesis that morphine inhibits DNA synthesis by direct action on proliferative cells within the germinal zone we have also examined the effects of morphine on [^{3}H] thymidine incorporation into brain slices. Brains were removed, sectioned coronally into .5mm coronal slices and incubated in an oxygenated Kreb's solution containing glucose. Incubations were performed in the presence or absence of morphine for varying periods of time followed by [^{3}H] thymidine (5 µCi/ml) for thirty minutes. Following incubations, the slices were collected and DNA was extracted. Morphine, in doses of .1 to 10 µM, had no effect on *in vitro* [^{3}H] thymidine incorporation into brain DNA over the time periods examined (.5 to 4 hr.) These results indicate that morphine administration *in vivo* inhibits DNA synthesis in neonatal rat brain. However, this effect may not be directly on proliferative cells in the germinal zone. (This work was supported by NIH grant NS 19319.)

143.9 TAIL-FLICK LATENCIES IN RATS ARE MODULATED BY NONNOCICEPTIVE PSYCHOLOGICAL FACTORS. R. Dantzer*, A. Tazi* and M. Le Moal (SPON: D. Beaubaton). INSERM U-259 Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

Variations in tail-flick latencies following exposure to a wide variety of stressors have been interpreted as reflecting activation of endogenous pain suppression mechanisms by stress. The present experiments were undertaken to investigate the possibility that this phenomenon reflects not only the physical properties of stressors, but also their psychological aspects, by varying the ability to control and predict reinforcement contingencies in food deprived rats.

Experimental and yoked animals differed in their possibility of controlling the delivery of food by lever pressing (controllability), predicting its occurence thanks to a prefood signal (predictability) or developing adjunctive activities (schedule-induced polydipsia, SIP).

In the controllability experiment, six experimental rats were submitted to a continous reinforcement schedule and six yoked animals received concomitantly the same amount of food. Over 6 successive daily training sessions, post-session tail-flick latencies were higher in yoked than in experimental animals (5.25 versus 4.68 sec, $p<0.01$, mean pre-session tail-flick latency : 4.06 sec). Presentation of massed food or withdrawal of reinforcement (extinction) induced a significant increase in post-session tail-flick latencies of experimental animals.

In the predictability experiment, 12 rats were exposed to a variable-time 60 sec schedule of food reinforcement with a 10 sec light signal predicting the delivery of food or unrelated to delivery of food. Over 9 training sessions, animals submitted to unsignaled food developed higher post-session tail-flick latencies than animals exposed to signaled food (4.53 versus 4.01 sec, $p<0.01$; mean pre-session tail-flick latency : 3.85 sec).

In the SIP experiment, 27 rats were submitted to a fixed time 1 min schedule of food reinforcement with (n=18) or without (n=9) water bottle. Nine out of 18 rats developed consistent drinking (about 10 ml in 30 min session) within 9 sessions. They had lower pre-session tail-flick latencies than rats which did not develop drinking (3.68 vs 4.33 sec, $p<0.01$). Development of SIP was accompanied by a significant reduction of the difference between post- and pre-session latencies so that SIP rats had lower post-session tail-flick latencies than animals without access to water (3.88 vs 4.11 sec, $p<0.01$). Removal of the water bottle induced a significant increase in tail-flick latencies of polydipsic animals.

These results demonstrate for the first time that tail-flick latencies are very sensitive to psychological aspects of nonnociceptive stressors.

143.10 VASOCONSTRICTOR ACTION OF METHIONINE AND LEUCINE ENKEPHALIN GIVEN INTRAVENOUSLY TO UNRESTRAINED RATS. J.A. Thornhill and W.S. Saunders*. Department of Physiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

Recent studies have shown that endogenous opioids (methionine enkephalin, ME and leucine enkephalin, LE) are co-released from the adrenal medulla along with catecholamines upon splanchnic innervation of the tissue. This finding has prompted investigators to seek out the possible physiological action of the adrenal enkephalins, including their possible modulatory role in affecting blood pressure regulation. The current study was undertaken to first determine the blood pressure (BP) responses of unanesthetized, unrestrained rats to intravenous (iv) administration of ME and LE and the possible mechanism(s) of action responsible for the resultant BP response. Under sodium pentobarbital anesthesia, male Sprague-Dawley rats had their femoral artery and veins cannulated for subsequent BP monitoring and drug administration, respectively 24-48h later. Random iv injections of ME or LE acetate (1.6, 16 or 48 nmoles) to unrestrained rats caused a rapid but brief dose-related pressor response (greater with LE) while heart rate (HR) was not changed from pre-injection control values. These responses were similar qualitatively to iv administration of norepinephrine (NE) HCl (.48, 2.4 and 4.8 nmoles) although NE caused a greater reflex bradycardia. Combination iv injections of NE (2.4 nmoles) with ME or LE (16 nmoles) did not significantly change the BP and HR responses from that when NE (2.4 nmoles) was given alone. Pretreatment antagonist studies were then conducted using atropine sulphate, propranolol HCl, phentolamine HCl, naloxone HCl and diprenorphine HCl in order to determine the possible receptor mechanism(s) involved in the mediation of the pressor response to LE and ME. IV injection of the antagonists (10 µg -> 1 mg/ml) 10 min before iv LE, ME (16 nmoles) or NE (2.4 nmoles) revealed that the pressor response of rats to LE and NE could be attenuated by phentolamine (α-receptor antagonist). Both naloxone and diprenorphine (mu and delta opiate receptor antagonists) caused a slight blockade of the pressor effect of LE. Antagonism of the pressor response to ME was not achieved with the doses used of any of the receptor blockers tested. The results suggest that LE and ME given intravenously causes an increase in BP in unrestrained, unanesthetized rats with no associated rise in heart rate. The pressor response elicited by LE is probably caused by vasoconstriction of the peripheral vasculature.

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143.11 μ AND k OPIATE RECEPTORS MEDIATE SPECIFIC BEHAVIOURAL SYNDROMES FROM THE SUBSTANTIA NIGRA.
M. Morelli and G. Di Chiara. Institute of Exp. Pharmacology and Toxicology, University of Cagliari, Cagliari, Italy.

Recent studies have indicated the presence of μ and k opiate receptors in the basal ganglia: μ receptors are primarily activated by morphine, while k receptors are activated by dynorphins. Moreover these subtypes of opiate receptors display different sensitivity towards naloxone antagonism, μ receptors being 10 to 20 times more sensitive than k receptors to its antagonism. No experimental evidence is available as far as regards the physiological role of these receptor subtypes in the basal ganglia; therefore we studied the effect of local injections, into the rat substantia nigra, of different compounds acting specifically on μ and k receptors. Agonists of μ receptors like morphine and different types of β-Casomorphins, when injected bilaterally into the substantia nigra produced stereotyped behaviour characterized by hypermotility, sniffing and gnawing depending on the doses used. Low doses of naloxone (0.5 mg/kg s.c.) effectively antagonize the gnawing but only high doses (10 mg/kg s.c.) were able to antagonize the hypermotility and sniffing, suggesting an involvement of μ receptors in the mediation of gnawing but not in the mediation of hypermotility and sniffing. On the contrary the bilateral intranigral injections of different types of dynorphins or k-agonists like bremazocine and U 50-488, which are known to be more specific than dynorphin at the k receptor, produced dose-dependent hypermotility and sniffing, but were unable to produce gnawing even at doses 10 times higher than those able to induce hypermotility and sniffing. High (10 mg/kg s. c.) but not low (0.5 mg/kg s. c.) doses of naloxone were able to antagonize this behaviour. These results suggest that gnawing is elicited by stimulation of μ opiate receptors in the substantia nigra while hypermotility and sniffing are due to stimulation of k receptors. Moreover, the administration of 0.05 mg/kg of haloperidol s.c. which blocks the dopaminergic receptors, antagonize the hypermotility and sniffing produced by the intranigral injection of k and μ agonists but is unable to antagonize the gnawing behaviour produced by stimulation of μ receptors indicating that the hypermotility and sniffing is mediated by dopamine while gnawing is independent from it.

143.12 OPPOSITE EFFECTS OF μ AND k OPIATE AGONISTS ON DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS AND IN THE CAUDATE OF FREELY MOVING RATS.
A. Imperato* and G. Di Chiara. Institute of Exp. Pharmacology and Toxicology, University of Cagliari, Cagliari, Italy.

The opiate analgesics are known to produce a variety of pharmacological and behavioural syndromes such as analgesia, physical and psychological dependence, euphoria and sedation. μ-Receptors agonists, like morphine, show addicting properties in animals, while pure k-agonists are apparently devoid of this property. Moreover, k-agonists, elicit sedation, while μ-agonists in low doses produce behavioural excitation. Brain dopamine has been related both to the reinforcing and the motor stimulating properties of opiates, but the evidence supporting this, is essentially indirect. Recently we have developed the technique of trans-cerebral dialysis for estimating the release and metabolism of DA in awake, freely-moving rats. Using this procedure we have studied the effect of morphine, μ-agonist, and bremazocine and U 50,488, k-agonists, on the in vivo release and metabolism of DA and we have correlated it, to the spontaneous behaviour of the animals. Systemic administration of a low dose of morphine (1.0 mg/kg s.c.) significantly stimulated DA-release in the n.accumbens but not in the caudate. DA-release in the accumbens was maximally stimulated (30% basal values) already 20 minutes and up to 80 min after administration. This dose of morphine elicited behavioural stimulation characterized by grooming, hypermotility and rearing. Administration of a low dose of naloxone (0.1 mg/kg s.c. 15 min before morphine) completely prevented the stimulation of DA-release as well as the stimulation of behaviour. Higher doses of morphine (5 mg/kg s.c.) produced frozen postures and immobility for about one hour, followed by behavioural stimulation for the next 2hr 20 min. DA-release increased maximally by about 80% in the accumbens and by about 50% in the caudate. After this dose of morphine, DA-release stimulation was initially associated with behavioural inhibition but out lasted it, and continued for the whole duration of the behavioural stimulation. In contrast to morphine, a dose of 5.0 mg/kg s.c. of the highly selective k-agonist U 50,488, produced a rapid and pronounced reduction of DA release in the accumbens and in the caudate. This dose of U 50,488, in agreement with the literature, reduced motility. Both the behavioural and the biochemical effects of U 50,488 were unaffected by low doses of naloxone (0.5 mg/kg s.c.) while rather large doses of naltrexone (5 mg/kg s.c.), a pure opiate antagonist were able to reverse the inhibitory effects of U 50,488 on behaviour and on DA-release in the accumbens and in the caudate. The present results provide evidence for the idea that the differential effects of μ and k opiate agonists on behaviour are dependent upon a differential effect on mesolimbic and mesostriatal DA-system. In particular our results suggest that the lack of behavioural stimulant properties and the low abuse liability of k-opiate agonists is related to the lack of stimulation of the mesolimbic DA system.

144.1 PURSUIT AND SACCADE ASYMMETRIES IN LATENT NYSTAGMUS. T.C. Hain*, S.E. Kelman*, and D.S. Zee. Dept. of Neurology, Johns Hopkins Hospital, Baltimore, Maryland 21205

We recorded eye movements (search coil) during monocular viewing in 3 patients with latent nystagmus (LN), congenital esotropia, and amblyopia. All patients had a conjugate nystagmus with the slow phase of the viewing eye directed toward the nose. The nystagmus changed direction when the patient moved a cover from one eye to the other, even in complete darkness. Slow phase velocities ranged from 2 to 6 deg/sec.

For pursuit tracking (triangular-wave, 30 deg/sec), viewing with the eye with better acuity evoked gains (peak eye vel/target vel) when the target was moving in the direction of LN drift that were 1.2-1.4x higher than gains for tracking against the drift. When tracking with the more amblyopic eye, gains were 2-4x higher for tracking in the direction of the drift than against the drift. For tracking step-ramp stimuli, similar directional asymmetries in gain and initial pursuit acceleration were seen. The direction of the initial acceleration of the eye and of the ramp were the same except when the step was in the direction of the drift and the ramp was against the drift. Then the eye initially accelerated in the direction of the step (e.g. 20-50 deg/sec^2 for a step of 3 deg and ramp of 10 deg/sec), followed about 40 msec later by an acceleration in the direction of the ramp.

Saccades in the direction of the drift were composed of a series of saccades with brief (20-100 msec) intersaccadic intervals during which the eye drifted onward with high velocity (e.g. 25-50 deg/sec). Saccades directed opposite the drift were larger, usually single and had a backward post-saccadic drift resembling dynamic overshoot. For example, in one patient, for 40 deg target steps with L eye viewing (drift to R) the average primary saccade to the R was 24.0 deg, to the L, 37.6 deg. With R eye viewing the average primary saccade to the R was 35.2 deg, to the L, 20.0 deg. Peak saccadic velocities were normal and appropriate to saccade size regardless of direction.

In conclusion, in LN there are large directional asymmetries in pursuit gain, initial pursuit acceleration, saccadic accuracy and post-saccadic drift. These asymmetries change direction according to which eye is viewing and consequently according to which direction the eyes are drifting. Also, during step-ramp tracking, when the step is in the direction of the drift (onto temporal retina) there is an initial smooth acceleration directed toward the position of the step even when the ramp is directed oppositely. Whether these directional asymmetries in saccade and pursuit tracking reflect differences in processing of visual information from nasal versus temporal retina or are determined by the underlying direction of drift of LN remains to be discovered.

144.2 EYE-HEAD COORDINATION: HOW ANIMALS HOLD THEIR NECKS. A. Berthoz, W. Graf, and P.P. Vidal. Lab. Physiologie Neurosensorielle, C.N.R.S., Paris, France; The Rockefeller University, New York; NYU Medical Center, New York (SPON: J.I. Simpson).

The orientation of the cervical vertebral column in the sagittal plane was determined quantitatively by X-ray photography of the head-neck region of nine unrestrained and awake species of vertebrates (man,monkey,cat,rabbit,guinea pig,rat,chicken,frog,lizard). In addition, the orientation of the horizontal semicircular canals was measured in three species from landmarks at the skull whose spatial relationship to the canals is known. In all vertebrates studied, with the exception of frog and lizard, the general orientation of the curved cervical vertebral column was vertical when animals were at rest, and not horizontal or oblique as suggested by the macroscopic appearance of the neck. The posture of the animal, whether lying, sitting or standing had little effect on this general vertical orientation, although some variability was noticed depending on the species. This variability prompts the definition of a resting zone, rather than of a resting position. The cervical column can take any orientation within ± 11° of a mean position. The objective vertical direction (gravity vector) was always within this resting zone. The vertical orientation of the cervical column causes the odontoid process of the axis (C2) to be oriented vertically, which thus provides the axis of rotation for yaw movements of the head. This axis is identical to that of the horizontal semicircular canals. However, at rest, animals did not hold their heads with the horizontal canals oriented earth horizontally all the time, but maintained them pitched up by 5 - 15°, as also reported for man. At other times, presumably when the vigilance level increased, the horizontal canals were brought into the earth horizontal plane. - The cervical vertebral column composes part of the S-shaped structure of the entire vertebral column, with one inflection around the cervico-thoracic (C7/Th1) junction. This particular configuration is interpreted to provide mechanical advantages by functioning as a shock absorber through its curvature, and by introducing a stable, energy saving balance of the head. Furthermore, when the head is lowered or raised, the atlanto-occipital and cervico-thoracic junctions are predominately involved, while the entire cervical column largely preserves its intrinsic configuration. - The vertical organization of the cervical vertebral column in birds and mammals, whether the animal is quadrupedal or bipedal points to a common organizational principle of head movement and oculomotor systems, including common neuronal elements for gaze control and related reflexes (vestibular, optokinetic, cervical), with collinearity of the rotational axes of the head around the odontoid process, the horizontal semicircular canals, and the horizontal extraocular muscles.

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144.3 SELECTIVE LABELLING OF BRAINSTEM AFFERENT NEURONES TO THE CAT ABDUCENS NUCLEUS BY RETROGRADE TRANSPORT OF [^{3}H]-GLYCINE. R.F. Spencer and R. Baker. Dept. Anat., Med. Coll. of Virginia, Richmond, VA 23298, and Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Neurones that utilize a specific neurotransmitter possess high affinity uptake and transport mechanisms that provide the basis for transmitter-specific retrograde labelling (Streit, 1980). In the present study, the localization and distribution of brainstem afferent neurones to the cat abducens nucleus labelled by retrograde transport of [^{3}H]-glycine has been compared to that of neurones retrogradely labelled by horseradish peroxidase (HRP).

Microiontophoretic injections of HRP into the abducens nucleus label neurones located in the ipsi- and contralateral medial vestibular nucleus (MVN), prepositus hypoglossi nucleus (PHN), oculomotor nucleus and overlying supraoculomotor region, and pontomedullary reticular formation (RF). Similar injections of [^{3}H]-glycine, however, selectively label by autoradiography only neurones located in the ipsilateral, and occasionally contralateral, MVN, and contralateral PHN and dorsomedial RF. In the ipsilateral MVN, small-diameter neurones are concentrated in the dorsomedial region lateral to the internal genu of the VIIth nerve, while medium-diameter neurones are located in the ventrolateral part of the nucleus. Small-diameter neurones in the contralateral PHN are located ventral in the nucleus, extending into the underlying RF. Near the caudal portion of the abducens nucleus, a third population of small- and medium-diameter neurones is observed in the contralateral dorsomedial RF, corresponding to the location of inhibitory burst neurones. The selectivity of uptake and retrograde transport of [^{3}H]-glycine is indicated by the absence of labelling of this third population of neurones ipsilateral and in close proximity to the injection site, where local uptake by diffusion could have occurred. The specificity of uptake and transport is demonstrated by the absence of retrograde labelling following injections of [^{3}H]-GABA or [^{3}H]-leucine into the abducens nucleus.

The findings suggest that glycine may be the putative inhibitory neurotransmitter of neurones that are related to the horizontal vestibulo-ocular and saccadic eye movement systems. By contrast, GABA may be the neurotransmitter of second-order inhibitory vestibular neurones that are related to the vertical semicircular canals and that establish synaptic connections with oculomotor and trochlear motoneurones.

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144.4 A COMPARISON OF CONNECTIONS OF THE PRIMARY OCULOMOTOR CORTEX (FRONTAL EYE FIELD) AND PRIMARY HAND/ARM MOTOR CORTEX (DORSOLATERAL PRECENTRAL GYRUS) IN THE MONKEY. G. R. Leichnetz. Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Bruce and Goldberg (1985) have shown that the frontal eye field (FEF) cortex of the rostral bank of the arcuate sulcus contains neurons which fire prior to saccadic eye movements, and further that large amplitude saccades are elicited from microstimulation of dorsal area 8 and smaller saccades from more ventral area 8. Eye movements were produced with currents as low as 8 μa and latencies as short as 25 msec. Schlag and Schlag-Rey (1985) have shown that a region in the dorsomedial prefrontal cortex (a possible supplementary FEF) has similar oculomotor characteristics. This pre-oculomotor physiology led us to ask whether area 8 should be regarded as the primary oculomotor cortex, and whether its connections, previously studied, resemble those of the hand/arm area of the dorsolateral precentral gyrus, area 4. We, therefore, made horseradish peroxidase (HRP) gel implants in area 4 and used the TMB protocol of Mesulam (1978) to define its afferents and efferents.

While area 8 receives its principal cortico-cortical inputs from inferior parietal (PG), superior temporal sulcal, cingulate, prestriate (MT), and medial parietal (PGm) cortices, area 4 receives its inputs from supplementary motor cortex, postcentral, superior parietal, inferior parietal (PF) and cingulate cortices. The ipsilateral SMA and area 5 connections were heavily reciprocal and columnar (retrogradely labelled lamina III cells were heaviest, but lamina V cells were also observed). Area 8 received a moderate input from the middle claustrum and basal amygdaloid nucleus, but area 4 received only a small input from the dorsal claustrum and no projections from the amygdala. Area 8 had heavier thalamic connections with paralaminar mediodorsal, ventral anterior and medial pulvinar nuclei, whereas area 4 had heavy connections with a vertical serrated configuration of VL and VPLo, and centromedian intralaminar nucleus. Both regions received considerable input from the central lateral intralaminar nucleus. The two regions also shared common inputs from the nucleus basalis of Meynert, lateral hypothalamus, locus ceruleus, superior central and dorsal raphe nuclei. The FEF projected to the nuclei of Darkschewitsch and Bechterew, whereas area 4 had a very heavy projection to ND but not NB. Area 4 showed a prominent projection field in the ventral parvicellular red nucleus. Area 8 projected to the ipsilateral dorsomedial basilar pons and bilaterally to pontine tegmentum, while area 4 had a more extensive circumferential corticopontine projection. Area 4 projections contributed to the medullary pyramidal tract and to the cervical spinal cord, whereas area 8 did not.

144.5 CONNECTIONS OF THE PHYSIOLOGICALLY-DEFINED FRONTAL EYE FIELD IN SQUIRREL MONKEYS. M. F. Huerta, L. Krubitzer, and J. H. Kaas, Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Combined physiological and anatomical methods were used to study the connections of the frontal eye field (FEF) in squirrel monkeys. Contralaterally-directed saccadic eye movements were elicited by intracortical microstimulation (40 msec train of biphasic 25-300 μA, 0.5 msec pulses at 300 Hz) within the oval-shaped FEF (1 x 1.5 mm) near the inferior arcuate dimple. Adjacent to the FEF, stimulation of up to 300 μA did not produce eye movements, but resulted in other movements, typically of the vibrissae. Borders of the FEF were marked with small electrolytic lesions, and injections of horseradish peroxidase conjugated to wheat germ agglutinin were placed in this field. After perfusion, cerebral cortex was separated from the brainstem, unfolded, flattened, and sectioned parallel to the flattened suface; the brainstem was cut coronally. Sections were stained for Nissl substance, myelin, cytochrome oxidase or reacted with TMB. Injection sites were confined to the physiologically-defined FEF.

The FEF is reciprocally connected with three zones in the ipsilateral frontal lobe. One of these is located 2 mm rostral, another 2 mm medial, and another 1 mm caudal to the FEF. Reciprocal ipsilateral connections are also present with a region in the medial parietal lobe on the ventral bank of the caudal cingulate sulcus, a region in the inferior parietal lobule on the ventral bank of the lateral fissure and a region in the temporal lobe on the rostral bank of the medial superior temporal sulcus. Callosal connections are with the FEF and surrounding cortex. Finally, the FEF is connected with numerous subcortical structures which have been implicated in eye movement functions including the paracentral, medial dorsal, ventral anterior and pontine nuclei, layer IV of the superior colliculus, and the paramedian pontine reticular formation. The location of the physiologically-defined FEF varied with respect to sulcal patterns. Thus, connectional studies are optimized by combining physiological and anatomical methods.

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144.6 FUNCTIONAL PROPERTIES OF TECTAL PROJECTION NEURONS IN THE MONKEY FRONTAL EYE FIELD. M. A. Segraves and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, Maryland 20205.

The monkey's arcuate frontal eye field (FEF) is the primary cortical area responsible for the control and initiation of purposive saccades. Bruce and Goldberg have recently shown that 54% of the neurons examined in the FEF discharged before eye movements. Forty percent of the presaccadic neurons examined in detail responded exclusively to visual stimuli, 20% discharged before purposive saccades regardless of the presence or absence of a visual stimulus, and 40% exhibited a combination of visual and movement responses. In the present study we have examined the activity of FEF neurons antidromically activated from the superior colliculus (SC) to determine the relative contributions of the various FEF neuron types to the output of the FEF.

In 2 awake behaving monkeys, 46 FEF neurons were antidromically activated by a monopolar microelectrode positioned within the ipsilateral SC using single negative-first biphasic pulses, 0.4 msec in duration. Current thresholds for antidromic activation varied from 6 μA to 1 mA, and the latency from 1.2 to 6.0 msec. Antidromic activation was verified using collision criteria.

Compared to the entire population of FEF neurons sampled by Bruce and Goldberg, the activity of a disproportionately large number of neurons that project to the SC was movement related (24 neurons, 52%). Eighteen of these neurons exhibited exclusively movement related responses, the remaining 6 also had a visual component in their activity. In addition, there was an unusually large number of neurons (13 neurons, 28%) with foveal receptive fields and/or anticipatory activity presumably important for the triggering process important for saccade initiation. The remaining 9 neurons did not fit the above categories. Their responses included visual, postmovement, tracking, and reward related activity. Only one unit was undrivable.

Testing at 250μ intervals with the stimulating electrode localized the site of lowest threshold for antidromic activation to the intermediate layers of the SC for the majority of neurons. The lowest threshold was obtained in the superficial layers for a single visual neuron, and was below the intermediate layers for a few additional neurons. Antidromically activated neurons were usually clustered within a narrow range in depth in a single penetration. Microlesions have localized these neurons to layer V. Eye movements were electrically evoked at low threshold from these FEF recording sites.

We conclude that the robust projection from FEF to SC does not provide substantial visual information; instead its primary purpose is to provide eye movement targeting and triggering information for the guidance of purposive saccades.

144.7 IMPAIRED TRACKING AND LOSS OF PREDICTIVE EYE MOVEMENTS AFTER REMOVAL OF THE FRONTAL EYE FIELDS. E.G. Keating, S.G. Gooley*, and D.V. Kenney*. Department of Anatomy and Cell Biology, SUNY-Upstate Medical Center, Syracuse, N.Y. 13210

Pathology of the frontal eye fields (FEF) slows visual search, makes it difficult to orient the eyes away from visual targets or foveate remembered target positions. The notion drawn from these findings is that the FEF supplies to eye movements a command derived from internal cognitive decisions. Foveal pursuit of moving targets often involves such an internal component, a prediction about the target's trajectory that is drawn from experience.

Two normal monkeys (M. mulatta) learned to pursue a target moving horizontally across a screen. The targets were sinusoidal (amplitude = 9.0°; freq. = 0.4 - 1.4 HZ.) or had a constant velocity (20-90°/sec). Repeated presentations of the same target trajectory produced predictive eye movements. The monkeys' pursuit sometimes preceded the target's motion. At occasional probe points the target was made to disappear for an interval or to move in unexpected ways. At 38% of these probe points the eyes moved away from the actual target and in the direction of the expected target trajectory. Both smooth and saccadic predictive movements were observed. The velocity of these smooth epochs and the amplitude of the saccades indicated that the monkeys were predicting both the direction and velocity of the target.

Ablation of FEF, first in one hemisphere and later bilaterally, degraded foveal pursuit. Tracking of targets that moved toward the lesioned side became increasingly saccadic. The gain of the remaining pursuit epochs (saccades removed) was reduced even for the low velocity sine and ramp targets. Some recovery occurred by 3 weeks after surgery, but reduced gain of the higher velocity targets persisted for the maximum 2.5 months of observation. The table shows for one subject the average gain of pursuit of two sine targets before and one week after removal of the left, then right FEF.

Targ. Freq.	Preop	l. FEF	r.FEF
0.4 Hz	.97	.87	.56
1.4 Hz	.79	.47	.20

Predictive eye movements were also reduced to a rarity for 1-2 wks following surgery. They returned thereafter but occurred uncommonly and were of small amplitude and short duration. The loss of predictive movements was not simply a reflection of the general lowering of pursuit gain. Predictive saccadic movements were also curtailed even though saccadic amplitude was not otherwise impaired by FEF ablation (USPHS EY04005).

144.8 FAILURE IN RECOVERY OF VISUALLY-GUIDED SACCADES IN HEMIDECORTICATED MONKEYS. R.J. Tusa, Johns Hopkins University, Baltimore, MD 21205.

Visually-guided saccades have been reported to recover in monkeys and human beings with lesions in striate cortex (blind sight). Although this recovery is believed to be mediated by the superior colliculus, little is known about the role of extrastriate cortex in this recovery process.

In this study visually-guided saccades were examined in 3 trained juvenile rhesus monkeys following hemidecortication. A 0.25 deg target light at an intensity of 3.0 ft L with a background intensity of 0.1 ft L was used (recovery of visually-guided saccades has previously been reported in monkeys with bilateral occipital lobectomies using this target light in our lab: Zee et al., 1982). In addition, a very bright target light was used in one of the monkeys (2,000 ft L, background 1 ft L).

Three different saccade paradigms were used: 1) Target Steps of 5-40 degs; 2) Step-Ramp target trajectories where the target was first stepped and then ramped 5-60 deg/sec; 3) Express Saccade paradigm where a 200 msec time gap occurred between the fixation light offset and the onset of the target light.

All 3 monkeys have been examined at least 6 months post-op. Accurate saccades were always made to targets stepped into the normal visual hemifield and latencies were at pre-op values for paradigms 1 and 2 and less than 100 msec for paradigm 3.

For targets stepped into the blind hemifield during all three paradigms, saccades were always made in the appropriate direction, but saccadic amplitudes were unrelated to target step size and mean saccadic latencies were 231 msec longer than pre-op. These saccades are likely to be searching saccades triggered by the disappearance of the fixation light as they are no different from saccades made when the fixation light was turned off without the onset of a target light (blank trials). Although saccadic amplitudes did not depend upon target step size, they did depend upon orbital positions of the eye prior to the saccade with the largest saccades evoked from orbital positions ipsilateral to the lesions.

In conclusion, subcortical regions are not sufficient for recovery of visually-guided saccades to stationary or moving targets in hemidecorticated monkeys. Zee, D.S., et al. Soc. Neurosci. Abstr. 8:291, 1982.

144.9 A QUANTITATIVE STUDY OF VISUAL PURSUIT DEFICITS FOLLOWING LESIONS OF THE FRONTAL EYE FIELDS IN RHESUS MONKEYS. J.C. Lynch and J.C. Allison*. Department of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216.

The frontal eye fields (FEF) have been implicated in the control of saccadic eye movements by electrical stimulation and single-neuron recording studies. However, experimental destruction of the FEF has consistently produced only mild deficits in the performance of saccadic eye movements. Furthermore, only a small percentage of neurons in the FEF are active during saccades, many have sensory receptive fields, and some are active during pursuit eye movements or head movements. Few quantitative studies of eye movement performance following FEF lesions have been performed. In order to investigate the effect of frontal eye field lesions on a broad range of eye movement tasks, two rhesus monkeys were trained on a group of visual fixation tasks, visually-evoked saccade tasks, and visual pursuit tasks. The monkeys were trained to fixate and follow a 0.1° target projected onto a 52° square screen. Oculomotor performance was measured during horizontal and vertical saccades ranging from 8° to 24° in amplitude; during visual pursuit of targets moving in the horizontal and vertical planes at velocities of 20°/sec to 40°/sec; and during the steady fixation of stationary targets at various locations on the screen. Eye position was monitored using chronic EOG electrodes. After performance on the tasks became reliable, bilateral surgical removal of the anterior bank of the arcuate sulcus and the banks of the posterior third of the principal sulcus was performed under sterile conditions using pentobarbital sodium anesthesia. The major finding of this study is that bilateral lesions of the FEF produced a severe deficit of visual pursuit performance in both the horizontal and vertical planes, whereas saccade performance was only mildly affected. For several weeks following the lesion, monkey R-5 was unable to match eye velocity to target velocity and could follow the target only by means of a series of small saccades when the target moved 20°, 30°, or 40°/sec. Eight days after the lesion, pursuit gain was 0.18 at 20°/sec and 0.12 at 40°/sec. Thirty-six days after the lesion, pursuit gain had increased to 0.51 at 20°/sec and 0.60 at 40°/sec. Horizontal pursuit performance of monkey R-6 was similar. Vertical tracking was tested in monkey R-6 and showed defects comparable to those seen in horizontal pursuit. Horizontal saccade velocity for 20° or 24° saccades decreased by about 10% following the lesions, while saccade latency increased by about 30%. These changes are similar to those produced by bilateral parieto-occipital cortex lesions. Vertical saccade performance was less severely impaired. These results suggest that the frontal eye fields are involved in the control of visual pursuit movements to a greater extent than has heretofore been recognized. [Supported by grants from NIH (EY-04159) and the Vaughan Stroke Research Trust]

144.10 UNEQUAL SACCADES DURING CONVERGENT EYE MOVEMENTS IN THE MONKEY. P.D.R. Gamlin and L.E. Mays, Dept. of Physiological Optics and the Neurosciences Program, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Studies in humans have demonstrated that saccades occurring during convergent eye movements can be markedly unequal in amplitude. For example, if a rightward saccade occurs during a convergent movement, the saccade in the left eye may be twice as large as the saccade in the right eye. This saccadic inequality is too large to be explained by the linear addition of the saccadic and convergent movements. We have observed a similar phenomenon in monkeys. These unequal saccades might result from saccadic size being programmed independently for each eye, or they might arise from non-linear interactions at the level of the oculomotor nucleus or plant. We have used two approaches to examine these possibilities in the monkey.

First, we trained monkeys to make symmetrical convergent movements in response to step changes in disparity. We were also able to produce predominately horizontal saccades by electrical stimulation of the superior colliculus. Neither the timing nor the occurrence of this stimulation could be predicted by the animal. Stimulation just before or after a convergent movement produced saccades of equal amplitudes. In contrast, when the colliculus was stimulated during a symmetrical convergent movement, saccades of different amplitudes were elicited in the two eyes.

In a second experiment, we recorded from vergence cells in the midbrain during both symmetrical convergent movements and those in which unequal saccades occurred. For symmetrical vergence, these cells exhibit a firing rate that is closely coupled to vergence angle (Mays, L.E., J. Neurophysiol., 51:1091, 1984). If the saccadic system were mediating changes in vergence by programming unequal saccades, then these cells might be expected to show a smaller change in firing rate for convergent movements in which unequal saccades occurred. However, we found that the behavior of these cells was similar regardless of the dynamics of the vergence movement.

These results suggest that the large inequalties observed in saccades during vergence eye movements are not the result of central programming. Rather, they appear to result from non-linear interactions at the level of the motoneurons and, or alternatively, as suggested by Kenyon et al. (Am. J. Optom. Physiol. Opt. 57:586, 1980), at the level of the oculomotor plant. (Supported by NIH grants EY03463 & EY03039)

144.11 QUANTITATIVE FEATURES OF PERCEIVED TARGET MOTION DURING SMOOTH PURSUIT EYE MOVEMENTS. J. Pola and H.J. Wyatt. Institute for Vision Research, SUNY State College of Optometry, NY, NY 10010.

During smooth pursuit tracking of a moving target in an otherwise dark environment, one perceives motion of the target. Since no target-background relative motion occurs (because of the dark), and there is little target motion at the retina, it is assumed that this perception comes from some sort of extraretinal signal, contingent on eye movement. Recently, it has also been suggested that such perception, perhaps arising from a corollary discharge, may be involved in the control of pursuit movements via positive feedback (Yasui & Young 1975; Wyatt & Pola 1979).

In spite of the role that this perception may play in normal pursuit, little is known about its quantitative features. We have performed the following experiment: To investigate the influence of an extraretinal signal on perception and avoid contaminating effects of retinal target motion, we used a complex stimulus. This consisted of a vertical bar within a square (4 x 4 deg), the bar jumping from side to side of the square at a given frequency. The bar-plus-square was retinally stabilized and centered at the fovea, so that the bar jumped from side to side of the fovea. Subjects, in an otherwise dark room, attempted to look at the jumping bar. The result of this was oscillatory smooth pursuit (to the foveal offset of the bar) at the same frequency as the jumping bar, occasional saccades, and concomitant movement (due to stabilization) of the bar-plus-square in space. The subjects task was to assess the peak-to-peak amplitude of motion in space of the square (which did not move at the retina and was perceptually distinct from the bar), and to compare it to the amplitude of a similar stimulus observed subsequently in the light.

We were able to vary the frequency of smooth pursuit and concomitant stimulus motion by varying frequency of bar motion (0.5 - 1.5 Hz). We found that at the frequencies studied, the perception of amplitude of motion of the square was less than the amplitude of smooth pursuit. Furthermore, as frequency increased, the ratio of perceived motion to eye movement decreased. For all subjects this decrease in perception was characterized well by a second-order transfer function; that is, the extraretinal signal responsible appears to pass through a second order lag prior to registration in perception.

Perception of target motion was also determined as subjects tracked sinusoidal motion of a small round target (3 deg pk-pk, motion retinally stabilized at fovea). Since in this case the assessment of motion included retinal target motion, the ratio of perceived motion to eye movement differed from the first experiment, increasing notably at high frequencies. These results, together with the results of the first experiment (above) can be accounted for if it is assumed that the perceived motion involves a time advance of 150 msec in addition to a second order lag.

144.12 Smooth-Pursuit Eye Movement Related Visual and Visuo-motor Responses in Dorsolateral Pontine Nucleus of Alert Monkey. D.A. Suzuki, E.L. Keller and R.D. Yee*. Jules Stein Eye Inst., UCLA Sch. Med., Los Angeles, CA 90024 and Smith-Kettlewell Inst. Visual Sci., San Francisco, CA 94115.

Physiological recordings and behavioral studies of monkeys with pharmacological lesions in the dorsolateral pontine nucleus (DLPN) implicate this structure as an important source of sensori-oculomotor information for the smooth-pursuit eye movement system. The determination of visual receptive field structure and interactive, visuo-oculomotor mechanisms was sought to further clarify the role of the DLPN in the regulation of smooth pursuit.

Extracellular activity was recorded in alert monkeys during the performance of a battery of visuo-oculomotor tasks. Visual responses were elicited by presenting discrete and large field visual stimuli while the monkeys were required to fixate a stationary fixation spot. Retinal image motion- and eye movement-related DLPN activities were studied during the initiation and maintenance of smooth-pursuit eye movements and also during pursuit of a moving target that was superimposed upon a random dot background pattern.

Results to date indicate that visual responses in DLPN are characterized by five receptive field (RF) types: (1) small, foveally centered and (2) large RFs that encode retinal image velocity, (3) large, retinal image acceleration RFs, (4) large, direction-only (non-velocity related) RFs, and (5) small, eccentric RFs that respond to discrete, stationary stimuli. All visual responses to moving stimuli were direction selective.

The responses of DLPN cells with RF types 1 and 4 could best account for the specific changes in eye acceleration that were observed during pursuit initiation. Eye accelerations were largest when retinal images were close to the fovea and RF type 1 would be activated. In contrast, eye acceleration was smaller and less correlated with target velocity for the eccentric retinal image motion that would stimulate activity in RF type 4.

Some DLPN cells exhibited both retinal image and eye movement-related responses. In some of these units, the eye movement-related modulations in discharge rate were facilitated when the monkeys tracked the target in the presence of a random-dot background pattern.

The results lend single-unit support for the behavior based conclusion that foveal inputs are more effective than eccentric visual stimuli for pursuit initiation (Lisberger, NSAbst 8). The facilitated pursuit response during conditions of visuo-oculomotor interactions suggests a role for the DLPN in optimizing pursuit in the face of anti-phase, relative background motion.
(Supported by NIH Grant EY05167.)

145.1 IN VIVO REGENERATION OF RHODOPSIN AND PORPHYROPSIN IN THE NEURO-RETINA. J.M. Flores* and A.T.C. Tsin. Division of Life Sciences, The Univ. of Texas at San Antonio, San Antonio, TX 78285.

The neuroretina of goldfish (Carassius auratus) contains a pair of scotopic (rod) visual pigments - rhodopsin and porphyropsin. The composition of these visual pigments changes in response to light and temperature and the mechanism of this visual pigment change is not known. In several in-vitro experiments, Suzuki, Makino-Tasaka and Miyata (Vision Res., 25: 149-154, 1985) found that rhodopsin regenerated faster than porphysopsin and therefore suggested that the rate of visual pigment regeneration provided a possible mechanism of visual pigment changes. In the present study, we have examined the regenerations of goldfish porphyropsin and rhodopsin in-vivo. Goldfish with porphyropsin-rich retinas were obtained from Grassyfork Fisheries, IN. Goldfish with rhodopsin-rich retinas were obtained by subjecting fish to a specific light and temperature regimen (16L/8D, delivered by a 7.5W light bulb, water temperature: 30°C; Tsin and Beatty, Exp. Eye Res., 29: 15-26, 1979) for one month. Before the experiment began, goldfish were placed in a dark room overnight and then illuminated by bright light (two 300W, two 100W and one 150W bulbs, in a 30 gal. aquarium) for one hour to bleach all visual pigments. They were then returned to the dark room and sampled at these time intervals (0, 30, 60, 90, 120, 180, 240, 300, 600 minutes of dark adaptation, n=3 to 6). The neuroretina was dissected out under dim red light and placed immediately in 1:1 mixture of phosphate buffer (0.1M, pH=7) containing hydroxylamine (0.1M). The visual pigments were extracted by 2% digitonin in 0.1M hydroxylamine (pH=7) and then analysed by a spectrophotometer. The initial bright illumination bleached 80-90% of these scotopic pigments. The porphyropsin-rich retina (92% porphyropsin) required 10 hours to completely regenerate all its visual pigments (maximum rate of regeneration: 14nmol/kg/hr at 20°C) and this time course is in agreement with Baumann's in-vivo study of the regeneration of porphyropsin in the crucian carp (Carassius carassius) performed at a similar temperature (15°C) (Nature, Lond., 233: 484-485, 1971). However, we found that the rhodopsin-rich retina (62% rhodopsin) required only 5 hours to regenerate all rod pigments (i.e. 29nml/kg/hr, at 30°C), thus the regeneration of rhodopsin seemed faster than that of porphyropsin. (Supported by NIH Grant No. RR-08195-05 and NSF Grant No. BNS-82-03064.)

145.2 CIRCADIAN ALTERATION OF DARK ADAPTATION IN LIMULUS LATERAL EYE. L. Kass, Eastern Virginia Med. Sch., Norfolk, VA 23501

Photoreceptors in the lateral eye of Limulus polyphemus have been shown to exhibit circadian rhythms in sensitivity, gain (response/photon), and spontaneous (dark) "bump" rates.[1] These circadian rhythms are mediated by about a dozen efferent optic nerve fibers that synapse in the retina.[2] I report changes that occur from day to night on parameters associated with a quantitative description for the time course of dark adaptation.

Electroretinographic recordings (ERGs) were elicited from the lateral eye in situ using the methods of Barlow.[3] The animal was restrained and partially submerged in a sea water aquarium within a light-proof cage. Light was projected onto a diffusing screen directly in front of the lateral eye. The adapting light and the ERG test flash had durations of 3 sec and 50 msec, respectively. The ERG was conducted to a differential amplifier via a corneal electrode and was recorded on a computer diskette. The ERG amplitudes were compared with the intensity response function measured in the dark adapted state. In this way, retinal sensitivity as a function of time after the adapting light was obtained. This defines the time course for dark adaptation.

A computer program was utilized to curve-fit the data. A "best-fit" analysis revealed that the sensitivity vs. time functions measured both during the daytime and nighttime states could be well described by the following equation:

$$S = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$$

where S is the sensitivity (in log units of light intensity) at time t after the adapting flash. A_1 and A_2 are the coefficients for the two exponentials, whereas τ_1 and τ_2 are the time constants. At 4-5 log units of intensity above threshold for the ERG, τ_1 and τ_2 are about 40 and 500 sec, respectively. During the day, A_1 and A_2 are about 0.8 and 1.1, respectively. At night, A_1 decreases in size by about 30%, A_2 increases in size by about 40%, and changes in τ_2 were not apparent. Thus, dark adaptation takes longer to fully recover after an equivalent number of photons are delivered to the eye at night, even though at earlier times ($t < \tau_1$) dark adaptation begins to recover more quickly. These circadian alterations in the time course for dark adaptation appear to come about primarily through changes in A_1 or A_2, rather than through alterations in the photoreceptor processes underlying the time (or rate; $k=1/\tau$) constants.

Ref.1: Kaplan, E. and R.B. Barlow, Jr. 1980. Nature 286:393-395.
Ref.2: Fahrenbach, W.H. 1981. Cell Tissue Res. 216:655-659.
Ref.3: Barlow, R.B., Jr. 1983. J. Neurosci. 3:856-870.

145.3 THE B-WAVE OF THE ELECTRORETINOGRAM AS A MEASURE OF CIRCADIAN ROD OUTER SEGMENT DISC SHEDDING IN ALBINO RABBITS. P. A. HOCK*, M. P. White, and M. F. Marmor*. Dept. of Ophthalmology, Stanford University and Veterans Administration Medical Center, Palo Alto, CA 94304.

The shedding of outer segment discs in rod photoreceptors is cyclic in nature. This has been demonstrated histologically as a daily increase in pigment epithelial phagosomal content soon after light onset in the morning (La Vail, M. M., Science, 194: 1071, 1976). With autoradiography it has been shown that mammalian rod outer segment turnover requires 9-11 days, thus 10% of the rod outer segment length must be shed daily (Young, R. W., Invest. Ophthalmol. Vis. Sci., 15:700, 1976). It cannot be determined from anatomical evidence how much of the daily complement of disc shedding is accounted for by the morning shedding event nor can the time course of disc shedding be determined. We have used the b-wave of the electroretinogram (ERG) to provide a new means to measure the shedding event.

Rabbits were maintained on a 12 hr light:12 hr dark schedule for at least 3 weeks. AC ERGs were recorded in the dark from anesthetized rabbits using a corneal contact lens electrode. The b-wave amplitude elicited by constant intensity, 50 ms, 500 nm, and 630 nm test lights, presented at 1 min intervals, was measured for a period of 1-2 hrs. The constant intensity stimuli were chosen at the beginning of each session to elicit a criterion response of 40 uV. Recording began either shortly before the usual time of light onset or in the middle of the usual light period of the entrained light-dark cycle.

30 min after the time of light onset we find a decrease in the amplitude of the b-wave obtained with a 500 nm test light. The calculated decrease in sensitivity required to produce this change in amplitude is approximately .1 log unit, which is the expected change if 10% of the discs are shed. We find no change in amplitude when ERGs are recorded in the middle of the light period. With histological examination of the pigment epithelium, we find the expected increase in large phagosome content shortly after light onset, and low phagosome numbers at midday (Bunt, A. H., Invest. Ophthalmol. Vis. Sci., 17:90, 1978).

We have also used the ERG to measure shedding in freerunning conditions, recording from animals housed in continuous darkness or with unilateral eyepatches. In these animals, the change in ERG amplitude shifts temporally with the changes in the lighting conditions. This is the first time that the ERG has been used to measure a shedding event in the living eye and has been correlated with the histology in the same species.

145.4 CYCLIC GMP CHANGES THE SOLUBILITY OF A PHOSPHORYLATED PEPTIDE, COMPONENT II, IN ROD OUTER SEGMENTS. H. Hamm. Dept. of Physiol. and Biophys., Univ. of Ill. Coll. of Med., Chicago, IL 60612.

Components I and II are rod outer segment (ROS) phosphopeptides of molecular weights 13 and 12 Kdaltons which are normally phosphorylated in the dark and are reported to dephosphorylate in the light. Cyclic GMP as well as cAMP can stimulate their phosphorylation in broken ROS.

This study addresses the subcellular localization of CI&II in ROS. In Percoll-purified, broken rods, incubated with γ-^{32}P-ATP, the phosphorylated peptides can be found entirely in the supernatant after brief centrifugation. Reincubation of the resultant membranes with γ-^{32}P-ATP again yields similar amounts of soluble phosphorylated CI&II, suggesting that the phosphorylated form of CI&II is soluble, while an unphosphorylated pool remains bound. This cycle of removal of soluble CI&II and rephosphorylation of membranes can be repeated at least four times with no apparent decrease of the non-phosphorylated membrane-bound pool. The released, phosphorylated form cannot be regulated by cGMP, cGMP-dependent protein kinase, or a monoclonal antibody to Gα called 4A, while the membrane-bound form is highly regulated by these agents. This suggests that CI&II are subject to regulation only when bound to the membrane, and phosphorylation causes release into the cytoplasm.

Cyclic GMP alters the solubility of one of these peptides, Component II, causing some phosphorylated CII to be membrane bound. This redistribution effect is half-maximal in the presence of 50 μM 8-bromo cGMP, or 250 μM cGMP. Cyclic AMP and 8-br cAMP can also support this redistribution, but 5'GMP, 5'AMP, GDP and ADP do not. It appears that cGMP has altered the ability of phosphorylated CII to bind to the membrane.

In an effort to determine whether similar redistributions occur in intact cells, rods were incubated with $^{32}P_i$ and allowed to synthesize γ-^{32}P-ATP. Dark-adapted rods contain some membrane-bound CII, and incubation with dibutyryl cGMP increases this phosphorylation. Bright light (1% or more rho bleached) causes the dephosphorylation of this membrane-bound CII. Conceivably it is this membrane-bound form that might participate in cGMP- and protein phosphorylation-mediated events in ROS.

145.5 **MONOCLONAL ANTIBODIES THAT DEMONSTRATE LIGHT DEPENDENT LABELING OF ROD OUTER SEGMENTS.** Grant W. Balkema, John N. Wood* and Ursula C. Drager, Dept. Neurobiology, Harvard Med. Sch., Boston, and Sandoz Inst., Univ. College, London*.

In retina sections treated with antibodies against the 200K neurofilament subunit we had observed variable labeling of photoreceptor outer segments. As outer segments are not known to contain neurofilaments, the labeling pointed to crossreactivity of the antibodies with unrelated compounds. Here we asked whether the variability represents different functional states in these compounds, by testing for light dependence of the labeling. We found two neurofilament antibodies--RT97 and R3--out of ten tested that labeled rod outer segments, only when the retina had been illuminated. The affinity for RT97 increased up to a saturated level over the duration of a bright illumination, whereas affinity for R3 appeared at the onset of the light stimulus, grew to a maximum and decreased with steady bright illumination. A third unrelated antibody, #16, recognized selectively dark-adapted rod outer segments. In addition to the illumination dependent labeling of rod outer segments all three antibodies recognized other components of the retina independent of the illumination history: RT97 and R3 labeled cell types known to be rich in neurofilaments and #16 labeled profiles associated with synaptic terminals.

To determine the molecular weights of the illumination dependent antigens, we removed retinas from light and dark adapted mice and prepared Western blots. So far we found a clear light/dark difference on blots only for RT97: in light adapted, but not dark adapted retinas, RT97 strongly recognized bands at 32-42K molecular weight, which lined up with bands labeled by RET-P1, a monoclonal antibody against rhodopsin (a gift from Colin Barnstable); when light adapted retinas were prepared under conditions that allow aggregation of rhodopsin, both RT97 and RET-1 labeled additional bands at 72-80K, presumably rhodopsin aggregates.

The 200K neurofilament subunit is known to be very heavily phosphorylated, and a major covalent modification of rhodopsin with light adaptation is phosphorylation. To test whether the modifications recognized by the two neurofilament antibodies are due to phosphorylation, we treated whole retinas, neurofilament preparations and isolated outer segment preparations with alkaline phosphatase, and tested antibody binding by solid phase RIA. In all preparations binding declined with such treatment, indicating that RT97 and R3 either recognize a phosphorylated site directly, or a conformational change brought about by nearby phosphorylation, which is in agreement with observations of others on 200K neurofilament antibodies. Antibodies selective for particular states of sites that undergo reversible covalent modifications may allow the study of physiological events with morphological means. Supported by EY 01938, EY 05716, and the Am. Fed. for Aging Research

145.6 A MONOCLONAL ANTIBODY THAT BINDS TO THE OUTER SEGMENTS OF CONES. V. Lemmon. Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Preliminary immunohistochemical experiments indicated that antibody 50-1B11 bound to the outer segments of a subset of photoreceptors in chickens. Subsequent tests showed that 50-1B11 also bound to a subset of photoreceptors in bull frogs, rats, rhesus monkeys and humans. Based on the distance between 50-1B11 positive photoreceptors, and a comparison with information available in the literature, it appeared that 50-1B11 might be binding to cones in monkeys.

Therefore, we performed a series of double label experiments based on those of de Monasterio et al. (Science, 213:1278, 1981). We observed that the procion yellow stained a subset of photoreceptors. The dye was present in highest concentrations in the outer segments but there was also label in the inner segments, soma and dendrites of some photoreceptors. When these same cells were examined for staining with 50-1B11, they were also found to be labeled but the staining was restricted to the outer segments. Examination of numerous sections from four different injected eyes indicated that there was excellent correspondence between the procion label and the 50-1B11 staining. Since de Monasterio has shown that procion yellow is selectively taken up by cones, our experiment gives strong evidence that 50-1B11 binds to the outer segments of cones in monkeys.

In other experiments, flat mounts of monkey retina were examined and 50-1B11 positive outer segments were observed to correspond perfectly to the pattern of cone ellipsoids. Tissue culture experiments indicate that the antigen is intracellular and associated with the membrane.

Since this antibody binds to such a wide variety of species, it should be a very powerful tool for studying cone differentiation in vivo and in vitro as well as in retinal transplants and in retinoblastomas. More importantly, it should allow, for the first time, relatively easy studies of human retinal degeneration where it should be possible to distinguish degenerating cones from rods.

Supported by NIH grant #EY05284.

145.7 **TRANSMITTER-GATED MEMBRANE CURRENTS RECORDED FROM SYNAPTICALLY CONNECTED AND FROM ISOLATED BIPOLAR CELLS OF THE SALAMANDER RETINA.** **M. Wilson, D. Attwell*, P. Mobbs*, M. Tessier-Lavigne*. Dept. of Zoology, University of California, Davis, CA 95616, and Dept. of Physiology, University College London, UK.**

Isolated bipolar cells obtained from the salamander (Ambystoma) retina by enzymatic dissociation have been examined using whole-cell patch-clamping. Two morphologically indistinguishable classes of bipolar cell may be discriminated by their response to applied L-glutamate, the probable photoreceptor transmitter (Slaughter and Miller, J. Neurosci. 5:224, 1985). One class, provisionally identified as hyperpolarizing bipolar cells, showed a conductance and current noise increase to transmitter, while the other, provisionally identified as depolarizing bipolar cells, showed a conductance and current noise decrease. Glutamate-induced currents in both types of cell reversed at approximately 0 mV with the extracellular and patch pipette solutions described previously (Attwell, Bevan, Mobbs, Tessier-Lavigne and Wilson, J. Physiol. 360:19P, 1985).

A comparison of membrane currents measured from isolated bipolar cells with those from synaptically connected cells has been made by whole-cell patch-clamp recording of bipolar cells in retinal slices (Werblin, J. Physiol. 280:449, 1978) prepared in dim red light and viewed under infra-red illumination. Unequivocal identification of these cells as bipolar cells was facilitated by the addition of Lucifer Yellow to the patch pipette solution. Bipolar cells in retinal slices and those isolated from the retina showed similar time-dependent, voltage-gated currents evoked by depolarizing steps, however the input resistance of cells in slices was much lower than for isolated cells.

In response to illumination with a small spot, depolarizing bipolar cells in slices showed an inward current that increased with hyperpolarization and nulled at 0 mV.

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145.8 COLOR-CODED HORIZONTAL CELLS IN THE TURTLE RETINA. R. Siminoff and T. Ohtsuka (SPON: C. Clemente) Dept. of Inform. Sci., Fac. of Eng., Tohoku Univ., Sendai and Dept. of Inform. Physiol., Natl. Inst. Physiol. Sci., Okazaki, Japan.

Three spectral response types have been found in horizontal cells (HCs) of many vertebrate retinas. These distinct responses, i.e., luminosity (L) type, and biphasic (B) and triphasic (T) chromaticity types, have been thought to be developed through a cascading pathway between three chromatic types of cone photoreceptors and three spectral types of HCs. Recently, however, morphological evidences against this cascading circuitry have been found in the turtle retina. 1) Spectral sensitivities of six morphological types of cones in the turtle retina were re-examined with 4 red, 1 green, and 1 blue cones (Ohtsuka, J. Comp. Neurol., '85). 2) Ribbon synaptic contacts between these 6 cones and 3 spectral types of horizontal cell were studied using intracellular injections of HRP and electron microscopy; both cell body and axon terminal of LHC receiving direct synaptic inputs from 30 to 60 cones of the three chromatic types, BHC receiving inputs from about 20 green cones, and THC receiving inputs from about 20 cones in the ratio 2 red and 1 blue cones (T. Ohtsuka & N. Kouyama, in preparation). 3) Furthermore, chemical synapses from the luminosity to the chromaticity type HC have also been shown (Kolb and Jones, J. Neurocytol., '84). Thus, based on these morphological pathways, a model of the cone-HC circuitry has been developed, and impulse responses simulated for various monochromatic stimuli at different mean illuminance levels. The linear model of the cone-HC circuit was developed, based on the retinal elements acting as low-pass filters (Siminoff, Biol. Cybern. '85). Intracellular responses were recorded from eyecup preparations of the freshwater turtles (Pseudemys scripta elegans and Geoclemys reevesii) using 20 msec monochromatic flashes covering the entire retina. The model gives simulated responses of the horizontal cells that are in excellent accord with the experimental data and (a) a simple wiring diagram consisting of direct hyperpolarizing inputs from the appropriate cones and a depolarizing input from the LHC acting as a voltage divider gives the basic responses patterns of the BHC and THC, and (b) a negative feedback circuit from LHC to cones and having the negative feedback gain directly proportional to the mean illuminance level are necessary features to account for deviations of the response patterns from the simple form. Thus, both BHC and THC are basically the same differing only as to the direct cone input.

145.9 RETINAL HORIZONTAL CELLS IN TURTLE:SPATIAL TRANSFER FUNCTION DEPENDENCE ON CONTRAST AND TEMPERATURE;ORIENTATION AND DIRECTIONALLY SELECTIVE CELLS. A.R.Adolph (SPON:E.Newman). Eye Research Institute and Harvard Medical School, Boston, MA 02114.

Luminosity horizontal cells(L-HC) of turtle retina are sensitive to stimulus spatial frequency. They exhibit two major classes of spatial transfer function(STF)in response to drifting sinusoidal gratings,low-pass and band-pass. High frequency asymptotes of both classes are similar,with upper corner frequency between ca. 0.3c/mm and 0.8c/mm. Slope of h.f. asymptote ranges from ca. -3dB/oct to -16dB/oct. The band-pass types are peaked in the frequency range of ca. 0.2c/mm to 0.5c/mm, and have l.f. aymptotes more gradual than their h.f.aymptotes, ca. 1dB/oct to 6dB/oct. The shape of STF is independent of contrast over a 20% to 60% range. High freq. asymptote,upper corner freq.,and band-pass peak freq. are relatively temperature insensitive. The major effect of temperature on STF is to increase l.f. asymptote slope as temperature decreases. STF may be modelled by the sum of two lowpass Gaussians, one positive wide band and one negative narrow band. STF exhibits lowpass or bandpass characteristics depending on relative widths and amplitudes of the two Gaussians. Line spread function(LSF) is the Fourier transform of STF and is a centrally peaked Gaussian with varying degrees of negative surround depending on the lowpass or bandpass character of STF. A retinal circuit which exhibits observed STF behavior includes surround inhibition of a central HC by lateral HC via a negative feedback pathway:surround HC-surround receptor-central receptor(via electrotonic coupling)-central HC. Since observed l.f. asymptotes have relatively small slopes, the surround effect is inferred to be small. As temperature decreases, synaptic responses are slowed and attenuated. There is a decrease in forward transmission, noticeable mainly in the central receptor-HC pathway, which results in increased l.f. asymptote slope. STF with 0.3c/mm corner freq.(1/2 power point) corresponds to LSF with 1/2 width of 0.6mm; STF with 0.8c/mm corner freq. corresponds to LSF with 0.5mm 1/2 width.

L-HC exhibit other spatially dependent properties, in some measure. Some are orientation sensitive with symmetrical, elongated response field patterns showing equal responses to sweeps in opposite directions. Others are directionally selective with unequal responses to sweeps 180° apart. (Supported by EY-03383).

145.10 **Rod suppression of cones and achromatic cone pathways in amphibian retina.** **Thor Eysteinsson* and T. E. Frumkes, Dept. of Psychology, Queens College of CUNY, Flushing, New York 11367.**

Intracellular recordings were obtained from all types of neurons in the superfused retina of mudpuppy and Xenopus. In general, a small test stimulus was used which was derived from a red light emitting diode which was was concentric with the recording electrode. Clearest data were obtained with sinusoidal flicker presented in the presence of diffuse background fields of different wavelength and irradiance. In dark adapted cones, achromatic horizontal and bipolar cells and in some third order neurons, backgrounds can enhance flicker responses by as much as 500%. This effect has an action spectrum which adheres quite closely to the pigment sensitivity of the green-absorbing (red-appearing) rods, and is maximal with flicker frequencies >5 hz and test stimuli 240-630 uM in diameter. This effect is much smaller or absent with much larger stimuli and/or with flicker frequencies <2 hz, and is unobserved in rods or in rod-driven responses from other neurons. The forgoing suggests that this effect at least partially reflects rod-modulation of horizontal cell feedback: by changing the ambient membrane potential of horizontal cells, rods control the horizontal cell influence upon cones. However, other types of evidence suggest the operation of a different and/or an additional mechanism: 1) although under mesopic conditions action spectra suggest that both rods and cones can mediate this background effect, the cone contribution is much smaller and the effect is totally absent in the light adapted retina; 2) in some individual hyperpolarizing bipolar cells and in cones, this effect can be observed both with background fields that depolarize and that hyperpolarize cones. Moreover in horizontal cells themselves, this effect is uninfluenced by large amounts of depolarizing and hyperpolarizing current injection; 3) This effect is not influenced by high concentrations of picrotoxin, although this substance is known to at least partially block horizontal cell feedback in Xenopus; 4) this effect cannot be observed in any second order neuron displaying a c-type s potential, even though these neurons receive input from the same cone-type as achromatic neurons showing the effect.

145.11 DOPAMINERGIC EFFECTS ON NEURAL MECHANISMS IN HUMAN VISION. S. Mitra,[1] I. Bodis-Wollner,[2] T. Hutton,*[3] and T. KRILE*[1]. Department of Electrical Engineering/Computer Science[1], Texas Tech University, Lubbock, Texas, 79409, Department of Neurology[2] and Ophthalmology, The Mount Sinai School of Medicine, New York, NY 10029 and Department of Neurology[3], Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

The effect of dopamine deficiency on the spatiotemporal contrast response suggests altered neural mechanisms in the retina. We studied the effect of dopamine on human vision (Bodis-Wollner and Mitra, 1984). Patients with Parkinson's disease have a dopamine deficiency in the basal ganglia. They also demonstrate dopamine-dependent visual abnormalities However, the site of this abnormality is unknown.

An approach to understanding the mechanisms of the spatiotemporal response function has been proposed by Kelly (1969). The essential feature of his model is a cross-connecting filter controlling the contrast gain in the retinal network. According to this model, we calculated the gain function from the flicker sensitivities to uniform fields and counterphase gratings of 2 c/d in normal and Parkinsonians. The cross-connecting filters of the Parkinsonians demonstrated marked deviations from a normal filter in the cut-off temporal frequencies. Moreover, the cross-connecting filters showed a gain over a wider temporal frequency domain under a condition of severe dopamine deficiency. These results suggest a specific role of dopaminergic neurons in contrast gain mechanisms in human vision.

145.12 ERG HYPER-RESPONSES AND DELAYS IN 6 HYDROXYDOPAMINE INDUCED DOPAMINE DEPLETION IN THE RABBIT RETINA. P. Olivier*, F. Jolicoeur, J.R. Brunette*, G. Lafond*, and A. Drumheller*. Deptartments of Ophthalmology and Psychiatry, Faculty of Medicine University of Sherbrooke, Sherbrooke, Quebec Canada J1H 5N4.

Adult pigmented rabbits weighing approximately 2.5 kg were used to study the effect on the ERG of a selective degeneration of dopaminergic neurons in the retina. 6 hydroxydopamine (6OHDA) 300 µg in 0.3 cc of a 0.9% NaCl solution which also contained 600 µg ascorbic acid was injected intravitreally into one eye; the fellow eye, used as a control, was injected with the same amount of the physiologic solution containing 600 µg ascorbic acid alone. After 7 days ERGs were performed. Subsequently animals were sacrificed and retinas isolated for biochemical analysis. Retinal dopamine (DA), norepinephrine (NE) and serotonin (5HT) concentrations were determined by HPLC with electrochemical detection.

DA content in the treated retinas was reduced by almost 68% (6OHDA treated 0.06 ± 0.05 ng/mg wet weight, control 0.187± 0.11 ng/mg) P <0.01, while 5HT and NE levels remained unchanged (5HT 6OHDA treated 0.008 ±0.002 ng/mg, control 0.018± 0.17 ng/mg)(NE 6OHDA treated 0.075 ±0.033 ng/mg, control 0.088± 0.038 ng/mg).

ERG recordings showed marked increases in amplitude of both a and b waves (a wave 6OHDA treated 45.7 ±15.5 µV, control 22.1± 5.9 µV, P <0.01) (b wave 6OHDA treated 217.1± 63.1 µv, control 65 ±11.2 µv P <0.01). This increase in amplitude was accompanied by a significant increase in the implicit response time of both wave forms (b wave implicit time 6OHDA treated 137.8± 5.6 ms, control 105.3± 4.8 ms, P <0.01)(a wave implicit time 6OHDA treated 24.85 ±3.9 ms, control 21.5 ±1.9 ms, P <0.05).

Our results demonstrate that 300 µg 6OHDA produces a selective reduction in DA retinal levels in rabbits and that this decrease is accompanied by concomitant increases of a and b wave amplitudes and implicit response time.

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146.1 NEURAL OSCILLATORS: PATTERN GENERATORS AND PATTERN INTERPRETERS. S.F. Gizster* (SPON: K.J. Thompson). Crump Inst. for Med. Eng., U.C.L.A., Los Angeles, CA 90024.

Behaviors have often been shown to be generated by multiple (and often apparently redundant or degenerate) oscillators, or single oscillators with several oscillatory mechanisms. The functional role of such multiplication of oscillators is sometimes unclear. In locusts a set of abdominal oscillators are found involved in abdominal ventilation. They are all phaselocked to a single master driving oscillator. The local oscillators sometimes misfire at random and hence seem to interfere with the execution of the simple motor program, in principle achievable by the metathoracic ganglion driver oscillator alone. What function do they perform which justifies these misfiring effects?

Arguing from engineering ideas, several possible functional uses of oscillators may be emphasized. Many of these uses are optimum when the oscillator is utilized as a phaselocked loop mechanism: (1) Oscillators are the best means for phase and frequency synthesis. (2) Real systems are affected by stochastic variations and circuitry involved in oscillatory behavior must cope with this problem. (3) Oscillators are an optimal way to detect and interpret periodic signals carrying AM, FM, or PM information. (4) Oscillators are the best way for timing integration of information from several sources.

Apparent redundancy of neural oscillators may often reflect these uses. Indeed these are possible functions of any neural oscillator, even if it acts alone. Neural oscillators may act as transponders i.e., systems to amplify and relay periodic information, either from central or peripheral signal sources. Oscillators may be used as demodulators for interpreting timing and phasing aspects of sensory or central signal sources. They may be used as synchronizers to time the collection and integration of signals from several sources. Finally, they may act as phase and frequency synthesizers (drivers of behavior), as in the classical central pattern generator view. A functional explanation of some multiple oscillator arrangements is possible in this framework, and suggests the possibility that some conventionally defined 'central pattern generators' may be peripheral pattern interpreters. In the freely behaving animal such oscillators might be under the complete control of afferent information.

The organization and functioning of the locust ventilatory system illustrates the transponder relay and integration notions of local oscillator function in a CPG. The local oscillators and their connections form components of a delay line multiplier circuit similar to those used in digital tape recorders. The ensemble optimally detects the metathoracic driver's ventilatory control signals, which have stochastic frequency flutter. The accuracy of this detection by the network outweighs the cost of occasional local failures of entrainment.

146.2 INTEGRATION OF EXCITATORY AND INHIBITORY SYNAPTIC DRIVE IN MOTOR NEURONS DURING PRODUCTION OF THE FICTIVE SCRATCH IN THE TURTLE. Gail A. Robertson and Paul S.G. Stein.
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Mechanisms involved in the production of the motor patterns for the three forms of fictive scratch in the turtle (J. Neurophysiol. 53:1533,1985) include both (1) synaptic excitation and inhibition of motor neurons and (2) intrinsic membrane properties that modulate motor neuron firing frequency. Intracellular injections of chloride ions (Cl) reveal that synaptic inhibition of each motor neuron is mediated by an increased conductance to Cl during the quiescent phases of its rhythmic motor output. In addition, synaptic excitatory and inhibitory conductances overlap in time during certain phases of the cycle such that the membrane voltage may be held depolarized relative to E(Cl) but below threshold, or depolarized relative to threshold but at a controlled, low frequency of firing. The control of membrane voltage at each point in the cycle in motor neurons of each pool is critical to the production of the appropriate motor pattern. Isolation of excitatory from inhibitory synaptic components, e.g. by local iontophoresis of strychnine, will reveal important aspects of the organization of premotor synaptic output during the production of fictive scratch motor patterns.

We observed membrane voltage trajectories during the fictive scratch in the absence of Na spikes by injecting QX222, a quaternary derivative of lidocaine that blocks voltage-sensitive Na channels (Strichartz, J.Gen.Physiol. 62:37, 1973), into hip protractor motor neurons (VP-HP). We found no evidence that QX222 affected synaptic conductances. For many scratch responses the depolarizing membrane voltage varied directly in time with the amplitude modulations of the integrated VP-HP motor pool burst. Stimulation of certain sites, however, elicited a dramatic depolarization that was poorly correlated in time with the more gradual amplitude modulations during the latter part of the integrated VP-HP burst. The motor output of the VP-HP pool does not reflect this putative synaptic drive, suggesting that intrinsic mechanisms may be important for limiting the firing frequency of the motor neuron and thus in shaping the output of the motor pool.

Some of the mechanisms by which motor neurons integrate synaptic drive to produce diverse motor patterns will follow from two lines of inquiry: (1) resolving synaptic drive into inhibitory and excitatory components in the absence of action potentials; and (2) altering intrinsic conductances, such as the Ca-dependent g(K), which serves to modulate firing frequency. These experiments may also lead to predictions regarding the organization of the interneuronal output underlying the different scratch motor patterns in the turtle, as well as changes in interneuronal activity that precipitate the transition from one motor pattern to another. Supported by NIH grant NS-15049.

146.3 AROUSAL BREATHING IN LAMPREYS: MODULATION OF THE RESPIRATORY PATTERN GENERATOR. D.F. Russell, C.M. Rovainen, and K.J. Thompson. Washington University School of Medicine, Dept. of Cell Biology and Physiology, St. Louis, Missouri 63110.

The breathing rhythm of lampreys is spontaneously active and drives brief synchronous bursts in the motorneurons of cranial nuclei VII, IX and X. These bursts can be modulated in amplitude, duration, and cycle period, either spontaneously or in response to a variety of sensory or central stimuli. Adult Lampetra fluviatilis, Ichthyomyzon unicuspis, Petromyzon marinus, and Lampetra lamottenii were used.

"Arousal breathing" is defined as an episode of enhanced frequency, amplitude, and duration of bursts in the respiratory motorneurons, continuing approx. 5 sec to minutes. It was studied in semi-intact preparations of the head, or the isolated brain or medulla. Arousal breathing was correlated with discharge in the medial reticular formation, in reticulospinal neurons, and in trigeminal motorneurons to the sucker. Episodes could occur spontaneously or in response to the following stimuli: (1) repetitive electrical stimulation of the floor of the isthmus; (2) stimulation of the optic nerve, or photic stimulation in head preparations; (3) repetitive stimulation of sensory branches of nerve V; (4) mechanical stimulation of the labyrinths or electrical stimulation of nerve VIII; (5) stimulation of the dorsal tracts of the spinal cord or intracellular stimulation of sensory dorsal cells in the rostral spinal cord. Arousal breathing is part of a general increase in brainstem activity, affecting a variety of motor systems.

Other types of modulation were also observed. (1) In head preparations from L.f., EMG bursts were sometimes absent in the caudal gill sacs (innervated by X motorneurons), or could wax and wane in amplitude over a time scale of minutes. In contrast, rostral gill sacs (innervated by VII and IX motorneurons) showed more consistent burst amplitudes. (2) Intracellular stimulation of 6/11 medullary dorsal cells suppressed or slowed fictive breathing in L.f.. (3) Stimulation of the IX-X roots, which contain afferents from the gill sacs, produced bilateral bursts of long duration ("coughs") and tended to suppress respiration, without producing prolonged discharages in the reticular and trigeminal systems.

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146.4 SWIMMING IN APLYSIA BRASILIANA: BEHAVIORAL AND CELLULAR EFFECTS OF SEROTONIN. D.W. Parsons* and H.M. Pinsker. Marine Biomedical Institute, Depts. of Physiology and Psychiatry, UTMB, Galveston, Texas 77550

Aplysia brasiliana swim by flapping their bilaterally symmetrical parapodia. Continuous swimming can be elicited by suspending an animal above the substrate, but swimming is abolished by a bilateral lesion of the cerebro-pedal connectives (CPC). CPC-lesioned animals will, however, swim during tonic electrical stimulation of a cut CPC or after injection of serotonin (5-HT; approximate circulating concentration 10^{-5}M). Putative primary motoneurons for parapodial opening and closing are located in the pedal ganglia. We now describe the effects of 5-HT on the swimming motor program (SMP) in intact animals and reduced preparations; and on the modulation of the activity of parapodial opener and closer neurons in isolated brains.

When monitored from parapodial nerves in intact animals, 5-HT injections elicited the SMP; bath-applied 5-HT also elicited the SMP in semi-intact preparations. In isolated brains, bath-applied 5-HT induced smooth bursting in identified parapodial opener neurons monitored intracellularly.

To determine whether 5-HT might be acting directly on these putative motoneurons, we examined the effect of localized iontophoretic application of 5-HT to opener cell bodies. Iontophoretic 5-HT induced smooth bursting in opener neurons, but had no effect on closer neurons. These effects of iontophoretic 5-HT were qualitatively similar to those of tonic stimulation of the CPC: typically a brief period of tonic firing in the opener neuron preceded the development of smooth bursting. In contrast, intracellular depolarization of an opener neuron produced only tonic firing.

The period of the bursting elicited by iontophoretic 5-HT application was temperature dependent. Iontophoretic 5-HT gave smooth bursting even when synaptic input was blocked in high Mg^{++}. In addition, a surgically-isolated opener neuron soma also produced smooth bursting. These findings indicate that 5-HT induced bursting in these normally-silent parapodial opener neurons is the result of an endogenous pacemaker mechanism that is released by 5-HT, and is not attributable to phasic synaptic modulation. Thus, parapodial opener neurons can be classed as "conditional endogenous bursters".

To further evaluate the role of 5-HT in normal swimming, we examined the effect of methysergide (a 5-HT antagonist) and 5,7-DHT (a "cytotoxic" analog of 5-HT) in intact animals. Acute administration of methysergide, and chronic administration of 5,7-DHT produced a selective decrease in the amplitude of parapodial flapping. The frequency of flapping did not change.

This research was supported by NIH and NSF grants to HMP.

146.5 DESCENDING AND PERIPHERAL MODULATION OF A LOCOMOTORY PATTERN GENERATOR. R.A. Satterlie. Dept. of Zoology. Arizona State Univ. Tempe, AZ. 85287

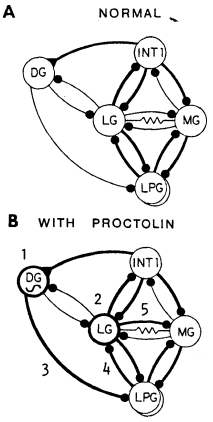
The primary circuit representing the pattern generator for swimming in the pteropod mollusc Clione limacina includes only four interneurons located in the paired pedal ganglia. The interneuronal circuit alternately excites and inhibits two antagonistic populations of wing motorneurons via monosynaptic connections. The central pattern generator will "free run" in the absence of cerebral and peripheral input, with a regular cycle frequency of 1-2 Hz. With intact cerebral ganglia, the swimming system will exhibit a variable cycle frequency ranging from 1 to 10 Hz. Increases above the basal frequency are associated with tonic depolarization of the pattern generator interneurons, and this input can be eliminated by severing the cerebro-pedal connectives.

Peripheral sensory input to the swimming system includes primarily inhibitory pathways activated during wing retraction (and associated swim inhibition). Inhibitory pathways include direct inhibitory inputs at both the interneuronal and motorneuronal levels and can be eliminated by severing the main wing nerve. Putative sensory structures include ciliary tufts and cones located on the leading edge, trailing edge and dorsal and ventral surfaces of the wings.

146.6 PROCTOLIN MODULATION OF THE GASTRIC OSCILLATOR IN THE LOBSTER STOMATOGASTRIC GANGLION. H.-G. Heinzel* and A.I. Selverston Dept. of Biol., Univ. of Calif. San Diego, La Jolla, CA 92093

The gastric oscillator of the lobster has previously been thought to operate as a network oscillator containing no endogenous bursters. It has been shown, however, that at least one neuron of the circuit (the dorsal gastric motoneuron DG) is capable of acting as a conditional endogenous burster. It shows, in rare cases, spontaneous slow regenerative depolarizations, which can also be induced by nerve stimulation (Hartline, D.K. and D.F. Russell, J. Neurobiol. 15:345,1984) or bath application of octopamine (Wadepuhl, M. and A.I. Selverston, in prep.). In all these cases, however, all other members of the gastric circuit are silent or show tonic firing, demonstrating that the weak output connections of DG are not strong enough to drive the network.

We show that bath application of proctolin to the stomatogastric ganglion, in a combined preparation, is able to influence DG so strongly that the whole gastric output pattern is changed. Nerve recordings and up to eight simultaneous intracellular recordings show that proctolin in concentrations as low as 10^{-12}M can change the normal gastric oscillator as follows: (1) DG which normally is mainly driven by the strong excitatory input from Interneuron 1, as confirmed by photoinactivation of INT 1, becomes an endogenous burster. (2) LG shows plateauing and (3) the DG to LPG inhibition becomes stronger leading to (4) disinhibition of LG from LPG. (5) The plateauing LG inhibits its synergistic motoneuron MG stronger than before causing a delay of its burst.



In addition, the action of proctolin on DG gives rise to output patterns where the medial tooth subsystem (DG and GM motoneurons) runs with different frequencies in relation to the lateral teeth subsystem (LG, MG, LPG) in vitro. Such patterns also occur in intact lobsters, as shown by endoscopic movie analysis of the teeth movement. The strong action of proctolin is also demonstrated by the finding that the gastric oscillator shows vigorous rhythmic activity, even with the stomatogastric nerve cut or blocked, a procedure which normally stops gastric cycling.

FIG. Proctolin induced changes in the gastric circuit.
strong, weak inhibition
strong excitation

146.7 NEURONAL TARGETS OF THE MODULATORY ACTION OF PROCTOLIN ON THE PYLORIC SYSTEM OF THE STOMATOGASTRIC GANGLION OF PANULIRUS INTERRUPTUS. S.L. Hooper and E. Marder. Biol., Brandeis University, Waltham, MA 02254.

The neuropeptide, proctolin, is present in input fibers to the stomatogastric ganglion (STG), and bath-application of proctolin modifies the pyloric and gastric mill motor patterns generated by the isolated STG (Marder, Hooper & Siwicki, submitted). Proctolin (10^{-9} to 10^{-6}M) increases both the frequency of the pyloric rhythm and the amplitude of the membrane potential excursions in the anterior burster (AB), pyloric dilator (PD), and lateral pyloric (LP) neurons. In a few cases the amplitude of the membrane potential excursions in the ventricular dilator (VD) and the pyloric (PY) neurons was also increased. Additionally, in about 60% of the experiments, periodic interruptions of the pyloric rhythm which are associated with activation of the gastric system were seen. These interruptions change the firing rate and/or membrane potential excursions of all the pyloric network neurons. This suggests that proctolin is a neurally released modulator of the STG. In order to characterize in greater detail the physiological actions of proctolin, we have attempted to determine which of the neurons in the pyloric network of the STG are responsive to proctolin. We have used two general strategies: a) individual neurons were deleted from the STG using the Lucifer yellow photoinactivation technique (Miller & Selverston, Science, 1979) to determine whether the proctolin effects required the presence of that neuron in the circuit b) individual neurons were isolated from electrical and chemical synaptic inputs (by photoinactivation and pharmacological agents) to determine which isolated neurons are sensitive to proctolin.

Deletion of no single neuron removes all of proctolin's effects on the pyloric motor pattern. The excitatory action of proctolin persisted after deletion of the AB, VD, LP, or two PD neurons. Thus the effects of proctolin on the intact circuit are due to actions on at least two different cell types. Isolated AB neurons responded strongly to proctolin. The amplitude of AB membrane potential excursions and burst frequency were increased. Isolated LP neurons were weakly excited by proctolin. Isolated PD and PY neurons were relatively unaffected by proctolin. The interruption of the pyloric rhythm associated with gastric rhythm activation remained after deletion of any single neuron of the pyloric network.

We conclude that in the intact ganglion the increased frequency of pyloric cycling and amplitude of membrane potential excursions in the electrically coupled AB and PD neurons is due to proctolin's excitatory effects on the AB neuron. The dramatic excitation of the LP neuron in the intact ganglion can be explained by proctolin's dual excitation of both the AB and the LP neurons. Research supported by NS-17813 to E.M.

146.8 SYNAPTIC TRANSMISSION IN THE 7TH ABDOMINAL GANGLION OF THE SCORPION, HETEROMETRUS FULVIPES. P. Murali Mohan*, K. Yellamma* and K. S. Babu* (Spon: R. L. Klein). Dept. of Pharmacol. Toxicol, Univ. Miss. Med. Ctr., Jackson, MS 39216, and Dept. of Zoology, S.V. Univ., Tirupati 517502, India.

In H. fulvipes the 5th (last) metasomatic segment, bearing the telson, is capable of only upward and downward movements, while the other metasomatic segments are capable of sideward movements also. The 4th (4n) and 5th (5n) metasomatic segmental nerves and the telsonic nerve (Te), arising from the 7th (last) abdominal ganglion, innervate the musculatures of the 4th and 5th metasomatic segments and the telson respectively. The mobility of segments could arise in part from the functional organization of the neurones in the CNS, corresponding to the heterogeneity of innervation between the 4th and 5th metasomatic segments. This aspect was examined in the present study.

For this purpose, the general features of synaptic organization in the 7th abdominal ganglion were studied by electrophysiological and anatomical methods. Electrophysiological recordings from 5n, Te and 4n were made by using extracellular platinum hook electrodes, while stimulating the ipsilateral and contralateral connectives anterior to the ganglion. The anatomical organization of neurones in the ganglion was traced by the extracellular cobalt chloride back-filling technique of Pitman et al. (1973).

Stimulation of the ipsilateral connective elicited a burst of activity with both large and small action potentials in 5n, Te and 4n (Thresholds 2.2-4.0 V; conduction velocities 1-7 m/s; synaptic delays 0.5-1.2 msec). In contrast, contralateral stimulation induced very little activity in 5n and Te, with one or two small and labile action potentials (Threshold 3-5 V; conduction velocities 1-1.5 m/s; synaptic delays 4-4.4 msec). However, contralateral stimulation induced large bursts of activity in 4n (Thresholds 2.4-3.0 V; conduction velocities 1.5-6.5 m/s; synaptic delays 1.2-1.4 msec). The anatomical organization of the 7th ganglion indicated the possibility of synaptic contacts for 5n and Te only ipsilaterally. In contrast, the dendritic arborizations of motor neurones of 4n extended also to the contralateral side of the ganglion. This explains the higher activity in this nerve for both ipsi- and contralateral stimulations. The existence of ipsi- and contralateral connections seems to bear significance in the multidirectional movement of the 4th segment. In contrast, 5n and Te innervating the 5th segment and telson respectively seem to restrict their synaptic contacts predominantly to the ipsilateral side of the ganglion.

146.9 IDENTIFICATION OF CANDIDATE PEPTIDERGIC FEEDING REGULATORY NEURONS IN HELISOMA. A.D. Murphy, W,K. Stell, and K. Lukowiak*. Depts. of Medical Physiology and Anatomy, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Feeding in the snail, Helisoma, largely results from cyclical alternating contractions of protractor and retractor muscles. The motoneurons for both sets of muscles, as well as the central pattern generator which drives the motoneurons, are located in the buccal ganglia. The basic feeding behavior is relatively stereotyped. Intuitively, however, one would expect regulation of feeding to be complex, reflecting a variety of external and internal environmental signals. Previously, it has been shown that serotonin and dopamine could initiate or increase feeding patterned motor activity (PMA) in Helisoma. Recently, we found that micromolar concentrations of small cardioactive peptide B (SCP_B) similarly initiated or increased PMA of the buccal ganglia. In addition, micromolar concentrations of FMRFamide reduced spontaneous PMA to approximately one-third the control rate and 10^{-9}M FMRFamide completely suppressed PMA. We began the search for peptidergic regulatory neurons by immunofluorescence staining utilizing antisera to FMRFamide (from T.L. O'Donohue) and a monoclonal antibody to SCP_B (from S. Kempf, B. Masinofsky and A.O.D. Willows). A number of candidate regulatory neurons, which display FMRFamide-like or SCP_B-like immunoreactivity, have been localized with either their somata in the buccal ganglia or with axons projecting to the buccal ganglia. On the ventral surface of each of the paired buccal ganglia, there is a single neuron with FMRFamide-like immunoreactivity which stains very brightly, and often two to four other somata that are less immunoreactive. A cluster of about 30 small FMRFamide-like neurons are located on the dorsal surface of the buccal ganglia. Usually seven or eight pairs of SCP_B-like immunoreactive somata were found on the dorsal surfaces and an additional 4 or 5 pairs on the ventral surfaces of the buccal gnaglia. In addition, there are both FMRFamide-like and SCP_B-like immunoreactive axons in the cerebro-buccal connectives and there are peripheral neurons on the gut which show immunoreactivity to one or both of these peptides and project axons to the buccal ganglia. Characterization of these candidate regulatory neurons is in progress. Several of the SCP_B-like neurons in the buccal ganglia are primary effector neurons and have no effects on PMA. These include protractor motoneurons B18 and B19 and the salivary effector neuron B4. Preliminary experiments suggest that an SCP_B-like immunoreactive neuron (designated C2) with its soma in the cerebral ganglia and an axon projecting to the buccal ganglia increases PMA. A definite demonstration awaits double staining of the physiologically active neuron with Lucifer Yellow followed by the antibody to SCP_B and a rhodamine conjugated second antibody. Supported by AHFMR and MRC.

146.10 POSSIBLE MODULATORY ROLES OF ARGININE VASOTOCIN ON FEEDING MOTOR OUTPUT IN HELISOMA. J.E. Richmond, A.G.M. Bulloch, A.D. Murphy and K. Lukowiak. Department of Medical Physiology, University of Calgary, Calgary, Alta., Canada T2N 4N1.

The importance of modulation of motor activity by neuroactive peptides is becoming increasingly evident. Our laboratories have examined the roles of peptides in the context of two well-defined molluscan behaviors: (1) the gill withdrawal response of Aplysia and (2) feeding, primarily in Helisoma. It was found that food satiated Aplysia had a suppressed gill withdrawal reflex. Superfusion of arginine vasotocin, (AVT) over the abdominal ganglion of Aplysia produced a suppression of the gill-withdrawal reflex which paralleled the effects of satiation. This suggested the possibility that AVT might be a molluscan "satiety factor" with effects on the expression of feeding behavior. Feeding in Helisoma occurs readily in dissected preparations and is known to be modulated by both peptides (SCP_B and FMRFamide) and monoamines (5-HT and dopamine). Therefore we began to examine the role(s), if any, of AVT on feeding in Helisoma.

In the present study immunohistochemical evidence suggested two possible pathways by which an AVT-like substance might influence feeding patterned motor activity (PMA). The central pattern generator underlying feeding motor output is known to be in the buccal ganglia. These ganglia receive descending inputs from the central ring of ganglia via the cerebro-buccal connectives (CBC) and receive peripheral feedback via the buccal ganglionic nerve roots. Indirect immunofluorescent staining with an AVT antibody (provided by K. Lederis) showed a single pair of brightly stained somata on the ventral surfaces of the pedal ganglia. These neurons have a dendritic arborization in the pedal ganglia and axons that circumnavigate the central ring. They also have extensive arborizations in the cerebral ganglion neuropil but no AVT-like processes were seen in the CBCs. Thus any effects of these cells on feeding PMA must be indirect. There are also AVT-like processes projecting to the buccal ganglia from the gut via the gastric nerves which could directly modulate PMA.

Preliminary pharmacological studies suggest that AVT may produce a long-latency stimulation of feeding PMA in Helisoma as indicated by intracellular recording from identified buccal neurons. These effects, however, were not mediated by activation of the identified modulatory serotonergic neurons C1. Further studies are in progress. Supported by AHFMR and MRC (Canada).

146.11 BURST-ORGANIZING MECHANISMS DIFFER IN TWO CLASSES OF NEURONS IN THE LOBSTER CARDIAC GANGLION. A. Berlind. Biology Dept., Wesleyan University, Middletown, CT 06457.

Cardiac ganglia isolated from the hearts of lobsters (Homarus americanus) generate repetitive bursts of nerve impulses by a mechanism which involves both network interactions and endogenous burst-organizing potentials ("driver potentials", DPs). Tazaki and Cooke have presented evidence that a DP mechanism exists in each of the constituent neurons of the system (4 small "pacemaker" cells and 5 larger motorneurons) and that the DP in motorneurons is a slow depolarizing potential, localized to a region near the soma, with most of the inward current carried by calcium ions. The data presented here provide indirect evidence, from experiments in altered ionic media, that the DP mechanism in lobster pacemaker neurons is qualitatively or quantitatively different from that previously described in crab and lobster motorneurons. When a lobster cardiac ganglion is superfused with saline containing 18 mM manganese, which completely suppresses DPs in the motorneurons, the ganglion continues to generate spontaneous bursts, which are reduced in amplitude and frequency as compared to the activity in normal Mn^{++}-free saline. When the level of calcium in the perfusion fluid was reduced from the normal level (26 mM) to 1.3 or 2.6 mM (5 or 10% of normal), motorneuron DPs were eliminated but very long duration bursts, characterized by high-frequency interburst action potential discharge, were generated. Clear bursting behavior persisted in the intact ganglion even when all Ca^{++} was eliminated from the perfusion fluid. Three experimental modifications of the isolated ganglion preparation suggest that the prolonged bursts observed in low Ca^{++} salines reflect the persistence of the burst-generating capabilities of the small pacemaker neurons under conditions which suppress the large cell DPs. 1) In ganglia in which the 3 anterior motorneurons are functionally separated from the small cells by a tight silk ligature around the ganglionic trunk, low Ca^{++} saline eliminated bursting anterior to the ligature; burst duration in the 2 posterior motorneurons, which retained connections to the pacemakers, was prolonged. 2) When the two ends of the ganglion were placed in two independent perfusion pools, bursting was prolonged when only the small cells were exposed to low Ca^{++} saline, but not when only the large cells were so treated. 3) If the discharge of the motorneurons was silenced by perfusion with tetrodotoxin in such a two-pool preparation, the resultant small cell bursts were prolonged and intensified in low-Ca^{++} saline. Thse results suggest that the inward current responsible for DP generation in the small cells, unlike that in the motorneurons, includes a substantial contribution from an ion other than calcium.

146.12 SLOW OSCILLATIONS IN MEMBRANE POTENTIAL OF INTERNEURONS THAT CONTROL HEARTBEAT OF THE MEDICINAL LEECH. Edmund A. Arbas and Ronald L. Calabrese Biological Laboratories, Harvard University, 16 Divinity Ave. Cambridge, MA. 02138.

The heartbeat rhythm of the leech arises from the activity of a network of interneurons, the HN cells, of which there is a pair in each of the first 7 segmental ganglia. HN neurons of the 3rd and 4th ganglia, HN cells (3) and (4), are elements of the timing oscillator that generates the beat rhythm, and also control the firing of motorneurons that deliver the rhythm to the muscular hearts. The HN(3) and (4) cell pairs oscillate through their endogenous properties and strong reciprocal inhibitory connections. The premotor HN(7) neurons follow the beat rhythm that is delivered to them synaptically but do not contribute to its generation. All of these HN cells have an activity rhythm in which "slow" depolarizations of membrane potential capped by firing of fast action potentials alternate with hyperpolarized silent periods dominated by synaptic inhibition from other HN cells of the network. The slow oscillation spans about 10 mV and is centered around a mean between -45 and -50 mV.

The ionic basis of the activites of these HN cells was studied by intracellular recording and stimulation in isolated ganglia bathed by salines of various compositions. Replacement of Na^+ by Tris in the saline eliminated the production of fast action potentials, led to hyperpolarization of membrane potential to values near -60 mV and revealed the ability to form a plateau potential with a threshold near -55 mV and a peak near -30 mV. The plateau potential is prominently activated upon release from hyperpolarizing injected currents. Plateau formation was found to be accompanied by an increase in membrane conductance. Plateaus were enhanced by elevation of Ca^{++} concentration in the saline or by replacement of Ca^{++} by Ba^{++}. They could also be formed when Sr^{++} replaced Ca^{++}, but were blocked by addition of Mg^{++} or Co^{++} to the saline with the latter ion being effective at lower concentrations. We conclude that a voltage sensitive inward current carried by Ca^{++} generates the plateau.

Both the HN(7) neurons and the timing oscillator interneurons exhibited plateau potentials. In elevated Ca^{++}, HN interneurons of the timing oscillator additionally exhibited stable oscillations of membrane potential that were independent of Na^+-mediated fast action potentials. They depended on the production of plateaus, graded reciprocal inhibitory transmission, and escape from inhibition by the hyperpolarized neuron. Transitions in the state of the two neurons in each cycle were effected by the activation of a plateau potential in the previously inhibited neuron that terminated the plateau and caused hyperpolarization in the previously depolarized neuron. Supported by NIH 5 F32 NS 06453 to E.A.A. & NSF BNS B1-21551 & PHS 1 R01 NS21232 to R.L.C.

146.13 **COMPUTER SIMULATION OF THE LEECH HEART MOTOR NEURON (HE-CELL).** E. De Schutter* (SPON: European Neuroscience Association) and R. L. Calabrese. Dept of Neurology, Univ. Hosp. of Antwerp, B2520 Edegem, Belgium and Biological Laboratories, Harvard Univ., Cambridge MA 02138.

A computer model of the motor neuron (HE-cell) that drives the heart tubes of the leech *(Hirudo medicinalis)* has been developed with the Nodus simulation software (*Neurosc. Lett., S. 18:* 154, 1984). The model contained 51 compartments, with 15 dendrites and one active site in the exon at a distance of about 300 µm from the soma. Nerve crush experiments showed that in contrast with other leech motor neurons, the HE-cell action potential is generated inside the ganglion. The passive membrane parameters were based on experiments by Peterson (*Biophys. J. 43:* 53, 1983), the specific membrane resistivity was 35 kΩ cm^2 and the capacitance was 2.0 µF/ cm^2, cytoplasmic resistivity was 300 Ω/cm. The voltage dependent conductances at the active site were described by Hodgkin-Huxley like equations. The equations for an inward Na^+-current and for two outward K^+-currents (an A-current and the delayed rectifier) were based on measurements by Connor and Stevens (*J. Physiol. 213:* 31,1971). The model made an accurate simulation of an isolated HE-cell possible.

The electrotonic coupling of bilateral HE-cells has been examined. Five dendrites of the HE-cells were connected through identically shaped compartments with a diameter of 0.75 µm. The frequency coupling of this model and its response to DC current was similar to the values obtained by Peterson (1983). The coupling had only a small influence on cells that were firing in phase (a small decrease in firing frequency). When the cells were not in phase, the coupling drove them in phase by decreasing the difference (at a rate of up to 10% of the phase difference per cycle). This tendency to couple the firing of the two cells was not observed in *in vitro* experiments (using a low Cl^- bathing solution to block the interneuron generated IPSPs). It is possible that the coupling was masked by the jitter of the cells or by their difference in firing frequency. The effect of a difference in firing frequency on the coupling will be simulated. The A-current could be important in the coupling of action potentials observed in the simulation, changes in ion currents will be examined.

Recent experiments have shown that a small Ca^{++}-conductance exists in HN-cells (interneurons of the circuit that generates the heart rhythm) and in HE-cells (Arbas, abstract in this volume), but it is not clear if Ca^{++}-currents play a role in the bursting behaviour of HE-cells in a quiet leech. Simulations of the bursting of HE-cells, caused by barrages of IPSPs, will be done to examine the importance of different ion currents during bursting.

TRANSMITTERS IN INVERTEBRATES II

147.1 ACETYLCHOLINE CAUSES SQUID IRIDOPHORE CELLS TO BECOME IRIDESCENT IN VITRO. R.T. Hanlon* and K.M. Cooper* (SPON: J.E. Bottenstein). Marine Biomed. Inst., Univ. Texas Med. Br., Galveston, TX 77550.

Color change in cephalopods is produced by neurally controlled dermal chromatophore organs that are underlain with iridophore cells. Based upon the first evidence that some squid iridophores may be physiologically active in vivo (Hanlon, R.T., Malacologia 23(1):89, 1982), we now show that acetylcholine (ACh) applied in vitro changes the optical properties of the iridosomal platelets to produce iridescence. Excised layers of iridophores were prepared from the dorsal mantle of the bay squid Lolliguncula brevis. Application of $2.75x10^{-7}$M ACh in artificial sea water (500mM NaCl, 10mM KCl, 12mM $MgCl_2$, 10mM $CaCl_2$, either unbuffered or buffered with 4mM Hepes pH 7.4) changed iridophore cells from colorless or non-iridescent blue to bright iridescent red and gold when viewed with incident light. TEM examination of the cells revealed that there are ultrastructural differences within the platelets that can explain these different optical properties. The effect of ACh-induced iridescence was reversed by washing with artificial sea water. Equimolar atropine blocked the ACh response, while tubocurarine did not, suggesting the involvement of muscarinic receptors.

The excised iridophore preparations also contained a muscle layer as well as some nerve fibers embedded in the connective tissue. Squid iridophores have never been shown to have muscle or nerve connections, but it is possible that ACh acts upon one of these other tissue components, with a secondary effect on the iridophores. However, KCl applied in a concentration (80mM) that caused chromatophore muscle contraction had no effect on the ACh response, nor did 10^{-7}M tetrodotoxin. We suggest that the iridophores themselves may be the target cells. Calcium appears to affect ACh-induced iridescence. Treatment of iridophores with ACh in Ca-free medium resulted in a brief appearance of red iridescence which quickly faded. Verapamil ($2x10^{-4}$M), a calcium channel blocker, inhibited to a great degree the effect of ACh on these cells.

ACh is present naturally in the dorsal mantle iridophore layers at levels of approximately 1mM ACh/mg protein. Our evidence suggests the possibility of cholinergic, non-synaptic control of the iridophores similar to that found in sea urchin tube feet (Florey, E.F. and Cahill, M.A., J. Exp. Biol. 88:281, 1980). Such a system would be a novel mechanism of color change and would complement the neurally controlled chromatophores in producing complex body patterns in cephalopods.

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147.2 INGESTIVE BEHAVIOR DECREASES THE SEROTONIN IN THE LEECH C.N.S. C.M. Lent. BioMed, Brown Univ., Providence RI 02912.

In the medicinal leech, the impulse activity of identified serotonin (5-HT) neurons is sufficient for pharyngeal peristalsis, biting and salivation: physiological components of ingestion. Neural 5-HT is necessary for these components and the expression of feeding behavior. Serotonergic effector neurons are synaptically excited into phasic activity by a stimulus which initiates ingestion: the localized warming of the prostomium by 2-5°C. To determine whether the simultaneous activation of these neurons measureably decreases 5-HT, ganglia were dissected from leeches (0.5±0.1g) before and within 3 hours of ingesting a blood meal. Serotonin was extracted and measured by HPLC-EC throughout the 32 segments of the leech. The C.N.S. comprises a supraesophageal ganglion (non-segmental), a subesophageal ganglion (SubEG, 4), iterated segmental ganglia (g1-g21) and a caudal ganglion (7).

Anterior ganglia from the SubEG to g10 contain 13-17 pmol 5-HT/ganglion, the level decreases to 8.5 pmols in g17-20 and to 2.5 in the caudal ganglion. The 2 fold rostro-caudal gradient in 5-HT cell number/ganglion accounts only partially for this 5-6 fold neurochemical gradient. Serotonin is reduced significantly following the 30 min blood meal in all ganglionic samples, except the caudal ganglion. The decreases range from 13 to 35% and are greatest anteriorly.

Warming the lip synaptically activates the Retzius cells (RZ) at all times, but in spring and summer, it evokes high-frequency impulse bursts. To examine the effects of bursting upon the 3-5 pmol of 5-HT in RZ, physiological preparations were thermally stimulated 8-10 times in 30 min experiments. Each 5°C stimulus lasted 2 min and evoked bursts, after which RZ somata were dissected. The 5-HT of 6 RZ from 3 leeches was reduced by at least 95%, suggesting that bursting rapidly mobilizes 5-HT from somata of effector neurons.

Ingesting blood averaging 890% of their weight distends leeches, terminates ingestion and inhibits biting for many months. Body wall distension tonically hyperpolarizes and inhibits RZ reversibly. To examine the effects of distension upon serotonin levels, 10 leeches were fed satiating blood meals. The body walls of 5 were cut and the blood removed from their crops, while the posterior suckers of the remaining 5 were cut similarly. After 6 weeks, 5-HT was extracted and measured from g4, g7 & g8. Leeches which were distended had 19.9±0.6(9) pmol 5-HT/ganglion. Leeches whose blood was removed and were not distended had 25% more, 24.2±1.2(9) pmols ($p<0.05$, t test).

The reduction in ganglionic serotonin by ingestion can be attributed primarily to decreases in serotonin from the somata of effector neurons. These data indicate that synaptic activation mobilizes their serotonin into the periphery and suggest that their inhibition during distension may hinder serotonin synthesis.

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147.3 DEMONSTRATION OF STEREOSELECTIVE L-[^{3}H]QNB BINDING SITES IN NERVOUS TISSUE OF APLYSIA CALIFORNICA AND PLEUROBRANCHAEA CALIFORNICA. T.F. Murray, G.J. Mpitsos, J.F. Siebenaller* and D.L. Barker. Coll. of Pharmacy, Coll. of Oceanography and Dept. of Biochemistry and Biophysics, Oregon State Univ., M.O. Hatfield Marine Science Center, Newport, OR 97365.

In order to ascertain whether muscarinic (M) cholinergic mechanisms may be present in the nervous system of gastropod molluscs, we have characterized the specific binding of the M antagonist L-[^{3}H]QNB in membranes from Aplysia and Pleurobranchaea. L-[^{3}H]QNB bound with a high affinity (K_d=0.77±0.08 nM) to a single population of specific sites (B_{max}=47±2.1 fmol/mg prot.) in nervous tissue of Aplysia. An investigation of the subcellular distribution of L-[^{3}H]QNB binding sites demonstrated that the specific binding was enriched in fractions consisting predominantly of synaptosomes together with mitochondria. The specific binding of L-[^{3}H]QNB was displaced stereoselectively by the enantiomers of benzetimide, dexetimide and levetimide. The pharmacologically active enantiomer, dexetimide, was greater than 100x more potent than levetimide as an inhibitor of L-[^{3}H]QNB binding. Moreover, the M cholinergic ligands, scopolamine, atropine, oxotremorine and pilocarpine were effective inhibitors of specific L-[^{3}H]QNB binding. In contrast, the nicotinic receptor antagonists, decamethonium and d-tubocurarine, were virtually inactive as inhibitors of specific binding. The pharmacological characteristics of the L-[^{3}H]QNB binding site provide evidence for classical M receptors in Aplysia nervous tissue. The physiological relevance of the dexetimide-displaceable L-[^{3}H]QNB binding site was supported by the demonstration of the sensitivity of the specific binding to thermal denaturation. We have also demonstrated dexetimide-displaceable L-[^{3}H]QNB binding sites in nervous tissue of Pleurobranchaea. At a concentration of L-[^{3}H]QNB of 1.8 nM, nervous tissue of Pleurobranchaea bound 62 fmol/mg prot. The characteristics of the Aplysia L-[^{3}H]QNB binding site are in accordance with studies of numerous vertebrate and invertebrate tissues indicating that the M cholinergic receptor has been highly conserved through evolution. Toward the goal of establishing a behavioral assay for M action, we have preliminary evidence showing that M agents, when injected into Pleurobranchaea in doses that do not affect normal feeding, affect the ability of animals to learn food-aversion behavior.

This work was supported by Air Force Office of Scientific Research contract F49620-83-C-0063 to G.J.M.

147.4 HPLC AND IMMUNOCYTOCHEMICAL ANALYSES OF SEROTONIN IN HERMISSENDA CENTRAL NERVOUS SYSTEMS. S.Auerbach, L.Grover*, and J.Farley. Prog. in Neurosci. and Behav., Princeton Univ., Princeton, NJ.

Associative pairings of light and rotation result in pairing-specific long-term reductions of two K^+ currents (I_A and $I_{K\text{-}Ca}$), and enhancement of a voltage-dependent calcium current (I_{Ca}), in Hermissenda Type B photoreceptors. Pharmacological evidence indicates that serotonin (5-HT) effects these same ionic conductance changes. We have attempted to determine whether 5-HT is a neurotransmitter which may mediate significant portions of the associative neural and behavioral changes. We have asked: 1) Is 5-HT localized within areas rich in fine processes and cell bodies presynaptic to B cells, 2) Is 5-HT released during training, 3) Do 5-HT reuptake inhibitors substantially reduce short-term electrophysiological changes of B cells resulting from in vitro simulations of associative training?

Optical ganglia were dissected from 6 animals, and monoamines assayed by HPLC-EC. Sensitivity was 5-10 pg for NE, DA and 5-HT. 5-HT content was 33 ng/mg protein in optic ganglia and 95 ng/mg in remaining brain (mean of 3 replications). DA was 11 ng/mg in optic ganglion and 287 ng/mg protein in remaining brain (mean of 4 replications). NE was below detection limits in samples pooled from 6 optic ganglia as well as whole brains. 5-HT release was measured after paired light and rotation of whole-mounted brains. Whole brains released 17 ng/mg protein during a 2 hr incubation in ASW containing 10uM imipramine a 5-HT reuptake blocker. Experiments to determine if associative and random pairings have different effects on 5-HT release are in progress.

Location of 5-HT cell bodies and processes was determined by whole-mount immunocytochemistry using a fluorescent 2° antibody. The higest densities of stained cell bodies were observed in pedal ganglia, including a giant cell (LPl). Processes were seen leaving all 3 major pedal nerves. Fewer cell bodies were stained in cerebro-pleural ganglia (PG). One large symmetrical cell body was stained which sent a process out the buccal nerve. Many fibers stained in the area of the CPG where photoreceptor terminals are located.

In vitro conditioning effects were substantially reduced in the presence of 10uM imipramine. Five in vitro conditioning trials in normal ASW produced a mean cumulative depolarization of 6.8 mV (n=4). In 10uM imipramine ASW, cumulative depolarization was 1.0 mV (n=3) but B cells were on average 6.0 mV more depolarized prior to conditioning. B cell EPSP and caudal hair cell IPSP amplitudes and durations appeared to be enhanced by imipramine. These results support the hypothesis that 5-HT is a transmitter involved in long-term training-induced changes in B photoreceptor light responses. Supported by NSF BNS 8316707 to JF.

147.5 CYCLIC AMP, A COMMON BIOCHEMICAL LOCUS FOR THE EFFECTS OF 5-HT AND SCP_B BUT NOT FMRFamide IN TAIL SENSORY NEURONS OF APLYSIA K.A. Ocorr, M. Tabata*, and J.H. Byrne, Univ. of Texas Med. Sch., Houston, Texas 77225.

Current evidence supports a role for serotonin (5-HT) and cAMP in modulating the electrophysiological properties of sensory neurons mediating defensive tail withdrawal in Aplysia. Specific serotonergic inputs to sensory neurons, however, have not been demonstrated. Recently, a neuroactive peptide, small cardioactive peptide (SCP_B), has been isolated from Aplysia neural tissue. SCP_B is present in the ganglion containing the tail sensory cells and has been shown to mimick the effects of 5-HT (Lloyd, et al, 1984; Castellucci, et al, 10, 510, 1984; Lukowiak, et al, 1984). It is possible that 5-HT is acting as an agonist for the natural, peptidergic transmitter; alternatively, there may be parallel aminergic and peptidergic pathways with similar actions. As a first step in distinguishing between these possibilites we previously compared the ability of 5-HT to enhance cAMP levels in isolated clusters of sensory neurons with that of SCP_B. Exposure of clusters to 5uM 5-HT or 5 uM SCP_B produced identical (34 ± 11%, N = 12; and 32 ± 12%, N = 16) increases in the cAMP content. 5-HT and SCP_B produced similar inward currents associated with decreases in input conductance in voltage clamped sensory neurons. In contrast, another neuroactive peptide, FMRFamide, had no effect on the cAMP content of isolated clusters but had opposite electrophysiological effects of 5-HT on membrane currents.

We have examined the effect of 5uM SCP_B on cAMP levels in the presence of 5uM 5-HT in order to determine whether these transmitters utilize the same receptors to stimulate cAMP production. Under these conditions SCP_B produced an additional 45 ± 22% (N = 10) increase in the cAMP content of isolated clusters compared to 5-HT alone. This increase is similar to that observed in response to treatment with SCP_B alone. By contrast, increasing the 5-HT concentration by 5uM (to 10uM) resulted in a 37 ± 9% (N = 12) increase in the cAMP content, a net increase of only 3% compared to 5uM 5-HT alone. These data indicate that 5-HT and SCP_B exert their effects via separate receptors and support the possibility of parallel pathways that regulate cAMP levels in the sensory neurons. We are examining the effects of their combined presence on the electrophysiological properties of these cells.

We also examined the combined effects of 5uM FMRFamide and 5uM 5-HT. While FMRFamide produced no change in basal levels of cAMP it was possible that it could modulate the effects of 5-HT on cAMP levels. We found that the addition of FMRFamide neither increased nor decreased the cAMP content expected in response to 5-HT alone (N = 10). Therefore, it appears that the electrophysiological effects of FMRFamide and those of 5-HT do not share a common biochemical basis.

147.6 **CO-LOCALIZATION OF α-BAG CELL PEPTIDE AND EGG-LAYING HORMONE IN BAG CELL NEURONS OF APLYSIA CALIFORNICA.** S.-M. Pulst^{+*}, D. Gusman$^{*=}$, B.S. Rothman$^+$ and E. Mayeri$^{+=}$. $^+$Dept. of Physiology, University of California, San Francisco, CA 94143 and $^=$Dept. of Basic Sciences, California College of Podiatric Medicine, San Francisco, CA 94115.

The bag cells neurons of Aplysia californica synthesize and release two or more peptide transmitters derived from a common 29 kD precursor protein. It is not known if all bag cells process this precursor uniformly or if alternative processing occurs. We studied the distribution of two of these transmitters, α-bag-cell-peptide (α-BCP)(1-9) and egg-laying hormone (ELH), using an immunocytochemical method that allowed simultaneous visualization of two antigens.

Highly specific antibodies (Ab's) were raised in goat against the N-terminal(1-12) portion of ELH and in rabbit against α-BCP(1-9). Both Ab's were affinity purified. Paraformaldehyde fixed tissue sections were incubated with a mixture of the two primary Ab's, rinsed and stained with a mixture of FITC-sheep-anti-goat-IgG and TRITC-sheep-anti-rabbit-IgG. Absorption controls with 50-100 uM ELH, α-BCP(1-9), α-BCP(1-8) and peptide A showed that the Ab's reacted only with ELH or α-BCP(1-9), respectively. The secondary Ab's reacted only with goat-IgG or rabbit-IgG. We also developed a solid phase assay for crossreactivity. Nanogram amounts of peptides were spotted on filter paper, stained with the primary Ab's and visualized with the direct peroxidase method. These studies confirmed the results of the absorption controls and also demonstrated that neither Ab reacted with the 29 kD precursor as purified from bag cell extracts.

The pattern of immunoreactivity (IR) in tissue sections was highly specific. Intense IR for α-BCP and ELH was seen in bag cell bodies and processes, but not in other cells of the ganglion. All cell bodies within the bag cell cluster stained, although some cells showed more IR for α-BCP or ELH. Immunoreactive processes extended into the connective tissue sheath overlying the abdominal ganglion, into the pleurovisceral connectives and into the neuropil of the ganglion. ELH and α-BCP IR were co-localized in most processes. However, a small percentage of processes stained for only one of the peptides. In the cerebral ganglion cells that were immunoreactive for ELH also showed IR for α-BCP.

These results suggest that most of the bag cells process the ELH/BCP precursor uniformly, but that alternative processing may occur in a small subpopulation. The precursor is probably also expressed in the cerebral ganglion.

Supported by NIH grants NS16033 and NS16490.

147.7 CO-RELEASE OF FIVE PEPTIDES, ELH, AP, α-, β- and γ-BCP, DERIVED FROM A COMMON PRECURSOR PROTEIN OF THE BAG CELLS OF APLYSIA. B.S. Rothman, K.A. Sigvardt and E. Mayeri. Dept. of Physiology, Univ. of Calif., San Francisco, 94143

A burst discharge of impulse activity in the bag cell neurons initiates egg-laying behavior in Aplysia. Based on biochemical and DNA sequence studies, the bag cells synthesize a precursor protein that encodes one copy each of as many as 11 cleavage products. Three peptides, ELH, AP and α-BCP are known end-products of the bag cell precursor. They are present in bag cell extracts, and ELH and AP are known to be released by bag cells. ELH and α-BCP are neurotransmitters in the abdominal ganglion, and ELH also functions as a neurohormone that releases eggs from the ovotestis. Other predicted end-products of the ELH/BCP precursor include β-, γ-, and δ-BCP.

To further define the end-products of precursor processing and explore the role of bag cell peptides as chemical messengers, we tested their release when the bag cells were electrically stimulated. An intact abdominal ganglion was placed in a low dead-volume chamber and perfused steadily with sea water containing protease inhibitors. Perfusates were pooled from 6 ganglia; they were analyzed and further purified by a reverse-phase HPLC system that can detect as little as 10 ng of peptide. Perfusate equivalent to a single bag cell burst, collected 0-30 min before the initiation of a burst, showed no material except protease inhibitors. An equal amount of perfusate collected 0-30 min after the start of a burst showed 20 additional peaks of material. Based on comigration on HPLC with standards, six peaks of material were identified: ELH, AP, α-BCP(1-9), its N-terminal fragment α-BCP(1-8), β-BCP and γ-BCP. The amount of each peptide recovered from 8 bag cell bursts was (nM): 4.6, 7.3, 0.5, 0.05, 0.9 and 2.0, respectively. Recovery of the α-BCPs, unlike the other peptides, was dependent on the presence of a specific aminopeptidase inhibitor in the perfusion medium. These six peptides were also present in bag cell extracts, and far more α-BCP(1-9) was found than α-BCP(1-8). Other, unidentified peptides were present in both perfusate and extracts. In contrast, δ-BCP was not found in perfusate nor in extracts.

This study shows that 1) five peptides, ELH, AP, α-BCP(1-9), β- and γ-BCP, encoded on the ELH/BCP precursor are co-released, 2) these five peptides, but not δ-BCP, are end-products of bag cell precursor processing; their locations on the precursor indicate that a minimum of 3 additional peptides are required to entirely account for precursor processing, and suggest that δ-BCP is processed as a C-terminally extended peptide, 3) peptides released by a single bag cell burst can be detected directly by analytical HPLC without resorting to more sensitive, but overly specific methods such as RIA, 4) large amounts of released peptide can be recovered by preparative HPLC; novel peptides can potentially be identified by amino acid sequencing. Supported by NIH grant NS16490.

147.8 **CO-LOCALIZATION OF THE SCPs AND FMRFamide TO MOTOR NEURONS INNERVATING APLYSIA BUCCAL MUSCLE.** **P.E. Lloyd*, M. Frankfurt, I. Kupfermann, K.R. Weiss.** Pharmacol. & Physiol. Sci., Univ. Chicago, Chicago, IL 60637; Neurobiol. Behav., Columbia P. & S., New York, NY 10032.

Three procedures were used to confirm the presence of authentic FMRFamide (Fa) in Aplysia buccal ganglia: i). Bioassay; fractions from reverse phase (RP)-HPLC of buccal ganglia extracts were assayed with a new and sensitive bioassay (isolated snail esophagus). The major peak of activity had the same retention time as Fa. ii). Radiolabeling; buccal ganglia were labeled with ^{35}S-methionine, extracted and run on one mode of RP-HPLC. The labeled material with the same retention time as Fa was then sequentially run through a second mode of RP-HPLC and ion-exchange HPLC. The retention time of the labeled peak in the final two HPLC runs was identical to Fa iii). Immunocytology; staining of buccal ganglia sections with Fa-directed antisera revealed immunoreactive fibers and varicosities, numerous small neurons, and several large neurons which are part of a cluster of cells known to be motor neurons to buccal muscle. Staining of fibers and varicosities in buccal muscle was also observed. These results confirm that authentic Fa is present in the buccal ganglia of Aplysia. The two Small Cardioactive Peptides (SCP_A & SCP_B) were originally purified from Aplysia tissue and SCP-like immunoreactivity has also been observed in the large buccal motor neurons (Lloyd et al., J. Neurosci., In Press). To confirm the presence of Fa and the SCPs in the large motor neurons, individual somata were dissected from ganglia labeled with ^{35}S-methionine in the presence of colchicine (added to prevent transport of labeled peptides to terminals on other cell bodies). The somata were extracted and run on RP-HPLC in the presence of excess unlabeled peptides. The results from this procedure confirmed that large individual motor neurons synthesized either the SCPs or Fa. In addition, some large neurons appeared to synthesize both the SCPs and Fa. Staining of alternate sections of the buccal ganglia provided independent evidence that individual neurons contained both SCP-like and Fa-like immunoreactivity. Neighboring neurons that contained only SCP-like or Fa-like immunoreactivity suggest that this staining is not due to cross-reactivity of the antisera. Although they have moderate similarities in sequence, Fa and the SCPs are members of two distinct peptide classes. In general, they are not found in the same neurons or fibers and often have opposite physiological actions. It will be interesting to determine the physiological interactions of these peptides at the muscles innervated by the neurons containing both the SCPs and Fa.

147.9 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF SMALL CARDIOACTIVE PEPTIDE B TO DENSE-CORE VESICLES IN THE BUCCAL GANGLION OF APLYSIA. W. Reed*, K.R. Weiss, P.E. Lloyd*, I. Kupfermann, M. Chen, and C.H. Bailey. Center for Neurobiology and Behavior, Columbia Univ., P&S, N. Y. S. Psychiatric Institute, New York, NY, and Dept. Pharm. Physiol., Univ. of Chicago, Chicago, IL.

Small cardioactive peptide B (SCP_B) is a potent gastropod neuropeptide that exerts modulatory effects both centrally and peripherally. SCP_B is distributed throughout the Aplysia CNS, but occurs in highest concentrations in the buccal ganglion (Lloyd et al., J. Neurosci., in press; Mahon et al., PNAS, in press). Previous immunofluorescence microscopy of the buccal ganglion revealed SCP_B immunoreactivity ($iSCP_B$) in fibers and varicosities of the neuropil as well as in a selected subset of cell somas, including the somas of two large identified neurons, B1 and B2 (I. Kupfermann et al., Soc. Neurosci. Abstr., 1984). Biochemical studies have demonstrated that SCP_B is synthesized and released by B1 and B2 (Weiss et al., Soc. Neurosci. Abstr., 1985). Here we report the ultrastructural distribution of $iSCP_B$ within the buccal ganglion and specifically in the somas of B1 and B2.

B1 and B2, identified by their size and location within the ganglion, were labeled by intrasomatic injection of ferritin permitting their unequivocal identification in thin sections. One hour after injection, the ganglion was fixed in glutaraldehyde, dehydrated in methanol at -15°C, and embedded in LR White resin. Thin sections were stained for $iSCP_B$ using the avidin-biotin complex-horseradish peroxidase method of Whitnall et al. (Science, 222:1137).

Immunostaining of ferritin-labeled B1 and B2 cell bodies reveals that $iSCP_B$ is found within the lumens of large somatic dense-core vesicles. No organelle other than the dense-core vesicle is labeled. Occasional neurites and synaptic varicosities in the neuropil, as well as a number of unidentified somas in the ganglion cortex are also labeled. In each case the reaction product is confined to dense-core vesicles similar to those found in the somatic cytoplasm of B1 and B2. Preabsorption of the primary antibody with SCP_B inhibits staining in a dose-dependent fashion suggesting that the staining is specific for SCP_B-like antigens. A number of neurons contain dense-core vesicles that show no $iSCP_B$. When a primary antibody to Phe-Met-Arg-Phe-amide (FMRF-amide), another bioactive peptide common in Aplysia, is used, the dense-core vesicles of some of the $iSCP_B$-negative neurons are stained. This demonstrates that the staining is dependent upon a cell-specific content of the dense-core vesicles. Together with the biochemical evidence, these data suggest that SCP_B in B1 and B2 is sequestered into dense-core vesicles.

147.10 CALCIUM DEPENDENT-RELEASE OF NEUROPEPTIDES (THE SCPs) EVOKED BY INTRACELLULAR STIMULATION OF SINGLE IDENTIFIED APLYSIA NEURONS IN CELL CULTURE. K.R. Weiss, S. Schacher, I. Kupfermann, and P.E. Lloyd*. Center for Neurobiology & Behavior, Columbia Univ., N.Y. State Psychiatric Inst., New York, N.Y. 10032; Dept. of Pharmacol. Physiol. Univ. Chicago, Chicago, IL. 60637.

A pair of large bilaterally symmetrical buccal neurons B1 and B2, in situ, contain and synthesize neuropeptides SCP_A and SCP_B. Individual B1 and B2 neurons, maintained in dissociated cell culture, were used to investigate whether these putative neurotransmitter peptides are released by spike activity. Immunocytological staining of cultured B1 and B2 neurons revealed the presence of SCP-like immunoreactivity in their somata, processes, and varicosities. The cell culture technique allowed us to separate neurites from somata, and bioassays of HPLC fractionated material demonstrated that these neurons contained authentic SCP_A and SCP_B, both in their neurites and somata. HPLC fractionation of material from cells cultured in the presence ^{35}S-methionine revealed that cultured neurons synthesize the SCPs and transport these peptides into their neurites. The release of ^{35}S labeled peptides, resulting from spike activity, was measured in the presence of normal and low levels of calcium. Cell activity was controlled by intracellular electrodes, which were used either to silence the cell or to stimulate trains of spikes (1000 to 2000 spikes per 10 minutes). Spike activity resulted in measurable release of SCP_A and SCP_B, and this release was calcium dependent. In favorable preparations, 3-5% of the total neuritic content of SCP_A and SCP_B was released. These results show that neurons grown in the absence of targets can release neuropeptides. The demonstration of spike dependent and calcium dependent release, taken together with other evidence for the subcellular localization and potent physiological actions of the SCPs, suggest that these neuropeptides serve as neurotransmitters or neuromodulators.

147.11 FMRF-AMIDE IN NEURON R2 OF APLYSIA: EVIDENCE FOR ITS ROLE AS A SECOND NEUROTRANSMITTER. R. T. Ambron, P. Lloyd, M. S. Flaster, and S. Schacher. Ctr. for Neurobiol. & Behav., Depts. Anat. and Cell Biol., Columbia Univ., College of P&S, and NYS Psychiat. Instit., New York, NY, and Dept. of Physiol. & Pharm., Univ. of Chicago, Chicago, IL.

R2, the giant neuron of the abdominal ganglion, is cholinergic (Giller, E. & Schwartz, J., J. Neurophysiol., 34:93, 1971). We recently reported that R2 in vitro can regenerate neurites and form chemical connections with a variety of identified abdominal ganglion neurons including R15 (Schacher, S. et al., J. Neurosci., 5:1985, in press). R2 elicits both typical cholinergic as well as noncholinergic responses in these cells indicating that R2 releases both ACh and another transmitter.

Recently, R.O. Brown et al. (Soc. Neurosci. Abstr., 10:691, 1984) have reported finding molluscan peptide Phe-Met-Arg-Phe-amide (FMRFa)-like immunoreactivity in R2. We have extended these studies using both the intact ganglion preparation, and R2 synaptically connected to R15 in vitro, and present additional evidence for the role of FMRFa as a second neurotransmitter in R2.

To demonstrate that R2 makes FMRFa, we first exposed the isolated central nervous system to ^{35}S-methionine for 24h. Examination of R2's cell body and peripheral axons by HPLC showed that each contained a single radiolabeled peak that eluted with authentic FMRFa. The peptide is conveyed to the periphery by rapid axonal transport since it moves at a rate exceeding 60 mm/day and its transport was blocked by 2.5 mM colchicine.

HPLC analysis also demonstrated that radiolabeled FMRFa was synthesized by R2 growing in vitro and was transported into the neurites. The distribution was examined more closely using anti-FMRFa antibody and indirect immunofluorescence. The neuritic varicosities and growth cones of R2 growing in vitro with or without targets showed intense staining while cell bodies and non-varicose segment of neurites stained weakly. Furthermore, bath application of 5×10^{-7} M FMRFa produced an increased conductance hyperpolarization of long duration in R15 in vitro that mimicked the noncholinergic response evoked by spike activity in R2.

Like other Aplysia neurons in culture, R2 synthesizes, processes, and transports peptide transmitter even when grown in the absence of target cells (Flaster et al. and Weiss et al., these abstracts). The electrophysiological data demonstrate that R2 releases a second transmitter with FMRFa-like activity. Thus, the cell culture of R2 with and without target cells from the abdominal ganglion will be useful for studying the formation and maintenance of a dual-transmitter synapse.

CNS NEURONS: VERTEBRATE AND INVERTEBRATE

148.1 PAIRED-PULSE ANALYSIS OF NEUROPILE UNIT DISCHARGE EVOKED BY SLOW CONDUCTING FIBERS IN THE VENTRAL NERVE CORD OF LUMBRICUS TERRESTRIS J.L. Johnson. Dept. of Physiol. & Pharmacol., USD School of Medicine, Vermillion, S.D. 57069

The isolated ventral nerve cord (VNC) was stimulated as a site rostral to the clitellum. Intracord recordings of evoked responses were obtained at varying distances caudally using glass micropipette electrodes. In all cases, the stimulus pulse duration was 0.2 msec. All neuropile unit responses recorded were dependent upon activation of slow conducting intersegmental projections, and were not obtained at all if the dorsal giant fibers alone were activated. The earliest intersegmental small fiber system had a conduction velocity of 0.7-0.9 m/sec., and were activated at a stimulus strength of 2-2.5 volts. Increasing stimulus strength applied to the cord to higher levels activated additional intersegmental projections which could induce neuropile activity lasting for 50-100 msec. Neuropile unit responses were analyzed using paired-pulse (P1 - P2) stimulation of the rostral VNC at strengths sufficient to excite the slow intersegmental projections. Contrary to previous data (J. Exp. Biol., 45:141,1966),facilitation of the P2 response was clearly in evidence for most of the recorded neuropile units. The observed forms of facilitation of neuropile unit discharge include: (a) decreased latency for the P2 evoked response, (b) increased duration of P2 induced burst discharge, (c) shifting of the P2 induced discharge to a longer latency in the relative refractory period of the P1 stimulus (equivalent to preservation of response at short P-P2 interpulse delays), and (d) the sudden ability of the dorsal giant fibers to evoke neuropile discharge at the P2 stimulus at specific P1-P2 interpulse delays. For a total of 9 different neuropile units, the decrease in P2 response delay was 2.60 ± 0.42 (M ± SD) msec. using a P1-P2 interpulse delay of 5-26 msec. At rapid stimulation rates applied to the intersegmental projections, this form of facilitation could account for a highly significant phase-shift of response for these neuropile units which could be of physiological significance. In other neuropile units (such as b above) the increased duration of burst discharge with sustained activation could enhance the effectiveness of transmission at selected reflex circuits. Only one neuropile unit was recorded which was profoundly inhibited after the discharge induced by small fiber projections. This unit had a peak discharge rate of 20-25 Hz, and gave a single spike discharge after a latency of 45-52 msec with a 0.9 cm conduction distance.

It is anticipated that the differing forms of temporal interaction uncovered above will prove to be of central importance in the regulation of locomotion by the massive neuropile network of the earthworm VNC.

This work was supported by Parsons Trust Fund.

148.2 SNAIL GANGLIA PREPARATIONS RELEASE PROSTAGLANDINS. K.S. Madden* (SPON: W.G. Van der Kloot). Dept. of Pharmacology, New York Medical College, Valhalla, NY 10595.

In the A-cell of the snail, Helix aspersa, the action potential (AP) configuration and firing frequency can be systematically influenced by treatments known to increase or to decrease extracellular concentrations of prostaglandins([PG]$_{out}$)(1). The implied release of PGs from subesophageal ganglia has now been confirmed by radioimmunoassay(RIA). Thus, endogenous PGs may normally modulate cellular mechanisms determining APs in the A-cell.

Snails were fed or unfed for at least two months preceeding experiments. Ganglia (n=1 to 6) were dissected free of most connective tissue and placed in a holding bath. They were transferred to fresh snail saline (4 or 5 ml) at the start of an experiment. Samples of bathing medium (0.5 ml) were stored at -20°C until assayed. Derivatives of arachidonic acid(AA) formed by the action of cyclooxygenase(CO) were studied using standard RIA techniques and antisera for PGE_2, $PGF_{2\alpha}$, thromboxane(TX)B_2 and 6-keto-$PGF_{1\alpha}$. Indomethacin-Na$^+$(INDO, 3-12 μM)(2), an inhibitor of AA metabolism, was used in some experiments.

PGE_2, $PGF_{2\alpha}$ and TXB_2 were detected but 6-keto-$PGF_{1\alpha}$ was not. Extracellular concentrations of the predominant derivative, PGE_2, increased with time and through a process that was blocked by INDO. CO appears to metabolize AA in subesophageal ganglia.

Ganglia from fed snails released doses of PGE_2 that could exceed 3 nM which can account for changes in the AP. Ganglia from unfed snails released 10-fold less PGE_2. In these cases, synthesis may be reduced owing to decreased availability of AA, an essential fatty acid. Reduced synthesis may help to explain alterations in the A-cell activity that are associated with extended estivation.

PG synthesis is a Ca^{++}-dependent process involving the rearrangement of membrane phospholipids. In snail ganglia, manipulating the PG biosynthetic pathway also affects APs in the A-cell. This may be owing to direct changes in the membrane mechanisms mediating the cell's electrical excitability, to indirect effects of altering $[PG]_{in}$ and/or $[PG]_{out}$ or to some balance of these effects.

PG release from human and other vertebrate central nervous system(CNS) tissues is well-documented, but its function is not clear. The results of these studies suggest that the activity of certain vertebrate CNS neurons may be similarly modulated by PGs. Conditions that alter the PG biosynthetic capacity may enhance or depress CNS processes influenced by the activity of these neurons.

1 Madden, K.S. and Van der Kloot, W.G. (1984) Endogenously synthesized prostaglandins may modulate pacemaker activity in the snail A-cell. In: Prostaglandins and Membrane Ion Transport, pp.235-239. Eds: P. Braquet et al, Raven Press, New York.

2 Madden, K.S. and Van der Kloot, W.G. (1985) Indomethacin, prostaglandin E_2 and transmission at the frog neuromuscular junction. J. Pharmacol. Exp. Ther., 232, pp. 305-314.

148.3 REGIONAL DISTRIBUTION OF CALCIUM INFLUX INTO BURSTING NEURONS DETECTED WITH ARSENAZO III. W.N. Ross and K. Graubard. Dept. of Physiology, New York Medical College, Valhalla, NY 10595, Dept. of Zoology, Univ. of Washington, Seattle WA 98195, and Marine Biological Laboratories, Woods Hole, MA 02543.

Absorbance changes of the metallochromic indicator Arsenazo III were used in conjunction with an array of 100 photodiodes to measure changes in intracellular calcium at many positions simultaneously in identified neurons of the crab stomatogastric ganglion. The ganglion was dissected, desheathed and mounted on the stage of a Zeiss Universal Microscope. In some preparations the esophageal and paired commissural ganglia were left attached to enhance the voltage oscillations and to aid in cell identification. Normal saline was used. Arsenazo III was injected iontophoretically into the soma and spread rapidly throughout the cell. Calcium changes were monitored as changes in absorbance at 640 ± 30 nm. Using a 25X water-immersion lens, each pixel in the array detected from an area of 63 x 63 μm^2, allowing a clear separation of signals from the soma, neuropil, and axonal regions. The exact shape of these regions was determined by filling the neuron with Lucifer Yellow after the experiment.

When stimulated intrasomatically, several neurons showed calcium changes all over the cell with approximately the same time to peak at each position. Since calcium diffuses slowly in cytoplasm the early changes in each position result from calcium entering through channels close to the location of the absorbance change. This indicates that calcium channels are distributed widely in the neuropil and on the soma. When cells were allowed to oscillate without stimulation, absorbance oscillations were detected all over the neuropil but not in the soma. A comparison between the membrane potential recorded in the soma and the calcium signal in the neuropil shows that the calcium changes followed the slow wave oscillation with peak calcium detected 50-150 msec after the end of the voltage plateau. In one pyloric LP neuron the calcium oscillations in the neuropil could be detected without signal averaging indicating that the magnitude of the calcium changes was substantial.

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148.4 EFFECTS OF MONOVALENT CATIONS ON SUBSTANCE P-SENSITIVE INWARDLY RECTIFYING CURRENTS IN NUCLEUS BASALIS NEURONS. K. Yamaguchi*, Y. Nakajima, S. Nakajima and P.R. Stanfield. Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Substance P (SP) produces a slow depolarization concomitant with an increase in the membrane resistance in cultured cholinergic neurons of the rat basal forebrain (1). The present study is concerned with the ionic mechanisms of this SP-induced depolarization. We used cholinergic neurons from the nucleus basalis in dissociated cell culture prepared as previously reported (1). The whole cell patch clamp was used for electrophysiology. Pressure ejection of SP (3 μM) onto the neurons (in TTX), while the cell was voltage-clamped at resting potential, produced a slow inward current. When the cell was clamped at a hyperpolarized level, SP elicited a decrease in the inward holding current, indicating a reversal in the SP induced current change. Current-voltage relations were measured before the application of SP (control), and immediately after the application of SP (while the SP effect was still persisting). The SP-sensitive current, obtained by subtracting the current during the SP-stimulation from the control current, revealed an inwardly rectifying property. This SP-sensitive current was nearly abolished by Rb^+. These results indicate that the SP-sensitive currents are inward rectifier K-currents (2). At large negative potentials, the inward currents started to decline during the hyperpolarizing pulse, suggesting a voltage- and time-dependent block of the rectification channels. SP application seemed to accelerate the time course of this blocking. However, the block of inwardly rectifying channels by hyperpolarization as well as the accelerating effect of SP on this block persisted without the presence of external Na ions (replaced by tetramethylammonium ions). When the external solution was replaced by isotonic 100 mM-K^+, 0 mM-Cl^- solution, large hyperpolarizing pulses produced large inward currents (presumably partly carried by inward rectification channels). SP decreased the magnitude of these inward currents. Application of Cs^+ (0.1, 0.5 mM) to the external solution diminished the inwardly rectifying currents. This effect became more pronounced as the membrane was more hyperpolarized, suggesting a voltage-dependent block of the channels by Cs^+. The SP effect became less in the Cs solution, and again this decrease of SP effects by Cs^+ became more pronounced at larger hyperpolarization. This result indicates that SP affects channels that are blocked by Cs^+ at large hyperpolarizations, strengthening the idea that SP has effects on the inward rectifier channels.

(Supported by NIH Grant NS-10457).

(1) Nakajima, Y., Nakajima, S., Obata, K. and Carlson, C.G. (1984) Soc. of Neurosci. Abstract, 10: 659.
(2) Stanfield, P.R., Nakajima, Y. and Yamaguchi, K. (1985) Nature, in press.

148.5 PROPERTIES OF A DEPOLARIZING AFTERPOTENTIAL IN RAT PIRIFORM NEURONS. N.W. Milgram and M. Segal*. Center for Neurosciences, The Weizmann Institute of Science, 76100 Rehovot, Israel.

Several lines of evidence have implicated the piriform cortex in the development of epilepsy. In the kindling model, spontaneous spiking appears first in the piriform region. Piriform cells also exhibit a special reactivity to excitatory neurotoxins as evidenced by the occurrence of extensive cell death in aminal models of status epilepticus. The present research sought to determine if the epileptogenic susceptibility of this region is reflected in unique cellular properties. Special attention was focused on depolarizing afterpotentials (DAP) recorded in these cells. Intracellular recordings were made from slices of rat piriform cortex cut parallel to the anterior-posterior course of the lateral olfactory tract and maintained in an interface chamber. Prominant DAPs were recorded in over 77% of 35 cells recorded in the superficial pyramidal layer. In every instance, the DAP originated off the falling phase of the action potential (AP). In most cases there were two distinguishable components: a small redepolarization to a level 14±1 mV above resting potential which had the appearance of a small bump and a slow decay (42.8±3.4 msec) back to the resting potential. The DAP was accompanied by an increase in membrane conductance, and had a sharply delineated reversal potential of 57.5±1.2 mV which was at the peak level of depolarization achieved. This DAP appears to represent an intrinsic property of piriform cells; the response has not been observed in the absence of an AP, and shows no decrement during high frequency firing. In the large majority of cases when APs were triggered by current injection, the DAP was the only afterpotential manifested. When action potentials were triggered by excitation of afferents, however, there were also hyperpolarizing afterpotentials. Experiments designed to analyze the ionic mechanism for generating DAPs indicate that neither Cl^- nor Ca^{++} are critical, but Ca^{++} is involved in the initial redepolarization.

Functionally the DAP increases the excitability of piriform cells as indicated by: 1. a decrease in AP threshold during the DAP; 2. the appearance of doublets or bursts off the DAP which could be triggered by brief pulses and which occurred spontaneously; 3. the emergence of spontaneous bursting in cells injected with Cs which produced a marked enhancement of DAPs.

148.6 MAUTHNER AXON-EVOKED INHIBITION OF AN INDENTIFIABLE FOLLOWER NEURON REQUIRING AN UNUSUAL SYNAPTIC MECHANISM. J.T. Hackett and L.J. Greenfield, Jr*. Dept. of Physiol., Univ. of Virginia, Charlottesville, VA 22908

A single impulse evoked in a goldfish Mauthner (M) axon can synchronize a unilateral contraction of the body and a bilateral contraction of the cranial muscles. Several neurons, each postsynaptic to both M-axons, relay impulses to the cranial motoneurons on one side. We now report a separate pathway through a follower neuron which is inhibited by the ipsilateral M-axon and excited by the contralateral one.

Two microelectrodes were used to obtain simultaneous intracellular recordings from a M-cell and a follower neuron. The latter were found under the facial lobe, parallel to and on the medial side of the M-axon, and overlapping the caudal extent of the cranial relay neurons (CRNs). Follower neurons were distinguished by a hyperpolarizing IPSP evoked by ipsilateral M-axon stimulation. The IPSP could be inverted by hyperpolarizing current injections or by injecting Cl^- into the neurons. Injections of depolarizing currents into these neurons evoked repetitive firing without a response in the cranial muscles. Impulses in CRNs failed to produce either the EPSPs or IPSPs in the follower neurons and measurements of synaptic delay were consistent with this finding. The synaptic delays measured from the rising phase of the M-axon spike to the ipsilateral IPSP was 0.51 ± 0.09 msec (n=14), to the contralateral EPSPs was 0.45 ± 0.035 msec (n=4), and to the CRN-EPSP was 0.44 ± 0.02 msec (n=8). These data indicate the possibility that the M-axon transmitter produces several synaptic actions, which we examined pharmacologically. The IPSP was blocked by strychnine applied either topically to the fourth ventricle (3 μl, 2.7 mM) or injected i.p. (3 μg/gm fish). At these concentrations, the EPSPs were not blocked. However, the EPSPs could be blocked by a 20 times higher dose. D-tubocurarine applied topically (3 μl, 14 mM) selectively blocked the EPSPs. Penicillin (3 μl, 250 mM) applied topically did not block the IPSP or the EPSP.

We conclude that a neuron can be individually identified which receives inhibitory and excitatory inputs from the ipsilateral and contralateral M-axons, respectively. The M-axon collaterals do not cross the midline; therefore, the follower neuron may be bilateral. The transmission time is too short for two chemical synapses, which raises three interesting possibilities: 1) the release of two transmitters from the M-axon, 2) a non-spiking inhibitory neuron excited by the M-axon via an electrical synapse, or 3) the differential sensitivity to pharmacological agents of functionally different postsynaptic receptors for the same transmitter. (Supported by NSF Grant BNS 84-06259)

148.7 CELLULAR AND SYNAPTIC PHYSIOLOGY OF TURTLE HIPPOCAMPUS. J.M. Shen and A.R. Kriegstein. Dept. of Neurology, Stanford University Med. School, Stanford, CA 94305.

The dorsal cortex of the turtle, Pseudemys scripta, consists of two principal classes of neurons that occupy three distinct layers. Morphological, pharmacological and physiological data suggest many similarities to mammalian neocortex, rendering it a favorable preparation for comparative studies. We have begun to investigate the cellular physiology of the turtle medial cortex, which is anatomically homologous to mammalian hippocampus. 500 μm-thick coronally vibratomed sections or intact cortical slabs were placed in a chamber and perfused with oxygenated saline at 22°C, and intracellular recordings were made from the region of the medial cortex with 3M K acetate-filled microelectrodes.

We found a group of spontaneously bursting neurons (mean membrane potential = -50 mV, mean input resistance = 84MΩ) confined to a restricted zone in the dorsal medial cortex (DMC). Cells with physiological properties resembling pyramidal or stellate neurons of dorsal cortex were also present in this region, but spontaneously bursting neurons were never encountered more laterally or medially. Bursts ranged from brief depolarizing events consisting of only two or three spikes to long (up to 3 seconds) depolarizations with multiple action potentials (APs). Interburst intervals (IBIs) also varied from cell to cell even within the same slice, lasting from 1 to 8 seconds; some cells had extremely constant IBIs. Most bursts seemed to be synaptically mediated since hyperpolarizing the cells revealed spontaneous underlying excitatory postsynaptic potentials (EPSPs).

Extracellular electric shocks elicited inhibitory postsynaptic potentials (IPSPs) in neurons medial to DMC that are reminiscent of dorsal cortical pyramidal cell responses. DMC neurons, however, responded to both medial and lateral stimulation with an initial burst of APs often followed by IPSPs lasting 1-20 seconds. IPSPs were usually more pronounced following medial stimulation and were often interrupted by bursts of APs or EPSPs.

Spontaneously active zones were marked with focal applications of pontamine sky blue. Subsequent histological studies localized these sites to a restricted area in DMC characterized by a loosely packed pyramidal cell layer. An antibody specific to rat limbic system (kindly provided by Dr. Pat Levitt) intensely labelled turtle DMC neurons. Neurons in more medial areas were lightly stained in comparison, while dorsal cortical neurons were completely unstained. Thus, turtle medial cortex contains distinct physiological, anatomical, and immunohistochemical regions remisiscent of subfields of mammalial hippocampus.

Supported by NIH Grants NS00887-01 and NS21223-01.

148.8 EXCITATORY SYNAPSES BETWEEN CA3 NEURONS IN THE GUINEA PIG HIPPOCAMPUS. R. Miles* and R.K.S. Wong (SPON: R.A. DiCaprio). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

We have examined the properties of recurrent excitatory synapses in the CA2-CA3 region of the guinea pig hippocampal slices using two techniques. 1. simultaneous intracellular recordings from pairs of CA3 cells. 2. antidromic activation of CA2 pyramidal cells by stimulating CA1 stratum oriens. Recurrent epsp's were elicited in CA3 neurons separated from CA2 by a cut made from the alveus to the edge of the mossy fiber layer. Picrotoxin was used to suppress synaptic inhibition.

The amplitude of unitary mono-synaptic epsp's was 0.8-1.8 mV (n=6). Their rise time was 6-12 ms and duration at half amplitude 20-30 ms. Recurrent epsp's initially decayed more slowly than either step somatic depolarizations or epsps' evoked by mossy fiber stimulation. In Cs loaded neurons they reversed at more positive potentials than mossy fiber epsp's. Thus, some recurrent synapses apparently are made onto distal apical dendritic sites.

A slow voltage-dependent decay and an undershoot following unitary and antidromic recurrent epsp's suggested that intrinsic currents were activated. The undershoot, a 0.2-0.4 mV hyperpolarization of time to peak 60-80 ms from onset of unitary epsp's was blocked by intracellular injection of Cs and TEA. In these conditions epsp duration was greatly prolonged in a subthreshold potential range. This behavior seems to depend on intrinsic inward and outward currents since the voltage dependence of 2 mV - 5 ms intradendritic, depolarizing pulses mimicked that of recurrent epsp's.

A pre-synaptic burst lead to considerable temporal summation of unitary epsp's. A second epsp at interval 8-12 ms was strongly facilitated. Later epsp's at similar interval were facilitated less, possibly due to the growth of the intrinsic hyperpolarizing conductance. Thus a burst evokes a summed epsp of amplitude 2-5 mV, time to peak 25-50 ms with a falling phase of similar duration followed by an undershoot. Events of this time course were also elicited via multi-synaptic connections (n=28) revealed in the presence of picrotoxin. They were evoked at variable latency by pre-synaptic bursts but not by single spikes.

(Supported by NS 1864).

148.9 LOGIC OPERATIONS COULD BE MEDIATED BY INTERACTIONS BETWEEN EXCITABLE DENDRITIC SPINES. G.M. Shepherd and R.K. Brayton. Sect. of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510 and IBM Watson Res. Ctr., Yorktown Heights, NY 10598.

As part of an ongoing study of distal dendritic microcircuits, we have tested the hypothesis that excitable dendritic spine interactions (Shepherd, G.M., Proc. Nat. Acad. Sci., in press) could mediate logic operations. Using an electrical circuit analysis program, we have extended our previous compartmental model to include 4 spines arising from a length of dendritic branch. Each spine head included a model for an EPSP and a Hodgkin-Huxley action potential; the spine stems and dendritic compartments were electrically passive.

Three types of elementary logic operations have been analysed. An AND gate was simulated by simultaneous EPSPs in 2 adjacent spines. EPSP conductances (gEPSP) of 1 nS were sufficient in the model to trigger action potentials in spines 1 and 2, which then triggered saltatory conduction to 3 and 4. An OR gate was simulated by an EPSP in spine 1 or spine 2. In this case, a larger gEPSP of 2 nS was necessary to trigger an action potential, which then triggered the remaining 3 spines in sequence. Finally, an AND-NOT gate was simulated by combining an EPSP in spine 1 with an IPSP at different sites. When the IPSP was sited on the stem of spine 1, the spine impulse sequence fired when gIPSP was 2 nS, and was blocked by 3 nS. When the IPSP was sited on the dendritic branch near the origin of spine stem 1, a larger gIPSP of 7 nS was necessary to block the spine impulse sequence.

The results suggest that logic operations are inherent in the nonlinear interactions between excitable spines. They are consistent with conclusions (Rall, W. and Segev, I., in New Insights into Synaptic Function, ed. Edelman et al, New York: Wiley, in press) obtained from analysis of interactions between clusters of excitable spines, suggesting that the proportion of active spines in a distal dendritic tree would be limited, because their combined current would tend to fire the tree and override IPSPs in their path. It is possible that spines undergo dynamic turnover, and that their functional maturation can involve both synaptic and voltage-sensitive membrane channels, together with related metabolic, second messenger and cytoskeletal elements. If the proportion of mature excitable spines is thus limited, inhibitory synapses can be very effective in controlling their interactions, especially in thinner, more distal, dendrites. Thus, despite small numbers of inhibitory synapses on dendritic branches, their presence at strategic locations would give them powerful roles in establishing dendritic logic gates. The interactions between excitable spines were exquisitely sensitive to small changes in spine stem resistance, further supporting Rall's suggestion that these changes could provide a mechanism underlying learning and memory.

148.10 MODULATION OF EXCITABILITY OF FUNCTIONALLY RECONSTRUCTED CORTICAL PYRAMIDAL NEURONS BY DISTAL SYNAPTIC INPUT. T.M.McKenna, D.H.Perkel, N.M.Weinberger. Theoretical Neurobiology Facility, Dept. Psychobiology and Center for the Neurobiology of Learning and Memory, U.Cal. Irvine

A multi-compartmental neuron was constructed based on morphological and biophysical measurements from published data obtained in sensory and motor cortices. Neuronal time constants were obtained by injecting current pulses at the soma and peeling 2 or 3 time constants from the membrane potential decay. These time constants were found to depend upon the distribution of voltage dependent Na and K channels in the neuron.

We examined the pattern of action potentials (AP) produced by trains of inputs at the proximal apical dendrite and the modulation of this pattern by synaptic inputs placed on the distal apical dendrites or the basilar dendrites. The modulating input consisted of independent Poisson trains in three or more synaptic afferents. For a range of membrane specific conductances, the influence of the distal apical inputs on the probability of eliciting AP's from a regular input train at the proximal dendrite was found to be critically dependent on the duration of the delay between the beginning of the distal and the proximal input trains. The number of AP's produced was increased when the distal and proximal trains began within 60 ms of each other (either leading) and was maximally facilitated (>2X) when the two trains began simultaneously. When the distal train led the proximal train by more than 60 ms, the mean number of AP's was unchanged but the variance greatly increased. On trials when no spikes were produced, "recordings" of ion channel gates revealed that the tonic depolarization produced by the distal input resulted in Na inactivation. Distal trains that began more than 60 ms after the proximal train had no effect on the neuron's output. By contrast, input trains on the basilar dendrites could produce AP's at any time during the proximal train, depending on the precise relative timing of synaptic potentials.

Thus, electrotonically long dendrites can smooth synaptic potentials sufficiently so that sustained depolarizations may lead to lowered neuronal excitability. Furthermore, the relative time of onset of input trains in distal and proximal dendritic synapses is critical for increasing discharge probability.

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148.11 TRANSFER FUNCTION AND INTEGRATION IN CENTRAL NEURONS. L. Moore, B. Christensen and K. Yoshii. Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

A first approximation to the integrative properties of a single neuron can be assessed by measuring the linear input-output transfer function for different regions of the cell. The transfer function at the soma is a critical determinant of output since the threshold level that triggers an impulse is generally reached here. In order to measure transfer functions at the soma and the tip of a dendrite a mouse neuroblastoma tissue culture cell (NG-108) was used. These cells have prominent somas with branching neurites containing large growth cones at their ends. A low resistance patch electrode was employed for whole cell voltage and current clamp admittance and impedance measurements (Moore & Christensen, 1985) from either growth cones or the soma of the cell. Morphological measurements were made from each cell and a branched cable model (Koch, 1984) containing voltage dependent conductances was used to interpret the frequency domain data from the same cell. These cultured cells show typical excitability properties for both somatic and growth cone recordings. Synaptic inputs were simulated by pressure application of transmitters or a high concentration of potassium ions through a microelectrode. Steady state depolarization of the cells activated the voltage dependent conductances which led to marked changes in the measured transfer functions. These results have consequences for neuronal integration and function. First of all, the neuron is not just a simple summing amplifier of synaptic input. The change in the bandpass characteristics during synaptic activation facilitates access to the soma those synaptic potentials with higher frequency components. The cell becomes "tuned" to conduct synaptic potentials with frequency components in a particular range and filter out the rest of the synaptic fluctuations. Also, the dendritic tree may either have different tuning curves or modulate the soma response depending on the synaptic input at any given time. Thus, the neuron has an inherent mechanism of filtering site specific synaptic input. In this sense, the neuron is also a spatial filter selecting preferentially for synaptic input located on particular portions of the dendritic tree.

Koch, C. 1984. Biol. Cyber. 50: 15-33.
Moore, L. & Christensen, B. 1985. J. Neurophysiol. 53: 636-651.

PEPTIDES: BIOSYNTHESIS AND METABOLISM I

149.1 PURIFICATION, IDENTIFICATION AND LOCALIZATION OF BOMBESIN-LIKE IMMUNOREACTIVITY IN BOVINE ADRENAL MEDULLA. S. Lemaire. Département de Pharmacologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.

Immunoreactive bombesin (ir-BB) was found in acid (HCl) extracts of bovine adrenal medulla at a concentration of 27 pmoles per g of tissue. It was purified by successive chromatography through Sep Pak C-18 cartridges, Sephadex-G-50, partition chromatography and high pressure liquid chromatography. The latter purification step gave rise to four peaks of immunoreactivity (fractions I, II, III and IV). The major peak (fraction II) eluted closely to the amphibian synthetic peptide bombesin and a minor peak coeluted with synthetic porcine gastrin releasing peptide (GRP). The cellular and subcellular distributions of ir-BB were also analyzed. Bovine adrenal chromaffin cells were isolated by successive digestions with collagenase. Subpopulations of these cells were obtained by centrifugation through stepwise bovine serum albumin gradients. Three distinct cell layers (I, II and III) were obtained at the interfaces of the gradient: cell layer I was enriched in noradrenaline (adrenaline/noradrenaline ratio: 0.63) and its content in ir-BB was relatively high (1.64 pmoles/10^6 cells). On the other hand, cell layers II and III were enriched in adrenaline (adrenaline/noradrenaline ratios of 1.3 and 3.3, respectively) and their content in ir-BB was much lower (0.28 and 0.17 pmoles/10^6 cells, respectively). The subcellular fractions (granules, microsomes, mitochondria and cytosol) were obtained by differential centrifugations of an homogenate of bovine adrenal medulla. ir-BB was mostly concentrated in the secretory granules (0.65 pmoles/mg protein) as compared with the other cell constituents (from 0.06 to 0.19 pmoles /mg protein). These data indicate that bovine adrenal medulla contains various forms of bombesin-like material and that this material is more specifically localized in secretory granules along with catecholamines (most probably noradrenaline). The putative neuromodulator function of bombesin-like substances at this level remains to be investigated.
(Supported by the Medical Research Council of Canada and the Canadian Heart Foundation).

149.2 EFFECT OF SHORT TERM ELECTRICAL AND CHEMICAL STIMULATION ON THE CONTENT OF METHIONINE-ENKEPHALIN IN THE GUINEA PIG MYENTERIC PLEXUS. A. R. Gintzler, D. Clouet* and J. Glass*. Downstate Medical Center, Brooklyn, N.Y. 11203, and Res. Lab. New York State Off. Drug Abuse Serv., Brooklyn, N.Y. 11217.

Although the structure of both the enkephalin precursor, proenkephalin, and the gene that codes for it has been determined, the various physiological parameters that influence the rate at which proenkephalin is processed to enkephalin remain obscure. This report provides the first demonstration that the rate of enkephalin production is not fixed but is dependent on the degree of neuronal activity. The isolated longitudinal muscle with adherent myenteric plexus (LM-MP) preparation of the guinea pig ileum was chosen as a convenient source of enkephalin-containing neurons. The content of enkephalin in the myenteric plexus was determined by using a highly sensitive and specific radioimmunoassay (RIA) in combination with reverse phase High Pressure Liquid Chromatography (HPLC).

In the presence of Krebs buffer containing 2.5 mM calcium, electrical stimulation (20 Hz for 30 sec) or stimulation by high potassium (60 mM for 4 min) produced a significant increase (approximately 55%, $P<.02$) in the content of enkephalin-like immunoreactivity (ELI) in the myenteric plexus. The content of myenteric ELI was not altered when electrical stimulation was applied in the presence of a calcium free buffer containing the calcium chelator EGTA. Therefore, it appears that the effect of electrical stimulation is dependent on the presence of extracellular calcium. Electrically induced increases in ELI could also be abolished by pretreatment with tetrodotoxin indicating the need for intact functional sodium channels and the propagation of action potentials. Fractionation of LM-MP extract by reverse phase HPLC prior to analysis by RIA indicated that the increase in ELI was due to an elevation in the content of authentic methionine-enkephalin and not of some spurious peptide that cross reacts with the enkephalin antiserum.

These results indicate that tissue levels of enkephalin can fluctuate in response to changes in neuronal activity via a calcium dependent mechanism. The very brief time course for the increase following such stimulation (30 sec and 4 min following electrical and chemical stimulation, respectively) suggests that the elevation in content is due to an increase in the rate of processing of enkephalin precursor(s) to enkephalin and not to an increase in the rate of de novo synthesis of proenkephalin. Supported by a grant to ARG from the National Institute of Drug Abuse #DA02893.

149.3 AGE-RELATED INCREASE IN NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN RAT ADRENAL GLANDS. Hiroshi Higuchi* and Hsiu-Ying T. Yang. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Neuropeptide Y (NPY) is a 36 amino acid peptide, located in neurons of central and peripheral nervous system. Immunohistochemical studies indicate that NPY often colocalizes in catecholamine-containing neurons, suggesting a possible participation of this peptide in catecholaminergic transmission. In the adrenal glands of various species, NPY has been detected mainly in norepinephrine-containing chromaffin cells and also in nerve fibers penetrating through cortex and medulla. Since NPY-like immunoreactivity (NPY-IR) is stored in chromaffin granules, it might be co-released with catecholamines into circulation and function as a cotransmitter in various tissues. Interestingly, NPY appears to modulate the contractions of vascular and cardiac muscles. We show that the NPY content in rat adrenal gland increases with age, regardless of sex. Furthermore, another NPY-like immunoreactive peptide, which is more hydrophilic than NPY, was found to increase in the adrenal glands of aging rats. In contrast, similar age-dependent changes failed to occur in the cerebral cortex and hypothalamus, two brain regions with the highest concentrations of NPY, though injected NPY appears to exert various effects on the blood pressure, circadian rhythm and release of neuroendocrinal peptides in the CNS. The age-dependent changes of NPY-IR occurs specifically in the adrenal glands. Subsequently, we investigated the effects of the splanchnic nerve transection, because splanchnic nerve activity is known to modulate the various chromaffin cell constituents including tyrosine hydroxylase and enkephalins. Interestingly, the transection did not affect the adrenal NPY-IR content during the first week following transection, but abolished the age-dependent increases of NPY and the additional NPY-like immunoreactive peptide in rat adrenal gland. The high NPY content in adrenal gland may have physiologicla implications in adrenal function, and its age-related increase might be related to functional changes in aging animals. The appearance of another molecular form of NPY-IR in aging rats may be an important factor for future study in aging. In addition, the splanchnic nerve activity seems to regulate the NPY-IR content in the adrenal gland. Further investigations are in progress to explore the biosynthesis and functions of NPY and the additional NPY-IR peptide in the adrenal gland.

149.4 CEREBROVENTRICULAR AND VASCULAR METABOLISM OF ANGIOTENSINS IN RAT. J.W. Harding, M.S. Yoshida, R. Dilts, and J.W. Wright. Dept. of VCAPP, Washington State University, Pullman, WA 99164

Much of the data gathered to support a central role for angiotensins in cardiovascular control and body water balance has relied upon the intracerebroventricular injection of angiotensins (AII and AIII) and antagonists. Data gathered using this experimental approach, especially with regard to dose-response relationships, suffers from the unknown perturbation of ventricular metabolism. Initial studies, designed to address this potential problem, involved injection of ^{125}I-angiotensins (AII, AIII, Sar^1 Ile^8-AII) into the lateral ventrical followed by CSF collection via the fourth ventrical. HPLC analysis of collected CSF indicated nearly total metabolism of ^{125}I-AII and ^{125}I-AIII while approximately 50% of the ^{125}I-Sar^1 Ile^8-AII (SI-AII) remained. This degradation was shown to be dependent upon passage of the angiotensins through the ventricular space and not from direct CSF mediated action. Incubation of ^{125}I-AII and ^{125}I-SI-AII at 36°C for up to 90 minutes yield no significant degradation. However, ^{125}I-AIII was degraded by CSF but at a rate much slower than that measured subsequent to ventricular passage. In order to more accurately establish the rate of ventricular metabolism, rats were injected with labeled angiotensins and rapidly killed with focused microwave irradiation. This technique allowed the analysis of time points as short as 5 seconds after injection. The results of this study showed extremely rapid ventricular metabolism of angiotensins. AII had a t 1/2 = 22.5 sec while AII exhibited a t 1/2 = 6.5 sec.

Vascular metabolism of ^{125}I-angiotensins was also monitored and shown to be rapid with t 1/2's for AII = 9.1 sec and AIII = 8.9 sec. HPLC analysis of degradation products is consistent with an obligatory conversion of AII to AIII during the metabolic cascade. In total the results of this study encourage a reevaluation of the potency of various angiotensins (especially AIII) under conditions of reduced degradation and imply a significantly greater potency for AIII at the receptor level.

149.5 SUBSTANCE P CONTENT AND RELEASE IN DISSOCIATED AND MICROEXPLANT CULTURES OF RAT NODOSE GANGLION (VAGAL SENSORY) NEURONS. D.B. MacLean, P. Gulley and S.F. Lewis (SPON: J. Toole), Dept. of Medicine, Wake Forest Univ. Med. Ctr., Winston-Salem, NC 27103.

Substance P (SP), the widely distributed undecapeptide, is synthesized in cell bodies of vagal sensory ganglia and transported bidirectionally towards the medulla oblongata and peripheral sites of sensory innervation. The factors regulating its synthesis in and release from vagal neurons remain largely unknown. Although tissue culture is one promising model to study that regulation, culture of vagal sensory neurons has been limited. Recently, a successful dissociation technique has been described (Mathieu et al, Neuroscience 13:1373, 1984).

Nodose ganglia were removed from neonatal rats and either cut into 5-7 fragments each using fine scissors or dissociated in neutral protease (Dispase). Explants were maintained in enriched F-12/L-15 medium supplemented with adult rat and human placental serum; dissociates were maintained in enriched L-15, 5% rat serum. Seven to 10 ganglia equivalents were plated per 16mm well. Plating efficiency in dissociates was 15-30% at one week (3000-6000 neurons per well) and did not deline over the ensuing 10 days. Specific immunocytochemical staining of substance P was present in 30-40% of dissociated neurons by one week in culture; staining of microexplants was more variable. In microexplants, immunoreactive SP content per plated ganglion rose from 4.7 ± 1.2 pg/ganglion (G) (mean ± SD) at 1 week to 14.1 ± 3.6 pg/G at three weeks. In dissociates, after 5 days in culture SP content increased linearly with time: 9.0 ± 3.6 pg/G at 6 days, 64.7 ± 6.8 pg/G at 10 days and 128.0 ± 9.3 pg/G at 16 days (851 ± 61 pg/well). In both systems, 30 min epochs in 50mM K+ Kreb's buffer resulted in minimal release by 5-7 days in culture, whereas 10-20% of SP content was released at 14-21 days. Calcium-free, 0.125mM EGTA Kreb's inhibited both basal (low K+) and high K+ stimulated release. In preliminary studies of regulation of SP content, the addition of dibutyrl-cAMP (1mM) for one week resulted in a 4-fold increase in content ($p<.001$); cell number was unchanged. Phorbol ester (PMA), 20μM, had no effect.

Dissociated cultures of vagal sensory neurons produce large amounts of immunoreactive SP and may provide a powerful tool for the study of its regulation and release. Further releasing studies and co-culture with central and peripheral target tissues are in progress to assess specific differentiation of these neurons in culture.

These studies were supported by grant NS18705 from the National Institutes of Health.

149.6 RELEASE OF CORTICOTROPIN-RELEASING FACTOR (CRF)-LIKE IMMUNOREACTIVITY FROM RAT BRAIN REGIONS IN VITRO. C. B. Nemeroff, M. A. Smith, T. A. Slotkin, and G. Bissette. Departments of Psychiatry and Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

Since the discovery of CRF in 1981 (Vale et al. Science 213:1394, 1981), considerable research has been conducted with this neuropeptide quite apart from its neuroendocrine role. It is of considerable interest to determine whether CRF fulfills the requisite criteria to be considered a neurotransmitter in the CNS. Thus, immunohistochemical and radioimmunoassay (RIA) studies have shown that the peptide is heterogeneously distributed in the mammalian CNS. After iontophoretic application, CRF produces alterations in neuronal firing rates in several brain areas. Moreover, intracerebrally administered CRF produces profound effects on the autonomic nervous system as well as on behavior. In the present study a sensitive and specific CRF RIA (Meth. Enzymol. 103:565-572, 1983), was used to measure in vitro secretion of CRF-LI from minced slices of hypothalamus, amygdala, cerebellum, midbrain and striatum. Minced tissue (8 mm^3) was incubated in modified Krebs-Henseleit buffer at 37°C that received 95% O_2/5% CO_2 and pH adjusted to 7.4. Addition of high concentrations (56 mM) of potassium resulted in a marked increase in CRF-LI release. This depolarization-induced release of CRF observed in all brain regions studied except cerebellum and frontal cortex, was calcium-dependent, as evidenced by the lack of effect of high (K^+) in both calcium-free media and in the presence of EGTA. Furthermore scorpion venom released CRF-LI and this release was also calcium-dependent. In the hypothalamic minces, neither 5HT (1-100 μM) norepinephrine (10 μM) or carbachol (0.1-100 mM) did not increase CRF release. The hypothalamus contained the highest concentrations of CRF-LI, but moderate concentrations were found in the midbrain and amygdala. Low concentrations were found in the striatum and cerebellum. These results confirm previous findings derived from use of CRF bioassay methods. The present findings provide further evidence for a neurotransmitter role for CRF in the mammalian CNS. (Supported by NIMH MH-39415)

149.7 DEVELOPMENTAL EXPRESSION OF THE RAT VASOPRESSIN GENE. H.H. Zingg, D. Lefebvre* and G. Almazan*. Laboratory of Molecular Endocrinology, Royal Victoria Hospital, McGill University, Montreal, Quebec, Canada, H3A 1A1.

The nonapeptide vasopressin (AVP) is synthesized as part of an AVP-neurophysin precursor molecule in magnocellular neurons of the hypothalamus. It has been demonstrated by immunocytochemical methods that these neurons acquire the capacity to synthesize AVP by embryonic day 16-18 (J. of Neuroscience 5: 98; 1985). On the other hand, the synaptic input connections regulating the activity of AVP neurons are only fully established by postnatal day 15 (Peptides 1, Suppl. 1: 27; 1980). To investigate the regulation of AVP gene expression during development, we determined the levels of mRNA encoding pre-proAVP-neurophysin in rat hypothalamus during embryogenesis and early postnatal life.

A 2.2 kb fragment encompassing the entire gene for pre-pro AVP-neurophysin was isolated from a rat genomic library (in collaboration with Drs. B.J. Cwickel and J.F. Habener, Boston). A 0.7 kb restriction fragment containing exon C of the AVP-gene was used as a probe. This sequence is specific for AVP mRNA and lacks homology with the oxytocin-neurophysin gene. The hypothalamus of early postnatal and adult Sprague Dawley rats and the midbrain region of fetal rats (7-20 days p.c.) was removed under a dissecting microscope and extracted in 4 M guanidine thiocyanate. RNA was purified by ultracentrifugation through 5.7 M CsCl. Following electrophoresis in 1.5% agarose, the RNA was transferred to a nylon membrane and hybridized with labeled AVP exon C probe. After autoradiography, the intensity of the bands was quantitated by densitometric scanning.

Using these methods, we found that a marked increase in AVP mRNA occurred postnatally: whereas AVP mRNA remained undetectable during fetal life, the levels attained at 0, 1, 2 and 4 weeks postnatally corresponded to <2%, 4%, 49% and 63% of the levels found in the adult. At all stages, a single band of AVP mRNA was detected, suggesting that only a single AVP gene is expressed throughout postnatal development.

The marked increase of AVP mRNA in hypothalamic magnocellular neurons following the 1st week of postnatal life coincides with the completion of synaptic development. These data therefore suggest that synaptic input plays an important role in the developmental regulation of neuronal AVP gene expression.

149.8 **CHARACTERIZING THE SITE OF IN VITRO CENTRAL AND PERIPHERAL PROCESSING OF PEPTIDE E TO ACTIVE FRAGMENTS.** G. L. Hoyer* and T. P. Davis (SPON: A. Dray). Dept. of Pharmacol., Univ. of AZ, Coll. of Med., Tucson, AZ 85724.

In previous studies we investigated the central and peripheral effects of the proenkephalin A derived opioid peptide, peptide E, and known processing fragments BAM-22P, BAM-12P, and met^5-enkephalin on gastrointestinal transit and contractility. These peptides increase intraluminal pressure when arterially perfused through canine small intestine segments and peptide E, BAM-22P and met^5-enkephalin inhibit gastrointestinal transit when administered i.c.v. to mice. Recent investigations have also shown that the opioid peptide β-endorphin may be processed both centrally and peripherally to peptide fragments with opposite effects in pharmacological tests. Therefore, a single peptide precursor, such as peptide E, may serve as a precursor for peptide fragments with different biological activities.

We have previously reported that crude membrane homogenates of whole rat brain and mucosa/muscularis of the canine small intestine process peptide E into biologically active fragments. The present study was designed to characterize the site of peptide E processing by studying various cellular fractions. Purified synaptosomal plasma membranes (SPM) were prepared by the method of Burbach et al (Biochem. Biophys. Res. Comm. 92, 1980). Brain microsomes were isolated by centrifugation of the SPM supernatant at 105,000 g for 80 min. Plasma membranes (mucosa and muscularis) were prepared by the method of Emmelot et al (Biochem. Biophys. Acta. 90, 1964). Each fraction was resuspended in 50 mM Na/K PO_4 buffer (pH 7.4) and time course incubated (37°C) with peptide E (8 nmol) (J. Pharm. Exp. Ther. 227, 1983). The supernatant was analyzed by HPLC for identification and quantitation of the peptide E related fragments (Peptides 5, 1984). Analysis of the data showed significant differences in the formation of peptide E related fragments between tissues. The purified SPM and microsomal fraction of the brain produce met^5-enkephalin preferentially as compared to other peptide E related fragments. The cellular fraction of the mucosa and muscularis of the small intestine produce leu-enkephalin at concentrations similar to met^5-enkephalin. All fractions produce BAM-12P and BAM-22P at significantly lower levels (pmol) than met^5- and leu-enkephalin except the muscularis plasma membrane where the concentration of BAM-12P is comparable (nmol) to that of met and leu-enkephalin.

The rank order of potency of i.c.v. administered peptide E and related fragments, on the inhibition of gastrointestinal transit was peptide E = BAM 22P > met-enkephalin, however BAM-12P was not active in this assay. In the arterially perfused intestinal segment the rank order of potency for increases in contractility was peptide E > BAM-22P = BAM-12P > met^5-enkephalin. It is of interest to note that BAM-12P showed significant activity in the intestinal segment model but not in the centrally mediated transit model. This correlates well with the finding that only the muscularis showed appreciable BAM-12P formation. In conclusion peptide E has been shown to be processed, both centrally and peripherally, to biologically active fragments by the SPM, microsomal fraction, and plasma membranes. The biological activity of these fragments differs from the activity of the parent compound, peptide E. Several other fragments of peptide E are currently being synthesized and tested to determine if peptide E, or one of the formed fragments is responsible for the potent opioid effects.

149.9 **EFFECT OF CENTRALLY ACTING DRUGS ON β-ENDORPHIN PROCESSING BY BRAIN SYNAPTIC MEMBRANE AND MICROSOMAL ASSOCIATED PEPTIDASES.** T. P. Davis and A. J. Culling-Berglund*. Dept. of Pharmacol., Univ. of AZ, Coll. of Med., Tucson, AZ 85724.

In recent years, evidence has accumulated to indicate that both in vivo and in vitro β-endorphin (β-E) serves as a precursor for various neuroactive β-E fragments and has a neurotramsitter or neuromodulator role also. The β-E 1-16 fragment, α-endorphin shows amphetamine-like effects in several pharmacological tests, whereas the β-E 1-17 fragment, γ-endorphin (γ E) shows antipsychotic effects in animal tests.

A previous study in our laboratory reported evidence that specific centrally acting drugs may alter in vitro β-E processing in crude, twice-washed membrane homogenates of whole rat brain after 8 day infusions by minipumps (Eur. J. Pharmacol. 100, 249. 1984). The infusion of the antipsychotic drugs haloperidol and chlorpromazine yielded a significant increase in the formation of γ-type endorphins but not α-type endorphins. Phenobarbital and promethazine showed no effect.

To determine if these drug induced enzymatic alterations were specific to the synaptosomal plasma membrane (SPM) or microsomal fraction we administered chlorpromazine (4.1 mg/kg/day), promethazine (5 mg/kg/day) and phenobarbital (20 mg/kg/day) for 8 days to male Sprague-Dawley rats (140-215 g; n = 5) using Alzet Model 2001 osmotic minipumps. The purified SPM was prepared using the method of Burbach et al (Biochem. Biophs. Res. Comm. 92, 2. 1980) and microsomal fraction was prepared by centrifugation of the final supernatant at 100,000 xg for 60 min. Each fraction was time course incubated (37°C) with β-E (10 nmol) for 30-120 min. Samples were boiled and centrifuged prior to HPLC analysis as previously described (Peptides 5, 6. 1984). Animals receiving the antipsychotic chlorpromazine had a significantly shorter half-life for β-E at the SPM (45.5 min) as compared to animals receiving saline (78.1 min), promethazine (65.8 min), or phenobarbital (83.7 min). The microsomal β-E half-life for promethazine (18.9 min) and phenobarbital (39.6 min) was significantly faster than chlorpromazine (85.5 min) or control (100 min). Together with the 2 to 3-fold increased production of both α- and γ-type endorphins, promethazine and phenobarbital had a significant influence upon the enzymatic activity or concentration in the microsomal fraction. After studying the formation rates of γ-type endorphins from purified SPM's it was further noted that chlorpromazine showed a significant decrease in the formation of des-tyrosine-γ-endorphin and γ-E when compared to either controls, promethazine, or phenobarbital treated rats. However, it was apparent that the formation of the putative antipsychotic peptide des-enkephalin-γ-endorphin was higher in the chlorpromazine treated animals. No significant difference was noted in formation of either α- or γ-type endorphins in the microsomal fraction as compared to controls.

These results suggest that the infusion of chlorpromazine alters membrane associated proteases at the purified synaptosomal membrane but not in the microsomal fraction. This alteration leads to a disruption in the formation of γ-type endorphins but not α-type endorphin although β-E turnover was significantly increased.

149.10 BRAIN EXTRACTS CONTAIN PEPTIDES RELATED TO THE NH_2-TERMINUS OF PROSOMATOSTATIN. R. Benoit and N. Ling. Montreal Gen Hospital Res Inst, Montreal, Que. and Salk Inst, San Diego, CA.

Four peptides derived from pre-prosomatostatin (pre-proSS) have been identified in nervous tissue and shown to be released in vitro: somatostatin-14, somatostatin-28, somatostatin-28(1-12) and pre-proSS(25-100). In order to detect new molecular forms derived from the NH_2-terminal portion of prosomatostatin, antisera were raised against pre-proSS(25-33)-Tyr conjugated to bovine serum albumin using bis-diazotized benzidine. From three antisera that were generated, the one with the lower titer (SMo5) detected the largest amount of immunoreactive material in brain and was selected as the primary antibody in radioimmunoassay (RIA). A sensitive RIA was obtained using pre-proSS(25-33)-Tyr as the cold antigen and pre-proSS (25-33)-Tyr labelled with ^{125}I as the tracer. Tissue from brain regions of adult rats (Sprague-Dawley, 345 g) were extracted with 2 M acetic acid containing pepstatin, PMSF and bacitracin. The extracts were lyophilized, the dried material dissolved in RIA buffer and the amount of proSS NH_2-terminus-like immunoreactivity (proSS-Nt-LI) was measured. Results obtained showed that the hypothalamus contained 7.1 ± 0.4 ng proSS-Nt-LI material per 100 mg wet weight. A uniform distribution was observed in different neocortical regions: 2.8 ± 0.3 ng in frontal cortex, 2.8 ± 0.2 in somato sensory cortex, 2.7 ±0.1 in visual cortex. Levels in pituitary and cerebellum were 1.7 ± 0.1 ng and 0.88 ± 0.04 ng.

In order to further characterize the proSS-Nt-LI, whole brains were extracted with 2 M acetic acid containing peptidase inhibitors and the extract was fractionated on Sephadex G-75. Monitoring of the column effluent by the above RIA revealed that the majority of the immunoreactive material did not coelute with prosomatostatin (M_r: 10.4 kilodaltons) nor with pre-proSS (25-100). It eluted mostly in three zones corresponding to peptides of 6, 3.5 and 1.4 kilodaltons. The 6 K peptide may correspond to pre-proSS(25-88) i.e. prosomatostatin without SS28 and the 3.5 K peptide to pre-proSS(25-56). However, the 1.4 K peptide represents a new entity. It is displaced from a carboxy-methyl cellulose cation-exchanger by 0.06 M ammonium acetate pH 4.6. On a reverse-phase HPLC system using a C_{18} column with 0.1% trifluoroacetic acid/acetonitrile as the mobile phase, the 1.4 K peptide is more hydrophobic than the pre-proSS(25-33)-Tyr standard and elutes at 27% acetonitrile.

These data suggest that post-translational modifications occur at the NH_2-terminus of prosomatostatin. Chemical characterization of the proSS-Nt-LI peptides is required in order to know their structure and their levels in brain and to confirm the precursor-product relationship between proSS and these peptides. Supported by FRSQ, Canadian MRC and NIH (AM18811).

149.11 THE ESTABLISHMENT OF A NOVEL RADIORECEPTOR ASSAY FOR THE MEASUREMENT OF NEURONAL CHOLECYSTOKININ. I.J.M. Beresford* C.R. Clark*, J. Hughes* (SPON: R.G. Hill). Parke Davis Research Unit, Addenbrookes Hospital, Cambridge CB2 2QB, UK

Cholecystokinin (CCK) is one of the most abundant neuropeptides. The measurement of CCK has relied on radioimmunoassay with significant problems experienced in the generation of specific CCK-directed antibodies due to the existence of multiple molecular forms. In view of this and related problems a novel radioreceptor assay (RRA) for CCK using the cerebral cortex CCK receptor is described.

Mice (CFLP) were decapitated, the cerebral cortex removed on ice and homogenised in 90% MeOH: 10% H_2O (10 vols). The supernatant was evaporated to dryness and the reconstituted aqueous extract subjected to C18 Sep-pak chromatography. The purified extracts were then resuspended in RRA incubation buffer (10mM HEPES; 130mM NaCl; 4.7mM KCl; 5mM $MgCl_2$; 1mM EGTA; 5mg/ml BSA; 0.25mg/ml bacitracin; pH 7.2). For the RRA, cortical membranes were homogenised in 10 vols Tris buffer (50mM; pH 7.4), washed twice by centrifugation and resuspended in RRA incubation buffer. A standard curve was obtained by incubating the membranes (10mg prot/ml) with [^{125}I]-CCK 26-33 (0.2nM) and increasing concentrations of CCK 26-33 (10^{-11} - 10^{-6}M) for 2h at 25°C. Reconstituted samples were incubated with membranes and [^{125}I]-CCK 26-33 under identical conditions. Bound and free radioligand were separated by vacuum filtration and the radioactivity counted. The extraction efficiency for CCK 26-33 was 68 ± 6% (n = 5). The RRA sensitivity was 50 fmoles per tube and linear between 50 and 1000 fmoles. The intra- and inter-assay coefficients of variation were 4 and 5% respectively. The inhibition constant for the CCK 26-33 standard curve was 3.13 ± 0.31nM (n = 6). Under these assay conditions, mouse cerebral cortex contained 318 ± 27 pmoles CCK/g tissue. Cerebellum, which lacks CCK, did not inhibit radioligand binding.

Assay of CCK by radioreceptor assay has a number of advantages but perhaps the most important is that the receptor assay will determine the amount of biologically relevant CCK activity in a given sample. This RRA offers the potential for measuring CCK in a variety of tissues, including brain, CSF and plasma.

149.12 ENKEPHALIN CONVERTASE LOCALIZATION IN SPECIFIC NEURONAL PATHWAYS. D.R. Lynch*, W. Clark Venable*, S.M. Strittmatter* and S.H. Snyder (SPON: S. Sato). Johns Hopkins Univ., Dept. of Neuroscience, Sch. of Med., Baltimore, MD 21205.

Enkephalin convertase (EC) is a carboxypeptidase B-like enzyme whose biochemical properties and tissue distribution suggest that it physiologically processes enkephalin precursors. It is specifically inhibited by guanidinoethylmercaptosuccinic acid (GEMSA), and autoradiography with ^{3}H-GEMSA has localized EC to enkephalin containing regions of rat brain. In the present study we have used lesioning techniques in conjuction with ^{3}H-GEMSA autoradiography to localize EC to specific neuronal pathways, some of which contain enkephalin and some of which do not. Previous studies have demonstrated the presence of enkephalins in the stria terminalis by showing that knife cut lesions deplete enkephalin from the stria anterior to the lesion and cause an increase in enkephalins posterior to the lesion. Knife cuts of the stria terminalis increase EC within the stria posterior to the lesion and deplete EC from the bed nucleus of the stria terminalis. These results show that, like enkephalins, EC is axonally transported in the stria terminalis. The hippocampal formation also contains high levels of EC but low levels of enkephalins. EC in the hippocampal formation is concentrated around the granule cells and around the pyramidal cells in layer CA 34. Selective lesions of the dentate gyrus with injections of colchicine into the area of the granule cells deplete EC in the dentate gyrus but do not affect EC levels in the hippocampus around the pyramidal cell layer CA 3-4. Lesions of the pyramidal cells with kainic acid deplete EC in CA 3-4. These results demonstrate the presence of EC in the granule cells, which contain proenkephalin B-derived peptides, as well as in the pyramidal cells, which express neither proenkephalin. The developmental course of EC in these cells also supports this conclusion. While the granule cells are not fully developed in the rat until after birth and do not contain enkephalin like immunoreactivity until approximately postnatal day 10, EC levels are high in the hippocampus several days before birth. This confirms that EC in the hippocampus is found within the pyramidal cells as suggested by the lesioning studies. We have also localized EC to sensory cells in the trigeminal ganglion, which are devoid of enkephalins but do contain the peptides substance P, VIP, and CGRP. Thus, EC distribution primarily parallels that of enkephalins and is found in enkephalinergic pathways, but also occurs in a few regions which do not contain enkephalins. Accordingly, EC may process other neuropeptide precursors in some regions of the nervous system.

CARDIOVASCULAR REGULATION: BULBAR MECHANISMS

150.1 **MECHANICAL CARDIAC INPUT ONTO MEDULLARY RETICULAR NEURONS IN THE CAT.** R.W. Blair. Dept. Physiology & Biophysics, Univ. OK Hlth. Sci. Ctr., Oklahoma City, OK 73190.

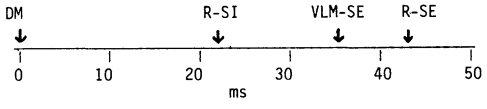
The medullary reticular formation is known to participate in cardiovascular "regulation", but most studies have concentrated on outflow from medullary reticular neurons (RN). Recent studies have indicated that RN also receive noxious input from the heart via sympathetic afferents. The present study was performed to determine whether innocuous stimuli applied to the heart affect the activity of RN. Cats were anesthetized with ketamine and chloralose, and were intermittently paralyzed with pancuronium. A bipolar electrode was attached to the left stellate ganglion (SG). Extracellular potentials were recorded from neurons in the reticular formation. The caudal medulla was searched with metal microelectrodes until a neuron responsive to stimulation of SG was found. These neurons were then tested for responses to aortic occlusion, to probing the heart, and to ventricular fibrillation. Of 41 RN tested, 8 (20%) increased their discharge rates, and 2 (5%) decreased their rates, during aortic occlusion. Of 16 RN tested, 9 (56%) were excited by touching the heart. Five of these RN had fairly distinct cardiac receptive fields. Probing one area of the heart would excite the cell, but the neuron would be insensitive to touching other areas. During fibrillation, 8 of 12 (67%) neurons were excited. Four cells responded at the onset of fibrillation, but the other four cells had a response latency of approximately 7 seconds. These data indicate that a variety of mechanical stimuli applied to the heart can influence the activity of RN. RN were also tested for noxious cardiac (coronary artery occlusion), somatic, auditory, and visual inputs. Various combinations of these additional inputs were found for different RN. In conclusion, RN receive innocous and noxious input from the heart, as well as input from other sensory modalities. (Supported by NIH grant HL 29618 and AHA Okla. Affiliate grant OK 83-G-25).

150.2 DORSOMEDIAL MEDULLARY NEURONS CONTROLLING SYMPATHETIC NERVE DISCHARGE. S.M. Barman and G.L. Gebber. Dept. of Pharmacol./Toxicol., Mich. State Univ., East Lansing, MI 48824.

The current study was initiated to test the hypothesis that the cat dorsomedial medulla contains the cell bodies of neurons involved in the control of sympathetic nerve discharge (SND). The spontaneous activity of 170 of 502 dorsomedial medullary (DM) neurons was temporally related to the 2- to 6-Hz cardiac-locked slow wave in inferior cardiac postganglionic SND. The discharges of these neurons remained synchronized to the 2- to 6-Hz component in SND when blood pressure, and thus baroreceptor nerve activity, were lowered to a level at which the phase relationship between the sympathetic nerve slow wave and the cardiac cycle was disrupted. Thus, the discharges of DM neurons were more closely associated with those of networks controlling SND than those of interneurons in the afferent limb of the baroreceptor reflex arc. Two observations indicated that the unit recordings were made from the region of the cell body. 1) DM neurons were excited by the iontophoresis of L-glutamate, an amino acid that depolarizes cell bodies but not axons. 2) An inflection on the rising phase of their extracellularly recorded spikes was observed. The inflection likely represents separation of the action potentials of the initial segment and soma-dendritic region.

Based on the results obtained using baroreceptor reflex activation, the dorsomedial medulla was found to contain a heterogeneous group of neurons with sympathetic nerve-related activity. DM neurons whose activity decreased in parallel with SND when carotid sinus pressure was elevated were considered to subserve a sympathoexcitatory (SE) function. Those DM neurons with sympathetic nerve-related activity whose firing rate increased during baroreceptor reflex activation were presumed to subserve a sympathoinhibitory (SI) function.

We compared the firing times of DM, rostral ventrolateral medullary (VLM), and raphe (R) neurons relative to the peak of the inferior cardiac sympathetic nerve slow wave. DM-SE neurons fired significantly earlier than their counterparts in the ventrolateral medulla and medullary raphe nuclei. DM-SI neurons fired significantly earlier than R-SI neurons. These data are summarized in the form of a time line which appears below.



These findings, together with the fact that VLM and R but not DM neurons project to the thoracic spinal cord (as determined by antidromic activation), support the view that DM neurons are closer to the region of origin of basal SND. (Supported by NIH grants HL-33266 and HL-13187.)

150.3 GABA and Serotonin Mediate the Sympathoinhibitory and Sympathoexcitatory Responses to Stimulation of Medullary Raphe Nuclei.
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Although it is widely appreciated that the medullary raphe nuclei play an integral role in the neural control of the cardiovascular system, there is surprisingly little data regarding the nature of the neurotransmitters which mediate the sympathoinhibitory and sympathoexcitatory responses elicited by stimulation of this area of the medulla. Recently, our laboratory found that serotonin antagonists blocked and serotonin uptake inhibitors potentiated the sympathoexcitatory response evoked from the medullary raphe nuclei (McCall, R.B., Brain Research, 311: 131, 1984). These data suggest that sympathoexcitatory responses elicited by raphe stimulation are mediated by serotonin. The present investigation was designed to extend this previous study and to determine the nature of the neurotransmitter mediating the sympathoinhibitory response evoked from medullary raphe nuclei. Computer summation techniques were employed to more accurately assess the effects of raphe electrical stimulation. The GABA antagonists picrotoxin (0.5-1.0 mg/kg, i.v.) and bicuculline (0.25-1.0 mg/kg, i.v.) blocked the sympathoinhibitory response evoked from the raphe in intact and mid-collicular transected, anesthetized cats. Mid-collicular transection blocked the high frequency spiking of sympathetic activity normally seen following GABA antagonist administration. Diazepam (0.3 mg/kg, i.v.) potentiated the sympathoinhibitory response elicited from the medullary raphe nuclei. Local application of picrotoxin to the dorsal surface of the lower brain stem, but not to the spinal cord, blocked raphe elicited sympathoinhibition. Picrotoxin often converted a sympathoinhibitory response into a sympathoexcitatory response. The sympathoexcitatory response to raphe stimulation could be blocked by intravenous or intrathecal administration of a serotonin antagonist. Finally, microinjections of L-glutamate into medullary raphe nuclei produced both pressor and depressor responses. These data suggest that the medullary raphe complex is heterogeneous with respect to autonomic function with sympathoinhibitory elements mediated at least in part by GABA and sympathoexcitatory pathways mediated by serotonin.

150.4 THE LINK BETWEEN NUCLEUS RETICULARIS GIGANTOCELLULARIS AND CARDIAC VAGAL MECHANISM IN THE RAT: ANATOMIC AND PHYSIOLOGIC STUDIES.
Samuel H.H. Chan, Julie Y.H. Chan, B.T. Ong* and Charles D. Barnes. Washington State Univ., Coll. of Vet. Med., Pullman, WA 99164-6520, and National Univ. of Singapore, Kent Ridge, Singapore 0511*.

Previous studies from our laboratory suggest that the nucleus reticularis gigantocellularis (NRGC) in the medulla oblongata may be intimately related to the physiologic and pharmacologic processes in circulatory regulation. That the cardiac vagal mechanism may serve as the output for this reticular nucleus to the heart is suggested by the observation that bilateral cervical vagotomy substantially eliminates the cardiovascular action of NRGC. The present study was undertaken to further investigate this NRGC-vagal link, using both anatomic and physiologic approaches. Since a controversy exists over the medullary origin of cardiac vagal innervations, we included nucleus ambiguus (NA), dorsal motor nucleus of vagus (DMV) and nucleus of tractus solitarius (NTS) in our experiments.

Wheat germ agglutinin-conjugated-horseradish peroxidase (WGA-HRP) was used in our anatomic studies to trace the connection between NA, DMV or NTS and NRGC in chloral hydrate-anesthetized rats. To ensure a discrete and localized injection, 15-20 nl of 1% WGA-HRP was infused into one of these cardiac vagal sites in each animal, via a micropipette (50-100 μm tip) over 5 min. Frozen 30 μm sections of the medulla were reacted with the TMB method and counterstained with thionin. Cells that conform with cytoarchitectural descriptions of NRGC neurons were heavily labelled with HRP granules 18-48 hrs following local application of the enzyme to NA, DMV and NTS. There was a bilateral representation, with a higher degree on the ipsilateral NRGC. HRP-labelled fibers could also be traced from the vagal sites to the vicinity of NRGC, signifying a reciprocal connection between them and the reticular nucleus.

Our physiologic experiments were performed on pentobarbital-anesthetized rats. A single NRGC locus in each animal capable of lowering the arterial pressure by at least 20 mm Hg upon electrical activation (10-s train of 1-ms, 20-50 μA rectangular pulses at 25 Hz) was first identified. The effect of stimulating this reticular locus, using single rectangular pulses (1-ms, 50-150 μA, 0.5 Hz) on NA, DMV or NTS was then evaluated. NRGC thus activated evoked field potentials or modified spontaneous single-neuron activities in the vicinity of these cardiac vagal sites. Furthermore, field potentials evoked by stimulating the cephalic end of the severed ipsilateral cervical vagus nerve in e.g. NA, were augmented, and the activated neuronal discharges enhanced, by NRGC.

The results from this study further reinforce the notion that the NRGC may utilize the cardiac vagal mechanism to exert its actions on the heart.

150.5 ^{3}H-PARA-AMINOCLONIDINE BINDING SITES IN VENTROLATERAL MEDULLA AND FRONTAL CORTEX DIFFER IN THEIR LIGAND SPECIFICITIES: IMIDAZOLE BINDING SITES IN THE VENTROLATERAL MEDULLA.
P. Ernsberger, M.P. Meeley and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine (CLON) is thought to inhibit vasomotor tone via alpha$_2$ receptors in the rostral ventrolateral medulla (RVL). However, the hypotensive effects of CLON in the RVL may, in fact, be mediated by a receptor type other than alpha$_2$, since depressor potency appears to depend upon an imidazole structure (Bousquet et al., JPET 232, 1984). We sought to establish whether CLON interacts with a putative imidazole binding site, as well as alpha$_2$ receptors, using radioligand binding.

Washed P$_2$ fractions from the ventrolateral quadrant of bovine medulla (VLM) and, for comparison, frontal cortex (FCx) were incubated in 50 mM Tris-HCl (pH 7.7) with 1.0 nM ^{3}H-p-aminoclonidine (^{3}H-PAC) at 25°C for 40 min prior to filtration; 10 uM phentolamine was used to define nonspecific binding.

(-)-Norepinephrine (NE) and epinephrine (up to 0.1 mM) inhibited approximately 75% of specific ^{3}H-PAC binding. However, the imidazolines CLON (> 100 nM), naphazoline (> 1 uM), and phentolamine (> 100 nM) displaced 100% of the specific binding. Thus, approximately 25% of specific ^{3}H-PAC binding sites as defined by phentolamine are non-adrenergic.

To further characterize ^{3}H-PAC sites, the competitive binding of a series of imidazole compounds was examined in VLM and in FCx. Unlike NE or CLON, the imidazoles histamine, cimetadine, and imidazole-4-acetic acid (IAA; a histamine metabolite) showed higher potency at ^{3}H-PAC sites in VLM versus FCx:

	IC_{50} in nM (% I_{max}) VLM	FCx
CLON	24 ± 8 (100)	5.6 ± 0.4 (100)
NE	166 ± 35 (70 + 1)	122 ± 31 (80 + 1)
Histamine	2,000 ± 1,000 (100)	38,000 ± 3,000 (100)
Cimetadine	480 ± 90 (68 + 9)	>30,000 (12 + 1)
IAA	22,000 ± 7,000 (72 + 3)	>300,000 (22 + 3)
*Extract	0.3 ± 0.1 U (100)	1.0 Units (100)

A methanol extract* of bovine brain (Meeley et al., this session) potently inhibited ^{3}H-PAC binding, particularly in VLM. In addition, studies of the binding specificity of non-adrenergic sites (10 uM NE added) in VLM showed that the extract had a high affinity for these sites (IC_{50} = 0.12 + 0.07 U), as did CLON (IC_{50} = 6 + 1 nM).

We conclude that: 1) CLON and related compounds may bind to a class of imidazole sites in brain; 2) these imidazole sites appear non-uniformly distributed in brain, being more apparent in VLM than FCx; and 3) an endogenous ligand present in brain may be involved in cardiovascular control through interaction with imidazole binding sites in the VLM. (Supported by NY Heart Association Fellowship and NIH Grant HL18974.)

150.6 ISOLATION OF CLONIDINE-DISPLACING SUBSTANCE FROM BOVINE BRAIN: FUNCTIONAL CHARACTERIZATION.
M.P. Meeley, P. Ernsberger, A.R. Granata and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Clonidine, a synthetic imidazole anti-hypertensive agent, has been shown to act centrally to lower blood pressure, specifically within the area of the rostral ventrolateral medulla (RVL) (Bousquet and Schwartz, Biochem. Pharm., 32, 1459, 1983). Recently, the presence of an endogenous substance in brain which potently displaces specifically-bound 3H-clonidine in rat brain membranes has been reported (Atlas and Burstein, Eur. J. Biochem., 144, 287, 1984). We sought to isolate clonidine-displacing substance (CDS) from bovine brain and further establish the physiological and neurochemical properties of partially-purified CDS.

CDS activity was determined using a 3H-p-aminoclonidine (3H-PAC) binding assay; unit activity is defined as that which displaces 50% of specific 3H-PAC binding (Ernsberger etal., this session). Fresh bovine brains (6) were homogenized in boiling distilled water, centrifuged at 100,000xg for 30 min, then heat-treated at 90-100°C for 5 min. The supernatant was dried and extracted with 20 vols of methanol, sonified for 3 min, filtered and concentrated. Further purification was obtained:

Purification Step	Spec. Activity (Units/g dry wt.)	Total Units/ 6 brains	Purif. (x-fold)	% Yield
Methanol extract	1565.	22,990.	---	100
Dialyzate	2553.	28,377.	1.6	123
$ChCl_3$/MeOH extract	3309.	19,776.	2.1	86

To determine if partially-purified CDS was physiologically active, rats were anaesthetized with urethane, paralyzed, and unilateral or bilateral microjections of CDS (0.02-2.4 Units/50nL vehicle) made in the C1 area of the RVL. CDS elicited a reversible (< 30 min) decrease in arterial pressure (AP) and heart rate (HR). Cardiovascular effects were dose-dependent (maximum Δ AP, -65+7mmHg; Δ HR, -40+8bpm; n=3) and restricted to the C1 area, as verified by PNMT immunocytochemistry. Neurochemically, an increase (2-fold) in the release of CDS activity over basal levels was observed when slices of rat ventral medulla were depolarized using Krebs' bicarbonate buffer, pH7.4 containing calcium and 56mM K+.

We conclude that (1) an endogenous clonidine-like compound (CDS) is present in brain; (2) partially-purified CDS, like clonidine, exhibits depressor activity when microinjected into RVL; (3) CDS in brain may be contained in nerve terminals and released with depolarization; and (4) CDS may be involved in neurotransmission related to central cardiovascular control. (Supported by NIH Grant NL18974.)

150.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF DOPAMINE-β-HYDROXYLASE, PHENYLETHANOLAMINE-N-METHYL TRANSFERASE, SUBSTANCE P AND 5-HYDROXYTRYPTAMINE CONTAINING CELL BODIES IN THE VENTRAL MEDULLA OBLONGATA OF THE CAT. L. Marson* and A.D. Loewy (SPON: E.J. Cole). Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Neurones responsible for the maintenance of vasomotor tone are present within the ventral medulla. To define the transmitters that may be involved, knowledge is required of the precise locations of the cell bodies containing these various putative transmitters in this area. The distribution of substance P (SP) and catecholamine-containing neurones within the ventral medulla of the cat were studied.

Following injection of colchicine into the 4th ventricle, the distribution of SP, dopamine-β-hydroxylase (DβH), phenylethanolamine-N-methyltransferase (PNMT) and 5-hydroxytryptamine (5-HT) containing neurones were mapped in the cat medulla using the peroxidase-anti-peroxidase technique. DβH containing cells were found in the A1 and A5 cell groups as described in other species. Cells containing both SP or 5-HT were found in the raphe obscurus and raphe pallidus, while cells containing 5-HT alone were also found in the raphe magnus. Two groups of SP containing cells were found in the ventral medulla. The rostral group lie ventral to the facial nucleus and the caudal group was found to lie lateral to the inferior olivary nuclei. 5-HT containing cells were also found in the region lateral to the inferior olivary nuclei, in addition a number of 5-HT containing cells were found overlying the pyramidal tract. PNMT-containing cells were found in the ventral medulla, at the rostral extent of the inferior olives, corresponding to the C1 cell group.

150.8 AUTONOMIC PROJECTIONS OF MEDULLARY ADRENALINE SYNTHESIZING NEURONS. D.A. Ruggiero, M. Anwar, D.H. Park and D.J. Reis. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

In medulla, neurons containing the adrenaline synthesizing enzyme, PNMT (phenylethanolamine-N-methyltransferase), are located in areas of autonomic regulation. In this study we have mapped axon pathways of neurons containing PNMT in order to identify potential anatomical substrates of adrenergic function. Fiber projections of PNMT neurons were first mapped in 20 rats; animals were anesthetized, injected with colchicine (150 ug/10 ul), and the peroxidase-antiperoxidase method was applied to 40 um sections exposed for 24 hr. to antibodies to PNMT. In a second series of experiments (n=30) the cells of origin of these projections were mapped by injecting wheat germ agglutinin-horseradish peroxidase conjugate into PNMT-labeled terminal fields and combining the method of retrograde transport with immunocytochemical localization of tyrosine hydroxylase or PNMT.

C1 neurons in the ventrolateral medulla (VLM) give rise to two bundles of axons: 1) Most axons arch dorsally and form the principal tegmental bundle (PT). Descending fascicles in PT enter dorsal parts of the lateral funiculus to innervate preganglionic cells throughout the thoracic spinal cord. Ascending axons of PT innervate the parabrachial complex (PBC) and pontine raphe and, via the median forebrain bundle, dorsomedial, perifornical, ventral and paraventricular hypothalamic nuclei, amygdala, nucleus of stria terminalis, septal nuclei and preoptic area; 2) a small bidirectional C1 bundle, paralleling PT, projects in the ventral tegmentum. Local projections of C1 neurons terminate in raphe pallidus and nucleus tractus solitarii-dorsal motor vagus (NTS-X); C1 dendrites directly contact the ventral surface of the medulla.

C2 axons descend and ascend along the periventricular and central grey. Descending periventricular axons (and intrinsic fibers of C2 neurons in NTS) ramify within NTS-X. Ascending axons innervate periventricular nuclei (including locus ceruleus and dorsal lateral tegmental nucleus) and in midbrain give rise to a dorsal limb ending in the midline thalamus and two ventral limbs both terminating in hypothalamus. C2 dendrites ramify in the medullary raphe, and contact intraparenchymal blood vessels and the dorsal surface. Double label experiments verify that both C1 and C2 neurons contribute to terminal fields in hypothalamus and NTS-X, whereas adrenergic spinal projections derive primarily from C1 neurons in rostral VLM.

In conclusion, C1 and C2 neurons preferentially connect with nuclei of visceral control and may be differentiated functionally. Mechanisms coordinating their proposed roles in afferent modulation, chemoreception, cortical, vasomotor and neuroendocrine activities remain to be elucidated.

150.9 NEURONS OF THE C1 AREA MEDIATE PRESSOR RESPONSES TO CENTRALLY ACTING CHOLINERGIC AGONISTS IN THE RAT. E.E. Benarroch, R. Giuliano, P. Ernsberger and D.J. Reis. Cornell Univ. Med. Coll., New York, NY 10021.

In the rat microinjection of cholinomimetic agents into the rostral ventrolateral medulla (RVL) produces a sympathetically mediated increase in arterial pressure (AP) and heart rate (HR) (Willette et al., J. Pharmacol. Exp. Ther. 231:457, 1984). The neural substrate for these effects has not been identified. Studies from our laboratory indicate that the area of the RVL containing C1 epinephrine neurons (C1 area) plays a major role in the tonic and reflex control of AP and in cardiovascular responses to electrical or chemical stimulation of a restricted area on the ventral surface of the RVL. We sought to determine whether neurons of the C1 area also mediate changes in AP elicited by cholinergic stimulation of the RVL. Rats were anesthetized (urethane), paralyzed and ventilated. AP and HR were recorded. The exposed ventral medullary surface was explored for evoked pressor responses to topical application of L-glutamate (L-Glu 10 nmole) to identify the area overlying C1 neurons (Benarroch et al., Soc. Neurosci. Abstr. 10:35, 1984). Agonists were applied to this "Glu sensitive" area of the ventral surface of the RVL. Lesions (500 uA, anodal d-c current for 30 sec) were made and their location correlated with the distribution of C1 neurons as assessed immunocytochemically.

Physostigmine or carbachol (100 nl solution 10^{-5} to 10 M) applied to the Glu-sensitive area elicited a dose-dependent increase in AP and HR. The threshold dose was 10 pmole, maximal effects were obtained with 10 nmole, and at higher doses a profound fall in AP was observed. Previous application of atropine (1 nmole), but not hexamethonium (1 nmole), to the same area prevented the responses to cholinergic agonists, but not to L-Glu (10nmole). The responses to carbachol (1 nmole) applied to the Glu-sensitive area were abolished by ipsilateral application of 100 nmole GABA (from 160 ± 5 to 104 ± 8 mmHg, n=5, $p < 0.05$) or by ipsilateral electrolytic lesion restricted to the C1 area (from 170 ± 8 to 98 ± 4, n=5, $p < 0.01$), but not the adjacent raphe. The effects of carbachol applied contralaterally to the C1 lesions were unimpaired. The pressor effect of intravenous injection (i.v.) of physostigmine (100 ug/kg) was abolished by either bilateral application of GABA (100 nmole) to the Glu-sensitive area (from 150 ± 8 to 60 ± 4 mmHg, n=4, $p < 0.01$) or bilateral electrolytic lesions of the C1 area (from 150 ± 5 to 54 ± 3 mmHg, n=4, $p < 0.01$). Sections (10um) from untreated rats were incubated with ^{3}H-quinuclidinyl benzilate (2nM). A moderate density of muscarinic receptors was identified in a region overlapping the C1 area of the RVL. We conclude that neurons of the C1 area a) mediate pressor responses to muscarinic cholinergic stimulation of the RVL and b) are critical for the pressor response to i.v. physostigmine.

150.10 HEMODYNAMIC CHANGES INDUCE VARIATIONS IN CATECHOLAMINERGIC METABOLIC ACTIVITY WITHIN THE VENTROLATERAL MEDULLA. J.Y. Gillon*, L. Quintin, G. Hilaire*, M. Ghignone* and J.F. Pujol* (SPON: J.J. BOUYER) Neuropharmacology, Roussel Uclaf, 93230 Romainville, France.

In vivo voltammetry has recently been shown to be a sensitive and reliable technique to monitor pontine noradrenergic (NA) activity (Buda et al., Brain Res., 1983, 273 : 197-206 ; Gonon et al., Brain Res., 1983, 273 : 207-216). This study was undertaken to assess whether such a technique would evidence changes in medullary catechol (CA) oxidation current (Suaud-Chagny et al., submitted) in relation to hemodynamic variations. Male rats (OFA strain) were anesthetized (chloral 400 $mg.kg^{-1}$), ventilated with oxygen/air mixture and paralyzed. Temperature was maintained to 37.5 + 0.5°C. Acidosis, as checked by frequent arterial blood gases, was buffered with sodium bicarbonate. Treated carbon fibre electrodes were lowered to the ventrolateral medulla (VLM) (L 1.9 mm, P 0.3 mm to the obex, DV -2.5 mm) with a 30° angle to detect a catechol oxidation peak (+50-75 mV, differential normal pulse voltammetry). Sixty minutes were allowed to elapse in order to stabilize the catechol peak height and mean blood pressure (MBP, 110 + 20 mmHg). Results are expressed as % of baseline values of catechol signal. Volume expansion (4-6 ml) decreased catechol peak height to 80% of baseline value while MBP increased 10 to 30 mmHg. In contrast, blood withdrawal (BWD, 4-6 ml) increased catechol signal to 150% while MBP decreased to 30-50 mmHg (absolute value). These variations in catechol metabolic index are in keeping with electrophysiological variations evidenced in the locus coeruleus during similar hemodynamic manipulations (Elam et al., Brain Res., 1984, 290 : 281-297). Infusion of sodium nitroprusside increased catechol signal to 160% while MBP decreased to 40 mmHg. Prazosin 1 $mg.kg^{-1}$ i.p. increased catechol signal to 125% while MBP decreased to70-80 mmHg. This is in agreement with increased unitary activity in the A5 nucleus during controlled hypotension (Andrade et al., Brain Res., 1982, 242, 125-135). Clonidine 100-200 $ug.kg^{-1}$ i.p. decreased the catechol signal to 60-70%, and MBPto 70-80 mmHg. This may be related to the inhibitory action of this molecule on CA VLM structure (Cahusac et al., Neurosci. Lett., 1983, 42 : 279-284). Clonidine 100-200 $ug.kg^{-1}$ injected 30 min before hemorrhage reduced the catechol peak increase, secondary to BWD, to 115%. This is in keeping with the inhibitory action of clonidine on central NA neurons under nociceptive stimuli (Marwaha et al., J. Pharm. Exp. Ther. 1982, 222 : 287-293) or during stress (Quintin et al., Soc. Neurosci. Abstr., 1984, 10 : I, 679). That medullary CA activity is related to hemodynamic variations should be interpreted in view of the close anatomical association of adrenergic excitatory and NA inhibitory VLM structures.

150.11 TWO DISTINCT MEDULLOSPINAL SYMPATHOEXCITATORY PATHWAYS ORIGINATE IN THE RAT NUC. PARAGIGANTOCELLULARIS LATERALIS (PGCL). Patrice G. Guyenet, D. Les Brown and Miao-Kun Sun*. Univ. Virginia School of Medicine, Dept. of Pharmacology, Charlottesville, VA 22908.

The retrofacial portion of PGCL contains neurons with spinal projections and a strongly pulse-synchronous rate of discharge. Below a mean arterial pressure of 75 mm Hg (MAP), these neurons exhibit a high level of discharge independent of AP and their activity decreases linearly to zero when MAP is increased from 90 to 160 mm Hg. This inhibition results from the activity of arterial baroreceptors and is mediated by GABA (see following abstract).

The axonal conduction velocities of these neurons exhibit a bimodal distribution with peaks at 0.68 and 3.1 m/sec respectively (N=170 cells).

Clonidine (clo, 11 μg/kg i.v.) exerts a selective inhibitory effect on slow-conducting units while fast-conducting ones are unaffected. By the iontophoretic route, clo (100 nA) also selectively inhibits slow-conducting neurons.

Single or twin-pulse stimulation of the PGCL (0.5 msec, 150 μA, 0.5 Hz) evokes two peaks of activity at the level of the lumbar sympathetic chain (L2-L3) with latencies of 98 ± 2.5 msec (N=7) and 206 ± 9 msec respectively. Similar stimulations but delivered in the lumbar spinal cord (L2-L3) elicit a peak of activity 65 msec after the stimulus. The sympathoexcitatory effect of PGCL stimulation therefore results from the activation of two medullospinal pathways whose latency from PGCL to the upper lumbar IML averages 33 and 141 msec respectively. These latencies correspond to intraspinal conduction velocities of 3.2 and 0.68 m/sec respectively, figures which are identical to those exhibited by the spinal axons of PGCL pressure-sensitive neurons.

These experiments are consistent with the following interpretations: first, SND is in a large part maintained via the tonic discharge of medullospinal excitatory inputs originating in the retrofacial portion of PGCL; second, the baroreceptor reflex inhibition of SND is a disfacilitation involving the inhibition of PGCL sympathoexcitatory neurons; third, the medullospinal sympathoexcitatory projection of PGCL consists of at least two neuronal populations, a slow-conducting clonidine-sensitive one and a fast-conducting clonidine-insensitive other. (HL 28785, HL 33513, HL 01128).

150.12 THE ACTION OF GABA ON SYMPATHOEXCITATORY NEURONS IN THE RAT VENTROLATERAL MEDULLA. Miao-Kun Sun* and Patrice G. Guyenet. Univ. of Virginia School of Medicine, Dept. of Pharmacology, Charlottesville, VA 22908.

The retrofacial portion of nuc. paragigantocellularis lateralis (PGCL) contains a population of neurons which project to the intermediolateral cell column, discharge spontaneously at low levels of arterial blood pressure and receive a powerful inhibitory input originating from arterial baroreceptors. The nature of the transmitter released by this inhibitory input was investigated with single-unit recording techniques and ionophoresis in halothane-anesthetized rats. All PGCL cardiovascular neurons recorded could be silenced by GABA (0.8M; ≤ 50nA n=20), glycine (0.5M; ≤ 60nA n=10) or inhibited by taurine (1M, 60nA, n=5). The effects of gly and taurine were completely antagonized by strychnine (50 mM; 100nA, n=10). Gly-induced inhibitions were unaffected by bicuculline methiodide (BiC, 20mM, 100nA, n=5). Conversely BiC completely antagonized the effects of GABA but did not alter the inhibition caused by gly.

BiC (100nA) blocked completely and reversibly the inhibition of PGCL cardiovascular neurons produced by raising mean arterial pressure (MAP) to 160 mm Hg (n=14). During the period when the cells were insensitive to MAP elevations, they were still inhibitable by gly but were insensitive to doses of GABA previously sufficient to produce 100% inhibition in the absence of BiC.

Strychnine, when applied in an amount sufficient to completely antagonize the effects of gly did not impair the blood pressure sensitivity of the units (n=6).

BiC in doses sufficient to block the inhibition induced by elevations in MAP also increased the basal rate of discharge of PGCL CV units by 50% on average. Similar increases in the units activity could be produced by local applications of l-glu (0.2M, 100nA). Yet such elevations in the cells firing rate did not modify the inhibitory effects produced by raising MAP (n=6).

It is concluded that GABA or a related substance mediates the inhibition of PGCL sympathoexcitatory neurons produced by the activation of arterial baroreceptors. Moreover PGCL sympathoexcitatory neurons may be tonically inhibited by a GABA-ergic input even at MAP below the threshold for arterial baroreceptor activation (HL 28785, HL 33513, HL 011128).

LIMBIC SYSTEM: DEVELOPMENT AND PLASTICITY III

151.1 EFFECTS OF EXTRACELLULAR FIELDS ON NORMAL AND EPILEPTIFORM ACTIVITY IN ADULT AND IMMATURE RAT HIPPOCAMPAL SLICES. S.M. Bawin, T.J. Purtzer*, M.D. Mahoney*, A.R. Sheppard, S. Ashwal* and W.R. Adey. Departments of Physiology, Neurosurgery and Pediatrics, Loma Linda University and VAMC, Loma Linda, CA 92357.

We previously reported that oscillating extracellular fields in the range of EEG rhythms and epileptic afterdischarges (5-130 mV/cm in tissue) induced long-term increases in population spikes and EPSPs recorded in the CA1 cell layer and apical dendrites of adult rat hippocampal slices. These post-field effects were independent of the passive and instantaneous polarizations which occurred during DC and sinusoidal fields with amplitudes larger than 50 mV/cm. We have now compared field effects in slices from 8-25 day old pup rats with those observed in adult slices, studied concurrently in the same recording chamber. The perfusing solutions contained either 2 or 2.5 mM Ca^{++} and 5 or 7 mM K^+ ($[K^+]/[Ca^{++}]$ = 2.5). Immature and adult slices responded similarly to polarizing fields, but the long-lasting increases in excitability that followed oscillating fields (5 and 60 Hz) in mature tissue were never observed in animals younger than 16 days, and were rarely seen in the older pups. Increasing $[K^+]/[Ca^{++}]$ ratio from 2.5 to 3.5 in either solution resulted in epileptiform evoked potentials and afterdischarges, and facilitated field-induced lasting potentiations in adult and 16-25 d pups, but not in slices from younger animals. Measurements with K^+ sensitive microelectrodes in the CA1 pyramidal cell layer and apical dendrites showed increased K^+ activity during and following repetitive field stimulation (5 and 60 Hz) in slices from adult and 18-25 d rats, but not in the 8-13 d pups studied so far. Altogether these results suggest that although passive neuronal polarization by strong (greater than 50 mV/cm) extracellular fields occurs readily in the immature brain, the K^+ response of the tissue and its capability to sustain long-lasting field effects appear slowly, concurrent with the development of synaptic excitability. Once established, these last two mechanisms of field interaction with the tissue may have a significant influence on excitability, especially when the extracellular $[K^+]/[Ca^{++}]$ ratio is elevated, such as during seizures or intracerebral hemorrhage.

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151.2 CYSTEAMINE SUPPRESSION OF KINDLED SEIZURES: DEPENDENCE ON INJECTION-TO-KINDLED SEIZURE INTERVAL. G.A. Cottrell and H.A. Robertson. Department of Pharmacology, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Higuchi et al. (Brain Res., 288:359, 1983) originally reported that cysteamine (β-mercaptoethylamine) suppressed amygdala kindled seizures at 24 and 48 hrs (with the suppression lasting up to 10 days in some animals) but not at 4 hrs. They attributed this suppression to brain somatostatin depletion. However, we feel that this explanation is unlikely as somatostatin depletion (Sagar, S.M. et al., J. Neurosci., 2:225, 1982; Srikant, C.B. and Patel, Y.C., Endocrinol., 115:990, 1984) and kindled seizure suppresson do not follow the same time course. Further, as we shall report, the injection to kindling interval is critically important for the suppression effect.

Male sprague-dawley rats were implanted with a twisted teflon-coated stainless-steel electrode in the left amydaloid complex or left dorsal hippocampus. The rats were kindled (2 sec, bipolar, 60 Hz, 340 μA to amygdala, 240 μA to hippocampus) on a daily schedule. Following kindling, rats were injected with cysteamine (200 mg/kg, IP). In the first experiment, we used the paradigm of Higuchi et al., i.e., injection 4 hrs prior to the kindling session. Our findings were essentially identical to those of Higuchi et al.: Four hrs after cysteamine administration, amygdala and hippocampal kindled rats exhibited kindled seizures. This suppression lasted for 4-10 days in individual rats. In the second experiment, we injected rats 2, 4 or 6 hrs after a kindled seizure. On the 24 hr kindling test, none of the rats in the 6 hr-post group exhibited suppression, i.e., they all had a stage 5 seizure. The effect of injection 2 or 4 hrs post was intermediate. In the 2 hr-post group, 2 out of 5 rats did not have a stage 5 seizure on the 24 hr test (the other 3 did). All these rats had a stage 5 seizure on the 48 hr test. In the 4 hr-post group, 3 out of 4 rats had a stage 5 seizure on the 24 hr test, 4 out of 4 on the 48 hr test. Thus, the percentage of rats exhibiting suppression and the duration of the suppression is dependent on the administration-to-kindled seizure interval. We therefore conclude that the suppression of kindled seizures following cysteamine administration is not due to the cysteamine per se, but must be due to an interaction of the cysteamine with the kindling process. One possibility is that it acts through glutamate receptors. Calcium-dependent glutamate binding is depressed by cysteamine (see poster of Robertson, H.A. and Cottrell, G.A., this meeting). (Supported by the Canadian MRC and the Dalhousie Medical Research Foundation).

151.3 THE EFFECT OF CHRONIC ETHANOL TREATMENT ON INHIBITION IN CA1 OF THE IN VITRO RAT HIPPOCAMPUS. C.J. Rogers and B.E. Hunter, Dept. of Neuroscience, Univ. of Fla., Coll. of Med. and V.A. Med. Cntr., Gainesville, FL. 32610

Chronic ethanol treatment (CET) produces an increase in the amplitude of the extracellular population spike (PS) with paired pulses in CA1 of the in vivo rat hippocampus (Abraham et al., 1981). The increase in PS amplitude was attributed to a decrease in recurrent inhibition (RI). It may also be possible that the increase in PS amplitude is the result of a decrease in recently described feedforward inhibition (FFI). Antidromic/orthodromic (A/O) and orthodromic/orthodromic (O/O) paired pulse techniques should allow for the relative dissociation of the influence of CET on RI and FFI. This study was designed to determine whether CET produced alterations in PS amplitudes in vitro as has been demonstrated in vivo and whether CET affects RI and FFI differentially.

Rats were fed a nutritionally complete ethanol (E) or sucrose-containing (S) liquid diet for 20 weeks. Ad lib lab chow and water were then given for at least eight weeks prior to acute electrophysiological in vitro manipulations. Stimulating electrodes were placed in stratum radiatum for orthodromic stimulation and in the alveus for antidromic stimulation. Extracellular field potentials were recorded at the pyramidal cell layer with micropipettes. Input/output (I/O) curves were collected followed by several paired pulse paradigms using different condition and test pulse current intensities as well as A/O and O/O configurations. Interpulse intervals (IPI) ranged from 20 msec to three seconds.

CET produced an enhancement of the PS using paired pulses in vitro as has been demonstrated in vivo. The increase in PS amplitude using O/O configurations is most pronounced with IPIs from 20 to 500 msec. A/O paired pulses at higher current intensities resulted in group S animals remaining moderately inhibited out to three seconds while group E animals showed only early inhibition. These results suggest that CET produces alterations in recurrent inhibition. The effect of CET on FFI is more difficult to interpret although it does not appear that the changes seen between groups with A/O paired pulses can account for the much larger differences observed between group S and group E animals with O/O data. These results suggest that CET alters the influence of FFI to a greater extent than it does RI.

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151.4 POWER SPECTRAL ANALYSIS OF THE HIPPOCAMPAL EEG IN THE NORMALLY DEVELOPING RAT. J.D. Bronzino, C.J. Siok*, K. Austin*, R.J. Austin-LaFrance* and P.J. Morgane. Trinity College, Hartford, CT 06106 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We have used power spectral analysis techniques to quantify the development of the EEG obtained from the frontal cortex and the hippocampal formation of normally developing rats during several different vigilance states. At 14,18,22,30 or 45 days of age, EEG recordings were obtained and computer analysis was performed during the vigilance states of quiet waking (QW), slow-wave sleep 1 (SWS1), slow-wave sleep 2 (SWS2) and REM sleep. In the power spectra derived from the hippocampal EEG it was found that in the 0.5-3.5 Hz frequency range during QW and REM sleep, the average power significantly decreased ($p<.02$ in QW; $p<.002$ in REM) as the animal matured from 14 to 45 days of age. A peak in the power spectra was also observed starting at 14 days of age in the 4-11 Hz (theta) frequency range during both QW and REM sleep. During REM the frequency at which the maxima of the peak occurred (peak theta frequency) significantly increased ($p<.001$) from 4.4 Hz at day 14 to 6.7 Hz by day 45. During QW the peak theta frequency at 14 days of age occurred at 4.3 Hz and its amplitude was 32% lower ($p<.01$) than that which occurred during REM. By day 45 the peak theta frequency during QW significantly increased ($p<.001$) to 5.9 Hz, but was significantly slower ($p<.01$) than the peak which occurred at the same age during REM. There was also a significant decrease ($p<.002$) in the amplitude of this peak when compared to the 14 day old animal. In the power spectra derived from the EEG of the frontal cortex, the average power of the predominant 0.5-3.5 Hz frequency band significantly increased ($p<.01$) during the states of SWS1 and SWS2 as the animal matured from 14 to 45 days of age. Our studies of normal electro-ontogenesis in these brain areas may serve as baseline values against which to assess the effects of neuronal insults on electrographic development in these regions. (Supported by NSF Grant ECS 8118440 and NIH Grant HD-06364).

151.5 AN INFORMATION/COMPUTATION THEORY OF HIPPOCAMPAL FUNCTION. W.B. Levy. Dept. Neurosurg., Univ. Virginia, Charlottesville, VA 22908.

It is the job of the nervous system to make good predictions about current and future states of the organism, the environment, and interactions between the two. Such predictions are constructed from predictions made by individual neurons. Indeed, parallel distributed theories of the nervous system interpret the activities of individual neurons as predictions. I.e., the activity of a neuron is its calculated probability that one of its best patterns currently exists. The calculation of this probability is conditioned on current afferent activity and the correlative history of the environment as stored in the connections between neurons. Additional neural circuitry predicts the overall current environment by selecting a subset of neurons that most strongly predict the existence of the features for which they are tuned. Optimally, the choice comes from predictions conditioned on all possible states of the environment.

Unfortunately, only a small subset of all possible conditional predictions can be generated by the brain because of the relatively limited number of neurons compared to possible configurations of the environment and the number of different ways predictions can be conditioned. This limitation is severe, i.e., most predictions will not be made. To minimize the damage of this constraint, afferents converging on a cell should be minimally redundant. Then each prediction is based on as much information as possible.

The hypothesis is that neurons of the dentate gyrus and CA3 remap the environment so that predictions made by CA1 neurons are based on minimally redundant information. This hypothesis restates the source coding problem in information theory, thus defining optimal performance: maximize the mutual information between the input and the output while minimizing the mutual information between the input lines with respect to themselves. In the model, the input lines are the layer II stellate cells of the entorhinal cortex which project to the DG and CA3, and the output lines are the CA3 pyramidal cells that project to CA1. The number of input and output lines is assumed equal. Septal inputs separate patterns in time. All other inputs are viewed as modulatory.

At least in one case there is a unique solution to this problem. For Gaussian environments the Karhunen-Loeve transformation is the optimal mapping. This transformation remaps the environment so that all output lines considered pairwise are independent and no higher order interactions exist. A more general solution suitable for optimally transforming more complex environments is desirable. The ultimate question is how and in what sense the natural constraints of anatomy and physiology produce a self-optimizing source coder.

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151.6 DEATH OF CA1 PYRAMIDAL NEURONS FOLLOWING ELECTROLYTIC LESION OF THE ENTORHINAL CORTEX. Y.Theoret* and M.R. Krigman, Dept. of Pathology, Univ. of North Carolina at Chapel Hill, NC 27514.

Unilateral lesion of the entorhinal cortex results in a partial deafferentation of the perforant pathway excitatory terminals in the outer molecular layer of the dentate gyrus. Although this technique does not affect the granule cells viability, we have observed a massive neuronal death of the CA1 pyramidal neurons.

Long-Evans rats (300-350g) were anesthetized with pentobarbital and the head was immobilized in a stereotaxic frame with the incisor bar set at -3.9mm. The parameters involved in the fabrication of the lesioning electrode and in the application of the direct current were similar to those reported by Nadler and co-workers (Brain Res.,131:241,1977). Electrolytic current was applied in nine locations of the entorhinal cortex. Animals were sacrificed at 6, 12, 24, 48 and 96 hours after the surgery and the brains were processed for either light or electron microscopy.

Light microscopy 48 hours post-lesioning revealed extensive neuronal necrosis of the CA1 pyramidal neurons on the ipsilateral side; rare scattered pyknotic neurons were observed on the contralateral side. There was no effect on CA4 and CA3 pyramidal neurons nor on granule cells on either side. Neurons located in the subiculum and in the regions deep and superficial to the CA1 cell body layer were spared. Ipsilateral to the lesion side, necrotic neurons were not discernible 6 hours after the surgery but increasing number of dying CA1 pyramidal neurons were seen at 12 and 24 hours. Bielschowsky staining showed a correlation between the degeneration of fibers in the stratum lacunosum moleculare (temporo-ammonic tract) and the necrosis of the CA1 pyramidal neurons. Electron microscopy at 6 hours post-lesioning revealed numerous electron lucent dilated structures in the CA1 neuropil ; these may be altered glia or degenerative terminal boutons. At the same time, CA1 pyramidal neurons exhibited swollen mitochondria in the apical dendrites and to a lesser extent in the soma, and vacuolation within the cytoplasm. At later periods, the mitochondrial changes were less prominent but the cytoplasmic vacuoles increased in size and number and were now visible in the nuclei. Microtubules were less organized and cytoplasmic dense bodies were seen.

Although a direct electrolytic effect could sometimes be observed on the most caudal portion of the hippocampal formation, our findings indicate the absence of such an effect on the rostral CA1 pyramidal neurons. We propose that the acute necrotizing process is related to the degeneration of the entorhinal cortex terminals (possibly temporo-ammonic tract) in the hippocampal formation via mechanisms which remain to be elucidated. (Supported by ES01104 and IRRSTQ Fellowship to Y.T)

151.7 EFFECTS OF CHOLINERGIC AGONISTS ON HIPPOCAMPAL NEURONS OF THE IMMATURE RAT. L. J. Reece* and P. A. Schwartzkroin (SPON: T. Kennedy). Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Cholinergic agonists are known to facilitate the firing of hippocampal pyramidal cells in the adult rat through binding to receptors which are primarily muscarinic. This facilitation apparently occurs through two mechanisms: one a post-synaptic suppression of a voltage-dependent potassium current, the other a pre-synaptic suppression of inhibitory influences. In the adult rat, the pyramidal cell response to muscarinic agents has been characterized as a slow depolarization with concurrent increase in input resistance. We have previously demonstrated that in immature hippocampus the response to another neurotransmitter, GABA, differs significantly from that in the adult. We have therefore begun to examine the response of immature hippocampal pyramidal cells to acetylcholine.

Transverse slices (400 um) were taken from the hippocampus of rats from 5 to 60 days of age. Carbachol (5 mM) was applied from a micropipette in droplet form to the surface of the slice in the area of the cell body layer and the proximal dendrites. Preliminary evidence indicates that the response of immature animals to applied carbachol, while perhaps differing from the response in the adult in some respects, is basically similar. The pyramidal cells in slices from immature animals undergo a membrane depolarization on the average of 10 mv; this depolarization is associated with an increase in the cell's input resistance. Thus, immature pyramidal cells respond to cholinergic stimulation before their normal cholinergic afferents are well established.

The adult response, seen in animals older than 20 days, sometimes includes a brief hyperpolarization of 1-3 millivolts preceding the more commonly observed depolarization. In the cells in which the early hyperpolarization is seen, a brief decrease in the input resistance correlates temporally with the onset of the hyperpolarization. This hyperpolarization may be mediated by inhibitory interneurons, for we have shown that interneurons are excited by applied carbachol. This hyperpolarization is less apparent in the immature hippocampus, perhaps due to the slow development of inhibitory circuitry in these animals.

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151.8 ORGANIZATION AND DEVELOPMENT OF THE HIPPOCAMPAL COMMISSURE IN PRIMATES: OVERPRODUCTION AND ELIMINATION OF COMMISSURAL AXONS IN INFANT RHESUS MONKEYS. A-S. LaMantia and P. Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

We have examined the cytological and axonal organization of the interhemispheric fiber tract classically referred to as the corpus psalterium or the hippocampal commissure (HC) using electron microscopic (EM) and immunocytochemical techniques in nine rhesus monkeys ranging in age from birth to four years. The number of axons in the developing and adult HC was estimated using a quantitative EM method employed previously for the corpus callosum and anterior commissure (LaMantia and Rakic, Soc. Neurosci. Abstr. 10: 1080, 1984).

Three morphological features distinguish the HC from the adjacent corpus callosum (CC). First, a glial capsule surrounds the HC and separates it from the overlying splenium of the CC in infant and adult monkeys. The cells and processes which compose this capsule have ultrastructural characteristics of astrocytes: they have numerous bundles of intermediate filaments, and display strong immunoreactivity with antisera directed against glial fibrillary acidic protein. These cells are extensively interconnected by numerous desmosomal junctions. Second, the distribution of astrocytes and their processes within the HC is quite different from that in the CC. In the HC, glial cells are more numerous and after the third postnatal month their processes form a trabecular meshwork which separates bundles of axons into fasicles. In contrast, the incidence of glial cells is considerably lower in the CC and the processes of these cells do not divide groups of axons into fasicles. Third, the mean diameter of myelinated axons in the HC (0.7 um) is half that of axons in the splenium (1.4 um). Furthermore, myelination of the HC proceeds at a slower rate; however, in the adult HC 95% of the axons are myelinated as in the overlying CC.

There is at least a fivefold overproduction of axons in the developing HC. At birth there are over 1,000,000 axons in the HC. During the first two postnatal weeks this number drops to less than 400,000, and elimination of HC axons continues at a slower rate until the second postnatal month when the adult number of 200,000 is reached. The major wave of axon loss in the HC overlaps with that in the CC and the anterior commissure (LaMantia and Rakic, 1984); however, each commissure loses axons at a different rate. The omnipresence of axonal elimination during infancy in these three commissures, which connect several anatomically and functionally distinct cortical regions, suggests that this process may play a significant role in the formation of interhemispheric connections.

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151.PO LACK OF PERSISTENCE OF ENVIRONMENTAL COMPLEXITY INDUCED INCREASE IN POSTSYNAPTIC RESPONSE TO PERFORANT PATH STIMULATION IN RAT DENTATE GYRUS FOLLOWING REHOUSING IN STANDARD LABORATORY CAGES. E. J. Green* and W. T. Greenough (Spon: C. L. Prosser). Neural and Behavioral Biol. Prog., and Depts. Psychol. and Anat. Sci., Univ. Illinois, Champaign, IL 61820

Several recent in vivo studies have found increased dentate gyrus field potential responses to perforant path stimulation following behavioral manipulations (e.g. Sharp et al., Soc. Neurosci. Abstr., 9(2), 1983). We recently reported that population epsp and spike responses were greater, in hippocampal slices maintained in vitro, from rats reared from weaning in a complex environment than in slices from individually cage-reared rats (Green and Greenough, Soc. Neurosci. Abstr., 10(1), 1984). The present study examined the persistence of this in vitro field potential response elevation during a subsequent period of re-housing in individual cages.

Rats in the environmental complexity initial condition (EC-I) were group reared from weaning in wire mesh cages containing a three dimensional lattice of wooden, metal and plastic objects which were changed daily, and were allowed free exploration in a separate play area containing a similar set of objects for 30 to 60 minutes each day. Rats in the isolated initial condition (IC-I) were housed singly in standard laboratory tubs. Following 30 days of differential rearing, both groups of rats were re-housed individually in plastic laboratory tubs for an additional 25-34 days. Hippocampal slices from littermate pairs were prepared for physiological investigation. Population responses were recorded in the stratum granulosum (ventral blade) of the dentate in response to stimulation of afferents in the middle molecular layer. Previously we found that slices from EC rats exhibited significantly larger population spikes and epsp's at the higher stimulus intensities in this region.

In contrast, in the present study, following 3-4weeks of additional housing in similar individual cage environments, there were no group differences in population spikes or epsp's across a range of stimulus intensities. Thus, the increased activation of granule cells in EC slices appears to be transient, persisting for less than 3-4 weeks in the absence of differential housing. Supported by NIMH 35321, PHS EY07005, System Development Foundation, and Univ. Il. Research Board.

151.10 STUDY OF INFLUENCES FROM HYPOTHALAMUS AND LOCUS COERULEUS UPON THE ELECTRICAL ACTIVITY OF THE OLFACTORY TUBERCLE. R. Guevara-Aguilar, L.P. Solano-Flores+, M. Nájar Joa. Departamento de Fisiología, Facultad de Medicina, División de Investigación, UNAM. 04510, México, D. F.

The interaction of locus coeruleus (LC) and lateral hypothalamus (LH) stimulation over the electrical activity of the olfactory tubercle (OT) were studied. The experiments were conducted in 30 Wistar rats of either sex, anesthetized with chloral hydrate (400 mg/kg). Evoked potentials and/or unitary activity were recorded in the polymorphic layer of the olfactory tubercle using a single barrel glass capillary electrode filled with 2 M NaCl. Bipolar parallel stainless steel electrodes were used for stimulation. The LC stimulation electrode was placed in P8 to 9.4; L 0.8 to 1.1 and V -5.5 to -6.3. The LH stimulation electrode was placed in P 1.0 to 2.0; L 1.0 to 2.0 and V -7.0 to 8.0. All the electrode position were determined from bregma in the horizontal rat's skull and from the brain surface.

LH stimulation evoked a biphasic (positive-negative) potential in the OT, a similar potential had been previously recorded in cats. In the same experiment the latency of the first wave of the OT potential evoked after LC stimulation was longer than that of the first wave of the OT potential evoked after LH stimulation. Repetitive stimulation of LC (800 µA, 0.2 ms pulse, 300 Hz maintained during 5 s) previously to single test shocks to LH showed: while the positive wave of the evoked potential in the OT by LH stimulation was reduced with respect to the control, the negative wave was enhanced after a transient deppression.

We had previously reported that LC stimulation decreased the discharge frequency of the OT units, when we studied the effect of LH stimulation over the same cells responded to LC stimulation we observed that: 38/56 neurons (67.8%) initially increased their activity, 12/56 neurons (21,4%) initially decreased their activity and only 6/56 neurons (10.7) exhibited no effects following LH stimulation. These results suggest a convergence of LH fibers and LC fibers upon the OT neurons.

This work was supported by CONACYT Grant PCSABEU-002187

151.11 **BEHAVIORAL ACTIVATION IN 3-DAY-OLD RATS PRODUCED BY ELECTRICAL STIMULATION OF THE NUCLEUS ACCUMBENS BUT NOT THE NEOSTRIATUM.** Eunice H. Lee* and Gordon A. Barr* (SPON: Robert L. Thompson). Biopsychology Program, Hunter College, City University of New York and Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461.

Young rat pups become behaviorally activated, emitting a series of responses, that includes mouthing, licking, pawing, and locomotion, following either reinforcing exteroceptive stimuli such as milk, or brief electrical stimulation of the medial forebrain bundle (Hall, *J. Comp. Physiol. Psych.*, 1979, 93: 977; Lithgow and Barr, *Behav. Neurosci.*, 1984, 98: 479; Moran et al., *J. Neurosci.*, 1983, 3: 10). To further characterize the role of striatal and mesolimbic systems in the behavioral activation of infant rats, we observed the behavioral response of 3-day-old rats following brief electrical stimulation of the caudate nucleus (CD) or the nucleus accumbens (NA).

Bipolar teflon coated stainless steel electrodes, bared at the tip, were implanted in the CD or NA of 3-day-old pups. Testing occurred 16 to 20 hours after surgery. Following a 5 minute adaptation period and a 2 minute baseline observation period, behavior was recorded over 6-one minute intervals during which direct current biphasic square wave stimulation (500 msec train of 50 pulses with a pulse width of 2 msec) was delivered every 10 seconds during every other minute. Current was varied between 30 and 150 μA, although inactive sites were tested up to 1000 μA. The behavior of the pup was recorded during each minute. Following testing, pups were overdosed with a barbiturate and perfused intracardially. Frozen sections were stained for Nissl substance and the electrode placements verified without reference to the behavioral data.

Stimulation of the NA resulted in behavioral activation. Stimulation of the CD and other areas such as the diagonal band of Broca or the lateral septum were negative. The elicited behaviors occurred immediately after the onset of stimulation and decayed rapidly when the stimulation ended. The predominant behavioral responses elicited by stimulation of the NA were mouthing, licking and pawing. Locomotion, in contrast, was seen following stimulation of both the NA and the CD.

To assess first if dopamine antagonists would block stimulation induced behavioral activation, and second if typical and atypical neuroleptics would differentially affect striatal and mesolimbic systems, subsequent studies tested whether the behavioral activation elicited by electrical stimulation of the NA could be blocked by the dopamine antagonists haloperidol and clozapine. Haloperidol (0.2 mg/kg) and clozapine (2.5 mg/kg) produced comparable effects and blocked stimulation induced activation in the NA and stimulation induced locomotion in both the NA and CD. At these doses the drugs did not affect reflex behaviors (e.g. righting) or produce catalepsy or sedation.

These results suggest the involvement of the mesolimbic system in the behavioral activation that accompanies reinforcement in infant rats, and suggest a possible role of the dopamine system in behavioral activation and reinforcement in neonates.

151.PO MODEL OF DELAYED NEUROLOGIC DETERIORATION FROM ANOXIA: BRAIN PATHOLOGY AND METABOLISM. R.E. Myers, K.R. Wagner, and G. de Courten-Myers. Research Service, VA Medical Center, Cincinnati, OH 45220 and Departments of Neurology and Neuropathology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Hyperglycemia at exposure to anoxia/ischemia provides the conditions for severe brain injury to develop. However, the time course and extent of deterioration of neurologic function and brain metabolism following anoxia/ischemia in such animals are less clear. We have respired hyperglycemic cats (30 to 50 mM serum glucose; control = 5 mM) with nitrogen for 8 minutes to produce a model of delayed neurologic deterioration. These animals initially develop fasiculations in tongue and facial muscles, hypersalivation and, in the absence of early cardiogenic shock, myoclonic jerks, seizures and coma. Widespread neuronal loss with diffuse capillary proliferation is present after several days survival. Cats that deteriorate earlier show moderate brain edema with cerebral and cerebellar herniation, and ischemic cell change, neuronal pyknosis and/or poor staining of nuclei. Similarly exposed saline infused cats show no such postexposure neurologic deterioration and no or substantially less marked lesions in brain.

At the end of exposure, glucose infused cats have significantly greater lactate concentrations in cortex (27 versus 20 μmoles/g), while the 2 groups show no differences in ATP (0.3 - 0.4 μmoles/g) or phosphocreatine (1 μmole/g). One hour following resuscitation, both groups have approximately control lactate concentrations, slightly reduced ATP (2 - 2.4 μmoles/g) but control phosphocreatine (5 to 6 μmoles/g). After 3 hours hyperglycemic cats trend toward elevated lactate and depressed ATP which reach significance by 5 hours (lactate = 8 and ATP = 1.7 μmoles/g). Saline and glucose infused control animals show no significant differences in metabolite concentrations. Electron microscopy 5 hours into recovery reveals severe cellular edema, with swollen mitochondria and damaged cellular membranes but only in hyperglycemic cats. In conclusion, hyperglycemia on exposure to anoxia leads to delayed development of severe neurologic injury, brain edema and widespread brain damage. Early following reoxygenation brain metabolism returns almost to normal, but, thereafter, in hyperglycemic animals slowly deteriorates with ATP declining and lactic acid accumulating in parallel with neurologic deterioration. (Supported by Veterans Administration Medical Research Service.)

NEUROETHOLOGY II

152.1 THE FEMALE'S OWN SINGINGS PROMOTE OVARIAN ACTIVITY: AUDITORY AND PROPRIOCEPTIVE FEEDBACK. M.-F. Cheng. Institute of Animal Behavior, Rutgers University, Newark, New Jersey 07102.

Hormonal control of the female reproductive system is strikingly similar among different species, including women. Similarly, social influence of the ovarian activity has been reported in many species. Most if not all the studies have been designed to ask which aspects of the social factors stimulate female reproductive functions. Clearly absent in these studies is a consideration of the dynamic nature of social interactions. In this study we report that in the ring dove it is the female's own singing in response to male courtship that directly stimulates her follicular development.

The male ring dove typically initiates courtship by singing two syllable notes (bow-cooing, nest-cooing) interspersed with chasing activity. The female joins in singing (nest-cooing) with the male at the nest site for a period of time. A few days later, the female lays a clutch of two eggs. Females stop singing abruptly after laying the 2nd egg.

Females muted by lesions in one of the neural control stations of nest-calls (medial portion of n. intercollicularis, mICo) or by cuts in motor nerves innervating syrinx blocked or reduced the effectiveness of the male's courtship to induce follicular response. These results led us to hypothesize that it is the female's own singing that promotes her follicular growth. To determine whether audiofeedback is involved in the female's endocrine response, a playback experiment was performed. Female doves were devocalized by inserting a 1½ inch polyethylene tubing (3/16" OD, 1/8" ID) into the interclavicular sac immediately surrounding the syrinx. Females were then assigned to the playback of different types of coos: male coos, the female's own coos, another female's coos; two other groups serve as controls, devocalized and sham-devocalized females without playback. The playback of the female's own coos resulted in significantly larger follicle size than if the playback was of male's coos. In another study, females were deafened by removal of both cochlea under anesthesia. Ten to twelve days after the surgery, these females and sham deafened females were paired with actively courting males, four out of 12 deafened females nest-cooed in response to male courtship; follicular development in these birds was comparable to sham-controlled females. Those deafened females that did not nest-coo showed little sign of follicular growth. We conclude therefore both auditory and proprioceptive feedback mediate the female's self-stimulation singing on follicular growth. Leucine autoradiography study suggests that efferent from mICo to n. periventricular region where LHRH neurons were found may mediate this self-stimulation effect. This work was supported by NSF grant BNS-8121495 and RSDA K02 MH70897.

152.2 IMPORTANCE OF MEDIAL FRONTOLIMBIC CORTEX IN PRODUCTION OF THE ISOLATION CALL OF SQUIRREL MONKEYS. J.D. Newman and P.D. MacLean. NICHD and NIMH, Bethesda, MD 20205

The isolation call may rank as the most basic mammalian vocalization, serving originally to ensure maternal-offspring contact. Our previous findings in the squirrel monkey showed that damage to the core of the brainstem at the thalamo-midbrain junction affected the structure and production of the isolation call. Since the medial frontolimbic cortex has proved to be the main cortical area for eliciting vocalization in the squirrel monkey, the question arises as to what effect damage to this area would have on the isolation call (reviewed by MacLean, P.D., Arch. Gen. Psychiatry, 42:405, 1985). For investigating this possibility, adult squirrel monkeys were tested for ability to produce isolation calls before and after ablations of different parts of the frontal lobe. Criterion performance was the production of 20 or more isolation calls during a 15-minute period of isolation in a sound-reducing chamber.

Whereas bilateral prefrontal lobectomy rostral to the cingulate gyrus had no effect on the production rate of isolation calls during a three-month period of testing, either a lobectomy or lobotomy just rostral to the knee of the corpus callosum resulted in an elimination of calls during a comparable follow-up period. Failure to produce isolation calls was also observed in monkeys in which ablations were confined to the medial walls of the frontal lobe, with the aspirations including the paragenual, subcallosal and preseptal limbic cortex, and adjoining presulcal medial neocortex. Cortical ablations restricted to the anterior supracallosal or subcallosal limbic cortex, or to the rostral supplementary area above the anterior cingulate gyrus, had either no effect, or only a transitory effect on rate of calling.

In the critical cases in which ablation of the medial frontolimbic cortex and adjoining neocortex eliminated the isolation call, there was but little retrograde thalamic degeneration, a finding perhaps owing to the preservation of sustaining collaterals of neurons innervating these areas.

152.3 FOREBRAIN INVOLVEMENT IN REPRODUCTIVE BEHAVIOR OF THE FEMALE FROG. C. Diakow, R. Shanley* and C. Scharff* Biology Dept., Adelphi University, Garden City, N.Y. 11530

When in breeding condition, female Rana pipiens are silent and show oviposition behavior if they are clasped by males. In contrast, unreceptive females emit a release call. The following experiments provide evidence that oviposition behavior is mediated by the forebrain but that inhibition of the call is not.

Exp. 1 tested for oviposition behavior in females with transections at the midbrain-diencephalic junction and in sham operates. Receptivity was induced in these autumn-collected animals with distension by cloaca ligation. There was a significant difference between the groups: 4/8 controls showed oviposition in response to clasps by male frogs; none of 14 transected females did. This indicates that the forebrain mediates oviposition.

Exp. 2 tested for oviposition behavior in POA-lesioned females and in sham operates. Subjects were collected in the spring and ovulation and receptivity were induced by administering exogenous pituitary glands and progesterone. There was no significant difference between the groups. All 6 sham-operates oviposited in response to clasp by male frogs, as did 4/8 POA-lesioned females. Histological analysis compares the lesion sites in females that oviposited with those that did not.

Exp. 3 tested for inhibition of the release call in females with lesions in the pre-optic area (POA) or with transections at the forebrain-midbrain junction. Sham-operated controls were also tested. Prostaglandin (PG) is known to silence intact females, so subjects received 5µ/gbw PGE_2(i.p.) and a control injection of the vehicle, deionized water (DI). Subjects were tested by means of a manual clasp around the trunk and PG and DI injections were administered in counter-balanced order 5 days apart. All lesioned and control females vocalized after DI injection but were silenced by PG, indicating that the POA and forebrain are not essential for the silence of the receptive female frog.

152.4 PHYSIOLOGICAL CHARACTERIZATION OF THE SEXUALLY DIMORPHIC LARYNX IN *XENOPUS LAEVIS* M. Tobias and D.B. Kelley, Dept. of Biol. Sci., Columbia Univ., New York, N.Y. 10027

Sex specific vocalizations coordinate reproductive behavior in the frog, *Xenopus laevis*. Male mate calls, used to attract and excite females, consist of alternating fast and slow trains of clicks. Female ticking, used to signal unreceptivity to the male, consists of a constant very slow train of clicks. Male and female calls are readily distinguishable on the basis of their temporal pattern. Production of the mate call depends upon circulating androgen in males. Females do not mate call. The purpose of this study was to determine whether male-like vocalizations could be elicited from an adult female larynx by stimulation of the laryngeal nerve with the appropriate temporal pattern. In addition, the effect of circulating androgens on laryngeal function was assayed in adult females.

We recorded electromyograms, tension, and actual sounds produced by an isolated larynx in response to stimulus trains delivered to the laryngeal nerve. The results reveal two sex differences in the physiological response of laryngeal muscle. One, there is a marked potentiation of muscle compound action potentials throughout the stimulus train in males but not in females. Two, male laryngeal muscle responds to stimulus trains identical to the mate call in frequency and duration by producing discrete twitches, each of which result in an audible click. In contrast, the female larynx produces a maintained contraction which results in a single click following the initiation of the contraction. Androgen-treated adult females produce a response intermediate between males and females; potentiation of compound action potentials and discrete twitch tension are both increased. However, discrete twitches are superimposed on a maintained contraction, which results in either aberrant clicks or no clicks.

In summary, stimulation of the laryngeal nerve with a train that mimics the temporal pattern of the mate call results in the generation of a "vocalization" comparable to an actual mate call in males. In females, even when androgen-treated, the same stimulus is ineffective in inducing the mate call. Thus, physiological characterization of the larynx has revealed that one rate limiting component in the production of male specific vocalizations resides in the periphery.

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152.5 GAIN CONTROL IN A PRIMARY SENSORY PROCESSING AREA, THE ELECTROSENSORY LATERAL LINE LOBE, BY MEANS OF DESCENDING INPUT FROM THE MIDBRAIN. J. Bastian. Dept. of Zoology, University of Oklahoma, Norman, Ok 73019

The Electrosensory Lateral Line Lobe (ELLL) of gymnotiform weakly electric fish is the sole recipient of the electroreceptor afferent projection, and the output of this region projects to the midbrain torus semicircularis and nucleus praeeminentialis (NP). This latter structure projects back to the ELLL forming a feedback loop and descending neurons can influence the behavior of most of the ELLL interneurons as well as two categories of ELLL output neurons, E and I-cells. E-cells respond to an increase in electrosensory input with a phasic burst of activity which adapts rapidly, I-cells respond to this same stimulus with a decrease in activity. Responses of both cell types are graded with stimulus intensity, show preferential responsiveness or tuning to sinusoidal amplitude modulations of the electrosensory input and respond well to moving stimuli such as small conducting or non-conducting objects. The normal responses of E and I-cells are dependent upon the integrity of the descending input from the NP. A major source of this descending input is carried into the ELLL dorsal molecular layer by parallel fibers originating in the overlying posterior eminentia granularis. The NP projects to the eminentia granularis via the praeeminential-electrosensory tract, and this tract can be silenced via microinjection of Lidocaine or by small lesions. Either technique modifies the behavior of the ELLL E- and I-cells similarly. Removal of the descending input to the DML does not alter the E or I-cells' spontaneous firing frequency, threshold, or tuning to AM stimuli. This treatment does, however, change the gain of the electrosensory system and the receptive field sizes of individual neurons for moving electrolocation targets. In normal animals E-cell responses increase at a rate of about 1.5 spikes/s per dB increase in stimulus amplitude and I-cell responses increase at a similar rate for decreases in stimulus amplitude. Following descending input removal, E-cell responses increase at a rate of 4.6 spikes/s per dB increase in stimulus amplitude and I-cell responses increase by 3.8 spikes/s per dB decrease in amplitude. Receptive fields, or areas lateral to the fish within which a 12mm metal cylinder caused increases of at least 100% of an E-cell's resting frequency, averaged 2 cm^2 in normal fish and this increased to 7 cm^2 in fish with the descending input to the DML removed. Likewise, receptive field sizes for I-cells, stimulated with a 12mm plastic cylinder, increase from 1.3cm^2 to 4.6cm^2. E and I-cell responses to moving electrolocation targets also decrease rapidly as a function of the target's distance lateral to the fish. Removing the descending input increased the size of the responses at any given distance and also increased the rate of response decay as a function of distance.

152.6 TEMPORAL HYPERACUITY: NONLINEAR CONVERGENCE OF ELECTRORECEPTIVE AFFERENCES. G. Rose and W. Heiligenberg. Neurobiology Unit, Scripps Institution of Oceanography UCSD, La Jolla, CA 92093

The mechanism by which the weakly electric fish, Eigenmannia, detects time disparities of several hundred nanoseconds was explored.

Eigenmannia produces a nearly sinusoidal electric signal of an individual frequency (range, 250-500 Hz) via a specialized organ in the tail. The fish detects this signal using electroreceptors that are located throughout the body surface. A fish's electric organ discharge (EOD) can be "jammed" by the discharge of another fish if the two signals are of similar frequency. These EODs sum to form a signal which is amplitude and phase modulated at a rate equal to their frequency difference. In its Jamming Avoidance Response (JAR), Eigenmannia increases or decreases its frequency if the EOD frequency of the neighboring fish is lower or higher than its own, respectively. To determine which direction to shift its EOD frequency, the fish must detect the small amplitude modulations and modulations in the relative timing of signals received by different regions of its body surface (i.e. time disparity). At threshold, Eigenmannia can detect amplitude modulations of 0.1% and time disparities of 400 nanoseconds. Timing information is provided in the responses of "T-type" primary afferents. These nerve fibers mark the occurrence of each EOD by producing a single spike. To assess the capacity of T-type afferents to lock their spikes to a particular phase of the stimulus cycle, recordings were made from individual fibers. Even the most accurate phase coders time-locked their spikes with a standard deviation of approximately 37 microseconds. Thus, for time disparities of several hundreds of nanoseconds, it would be impossible for a fish, using the information gathered from any single afferent over 300 milliseconds (latency of the JAR), to reliably shift its EOD frequency in the correct direction. When the amplitude ratio between the jamming stimulus (S2) and signal that served as a replacement for the fish's own EOD (S1) was small (i.e. 0.0016), corresponding to a maximum time disparity of 1.8 microseconds, no JAR could be observed upon stimulation of specific body regions posterior to the gills; JARs could be elicited by stimulating only the head region using an S2/S1 ratio of 0.001. Simultaneous stimulation of three posterior regions with the S2/S1 ratio of 0.0016, however produced a strong JAR. Thus, our results indicate that electroreceptive afferent inputs converge in a nonlinear manner to generate sensitivity to time disparities of several hundred nanoseconds. In this process, inputs which are individually insufficient for eliciting a measurable JAR produce a strong response when combined.

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152.7 EFFECT OF ABLATING THE CERCAL CHORDOTONAL ORGAN ON ESCAPE TURNS OF COCKROACHES. E. Gerzog,* A. Hahari* and J. Camhi. Dept. Zool, Hebrew Univ, Jerusalem, Israel. SPON: M. Devor

The cockroach Periplaneta americana turns away from the source of a wind puff, a response mediated by hair-like receptors on the cerci, paired posterior appendages. Rotation of one cercus medially by 60° from its normal lateral angle causes turning behavior to be shifted roughly 30° (mean of the one rotated and the one unrotated cercus). Underlying this behavioral change, the directional response curves are shifted for specific giant interneurons known to be heavily involved in mediating the turns (Comer and Camhi, J. Comp. Physiol. 155:31, 1984).

At the base of each cercus is a stretch-receptive chordotonal organ (CO) which senses cercal position (Bernard et. al., J. Comp. Physiol. 153:377, 1983). Given that cercal rotation leads to corresponding rotations of GI directional curves and turning behavior, the cockroach does not compensate immediately for imposed cercal rotations, respecifying wind angle with respect to body axis as occurs in crickets (Rozhkova et. al., J. Comp. Physiol. 137:287, 1980). Thus the CO, though apparently designed for registering the required sensory information, appears not to serve this function. It would seem, then, that the encoding of cercal position by the CO does not play a role in specifying the direction of the escape turn.

To test this idea, we ablated the CO from the right cercus and tested the cockroaches' turning behavior over the next 2-30 days. Wind stimuli received from within the angular range from head-on to 30° right of head-on evoked 69% turns to the right (wrong direction). Turns for wind from other angles were normal. The wrong turns persisted for at least 30 days. Sham operated controls gave significantly fewer wrong turns, 39% ($p<0.02$). CO ablation, but not the sham operation, caused large areas of degeneration in the lateral cercal nerve, through which the CO axons run. Neither operation produced any visible degeneration in the medial cercal nerve, through which the axons of cercal wind receptors run. We also counted the axons in the left and right medial cercal nerves. There was no significant difference on the two sides following either CO ablation or sham operation. The pronounced effect seen after CO removal was not produced by ablating other nearby sensory structures, such as the stylus. Thus CO removal appears to have a specific effect on the direction of escape turns. The substantial percentage of incorrect turns in sham-operated controls may result from a mechanical disturbance of the cercal base. We are testing effects of CO removal on wind responses of the GI's.

152.8 EFFECT OF ABLATING NON-CERCAL RECEPTORS ON CERCAL-MEDIATED ESCAPE BEHAVIOR IN THE COCKROACH. J. Camhi and L. Leibowitz.* Dept. Zoology, Hebrew Univ., Jerusalem, Israel

The cockroach Periplaneta americana responds to wind puffs, such as those resulting from the rapid approach of a predator, by turning away and running. The wind is detected by filiform hair receptors on the cerci, two posterior appendages. The cercal sensory axons project to the last abdominal ganglion (A6), where they excite giant interneurons (GI's), known to largely mediate the escape turns. If one ablates the cercus of one side, wind puffs from near the front on that side evoke turns toward, rather than away from, the wind source. Underlying this, the GI's on the side of the ablation are now almost silent in response to wind. Within one month, the turning behavior is largely corrected, and the GI's of the ablated side are now strongly activated by the opposite cercus (Vardi and Camhi, J. Comp. Physiol 146:299).

In studying such directional errors and their corrections, we ablated, in control animals, other posterior sensory structures but not the cerci. Ablating some structures, such as an anal stylus, caused little or no change in the turning behavior. However, after ablating unilaterally the tergites and sternites of abdominal segments 7-10 (whose sensory innervation derives from ganglion A6), wind puffs from a 40° frontal angular range on the ablated side evoked turns toward the wind source (incorrect turns) on 60% of the trials. This was significantly different from the 6% incorrect turns for normal animals. It was as though the cerci themselves had been ablated, though these were actually intact. The ablated areas contain no filiform hairs, and no receptors which, when stimulated by wind, evoke any action potentials in the GI's. Retests after two weeks showed that the incorrect turns persisted (65% wrong turns), though by 4 weeks there was significant correction (25% wrong turns). The corrected behavior persisted till at least 6 weeks. This time course parallels that of behavioral correction following unilateral cercal ablation. Ablating unilaterally just one segment (No. 7) leads to a similar number of wrong turns on day 1 (57%) and similar correction at 4 weeks (25%).

To determine whether these specific behavioral deficits and their corrections involve changes in the GI responses, we have recorded simultaneously from the GI's of both sides, both before and after ablating unilaterally segments A7-10. The ablation grossly alters the balance in left vs right GI activity, a property important in the motor decision whether to turn left or right.

152.9 TEMPORAL PATTERN RECOGNITION AND 'TRADE-OFF' PHENOMENA IN ACOUSTIC COMMUNICATION IN CRICKETS (GRYLLIDAE). J.A. Doherty* (SPON: M. Nelson), Sect of Neurobiology and Behavior, Cornell University, Ithaca, New York 14850

Behavioral studies of animal communication systems often have sought to specify the minimal physical properties of the sender's signal that are both necessary and sufficient for eliciting responses in the receiver. In systems where relatively few properties are involved, this strategy can be useful for directing studies of the neural correlates of signal detection and recognition. However, in more complex systems in which several signal properties are involved, such a strategy may not be adequate for elucidating more complex neuronal mechanisms of signal recognition. In some cricket acoustic communication systems, for instance, the recognition of male calling song and the elicitation of positive phonotaxis in responsive females during mating appears to involve the evaluation of several calling song temporal properties that have different weightings in the recognition process. These interactions can be described by a 'trade-off' in which several temporal properties contribute to the total attractiveness of the calling song. When the phonotactic efficacy of one temporal property is lowered, the attractiveness of the entire acoustic stimulus can be maintained by raising the attractiveness of other temporal properties. Some properties may have much higher weightings than others, leading one to believe that these other properties are 'nonessential' in the recognition process. However, when the attractiveness and weightings of the most potent temporal properties are lowered, the effects of other, less attractive properties become more evident. In this paper I will present results of ongoing behavioral investigations into trade-off phenomena and weighting factors in several species of crickets that have different calling song morphologies (i.e. trilling, chirping, and chirp-trilling species of crickets). The results of several phonotaxis asseys will be considered, including phonotaxis in arenas, in flight, and on a locomotion compensator. The behavioral results indicate that in addition to searching for necessity and sufficiency of temporal properties in acoustic communication systems, we should also consider how the nervous system encodes and recognizes certain combinations of temporal properties, each of which has a different weighting in the recognition process. (Supported by a stipendium from the Max-Planck-Society and an NIH postdoctoral research fellowship)

152.10 PREDATOR EVASION AND THE ORIGIN OF INSECT FLIGHT: AN EXERCISE IN EVOLUTIONARY NEUROETHOLOGY. John S. Edwards, Department of Zoology, University of Washington, Seattle, WA 98105

One of the major events in the evolution of terrestrial life was the monophyletic origin of flight in the Insecta. Current hypotheses concerning the origin of flight emphasise the emergence of a capacity for dispersal in patchy environments of the Palaeozoic. An alternative view stresses predation as a factor favoring the acquisition of flight. Paleontological evidence indicates that arachnids (scorpions and spiders) were present in the Devonian terrestrial environment when the first terrestrial hexapods arose. Predation pressure must thus have been a significant factor in the early evolution of the hexapods, favoring evasive strategies.

Our studies of the cercal sensory system and associated giant interneurons in the primitively wingless insect Thermobia domestica (Thysanura), the firebrat, and other apterygote insects indicate precise homology of their cercal sensory-giant interneuron systems with that of the well known orthopteroid insects. The system is a major component occupying comparable fractions of the total volume of the abdominal CNS (ca. 25%) in both groups.

Some apterygote insects and some orthopteroid insects run in response to cercal stimulation. Others jump. The critical introduction of a motor pattern in which the legs were extended simultaneously, to jump rather than sequentially, to run, was the predisposing factor for flight. The same muscle sets are used in running, jumping and flying; a longer jump is a better evasive response. Flight is generally preceded by a jump in modern insects, and it is plausible that in the refinement of the evasive jump, aerodynamic properties of the thorax were modified. Whatever the source of aerofoils leading to active wing surfaces, it is proposed that they arose inthe refinement of evasive jumping primed by cercal sensory input to abdominal giant interneurons. The emergent capacity for aerial exploitation of patchy environments was a consequence which led to the elaboration of active flight. Flight and vision subsequently interacted to drive the visual coordination of flight and replaced the original, slower mechanoreceptive cercal sensory system with descending visual giant interneurons, as found in some orthopteroids with minimal cercal systems and in most Holometabola.

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152.11 FROM FEATURE DETECTION TO BEHAVIORAL ACTION: A CELLULAR ANALYSIS IN THE LOCUST.
H. Reichert and C.H.F. Rowell*. Dept. of Zoology, Univ. of Basel, CH-4051 Basel, Switzerland.

Animals abstract relevant information from the profusion of diverse stimuli they encounter and then organize appropriate behavioral responses. How is this done? A partial answer comes from studies showing that the CNS of higher animals are equipped with feature detectors. These are individual neurons, each tuned to a complex stimulus configuration indicative of a relevant natural situation. In a few cases, some insight into the neural mechanisms involved in feature extraction has been gained. However, we know little about the subsequent processing of feature detector activity.

Here we report the identification and characterization of several unique feature detector interneurons in the locust CNS, which integrate information from the compound eyes, ocelli and cephalic windhairs to detect course deviations in flight. Furthermore, we describe the cellular organization of the subsequent integrative circuitry, which translates feature recognition into appropriate behavior. Our studies show that segmental interneurons play important roles in the two neural systems which transform deviation detector activity into corrective steering behavior. First, thoracic interneurons are central to the fast course correction system which acts directly on wingbeat. There these interneurons, gated by the central pattern generator for flight, convert the deviation signal of the descending detector interneurons into phase-coupled premotor drive and apply this drive to appropriate flight motoneurons. Second, similar segmental interneurons are key elements of the slower feedback system for course correction. There premotor interneurons are involved both in the translation of course deviation information into corrective repositioning of the head and in the transformation of the resulting proprioceptive mismatch between head and thorax into compensatory flight steering behavior.
Supported by the SNF.

152.12 HEAD-BODY COORDINATION IN FREE FLYING HOUSEFLIES: AERODYNAMIC PERFORMANCE AND VISUAL FLOW.
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Houseflies were simultaneously filmed from two sides at 75 frames/s and the angular movements of the body were analyzed with respect to the three orthogonal body axes (long (roll), transverse (pitch) and vertical (yaw) axis). In some cases also the position of the head relative to body could be seen.

The flights are characterized by quick, saccade-like body rotations about the vertical and transverse axis separated by much longer periods of little or no turning. Smooth, long-lasting angular movements about the vertical as well as the transverse axis do not play an important role. Rotations about the long axis are more frequent, may last longer and may be smoother than the rotations about the other two axes.

While the fly turns its body during a banked turn the head may compensate for the roll movements of the body up to about 90 degrees (see also Hengstenberg, in: Varju and Schnitzler, (eds) Localization and Orientation in Biology and Engineering, Springer, Berlin, 1984). The head's movement about the vertical axis is restricted to about +/-10 degrees relative to the body (Geiger and Poggio, Biol. Cybern. 25: 141, 1977). Thus the head is forced to make similar yaw movements as the body. The orientation of the vertical axis to gravity easily allows 360 degrees and more rotation about this axis, whereas roll movements seem to be restricted to about +/-90 degrees. The different orientation of these two axes to gravity may be the reason for both, the different time course of the turns and the different restriction of the movement of head and body relative to each other.

We speculate that on the one hand the movements in flight are coordinated to achieve maximal adaptation of the body movements to the requirements determined by the aerodynamic forces during flight. On the other hand the movements of the head (which is aerodynamically not very important) seem to be adapted to the constraints posed for the eyes which are fixed to the head to perceive the visual flow generated by the translational displacement of the fly in space with as little rotatory disturbances as possible.

152.13 A BIOCHEMICAL FEATURE GENERATOR FOR A FIXED ACTION PATTERN OF BEHAVIOR.
R.Gillette and D.J.Green. Department of Physiology and Biophysics and the Neural and Behavioral Biology Program, University of Illinois, Urbana, IL 61801.

Fixed action patterns (FAPs) are stereotyped and rigid episodes of behavior usually triggered by specific sensory releasors, and are characteristically insensitive to further sensory stimuli during their expression. In the predatory mollusk Pleurobranchaea we have studied a FAP whose distinctive features are encoded in enzymic interactions with intracellular ion fluctuations and modifiable conductances in single command neurons.

The paired ventral white cell (VWCs) of the buccal ganglion are activated by tonic food stimuli in the buccal cavity to fire minutes-long recurrent bursts of broadening action potentials, driving recurrent episodes of vigorous ingestion. The effects of food stimuli are mimicked by cAMP, its agonists and its inhibitors of degradation; presumably neuromodulatory pathways from the buccal cavity cause enhanced cAMP synthesis in the VWCs. The burst episode is initiated and sustained by cAMP- stimulated voltage dependent Na^+ current.

We find that the duration, intensity, and interval between recurrent VWC bursts is in large part influenced by a pH sensitive and Ca^{2+}/calmodulin-activated cAMP phosphodiesterase (PDE). PDE activity increases during the burst and falls during the refractory, interburst interval over a time period paralleling the recovery of the neuron's ability to generate another burst. Both in vitro (Brain Res. 271, 371) and in vivo assays (Green and Gillette, this volume) show that PDE activity is a sensitive function of intracellular pH and Ca^{2+} fluctuations such as occur during the burst cycle. Calcium-activated K^+ currents are not demonstrable in the VWCs and probably have no role in the burst.

Thus, the features of the intensity, decay, duration of the burst, the interburst interval, and the consequent behavior of the FAP are satisfied in the following model. An increased level of cAMP causes increased inward current and burst initiation., Intracellular accumulation of Ca^{2+} and H^+ due to spike calcium current and proton generating processes lead to net increasing PDE activation which causes a fall in cAMP and consequent fall in inward current, leading eventually to burst termination. The slow recovery of Ca^{2+} and H^+ levels through the buffering processes of the cell determines interburst interval through the rate of fall of PDE activity. The fall of PDE activity itself is the major factor in the rate of recovery of cAMP levels to the next burst threshold, given a constant rate of cAMP synthesis. The interactions of cAMP, PDE, Ca^{2+}, and H^+ form a negative feedback circuit with the basic characteristics of a single phase oscillator.

153.1 **IN VIVO MEASUREMENT OF CATECHOLAMINE RELEASE IN PRIMATE VISUAL AND SOMATOSENSORY CORTEX.** J.W. McClurkin, C.D. Blaha, R.F. Lane, and R.T. Marrocco, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403

We have used electrochemical techniques in combination with standard electrophysiological recording methods to assess visually evoked catecholamine (CA) release from nerve terminals in the striate (area VI) and somatosensory (area 3B) cortex of N_2O anesthetized macaques. Chronoamperometric measurements using stearate-modified graphite recording electrodes (Blaha and Lane, Brain Res. Bull. 1983, 10:861) selective for the CAs were used to monitor changes in CA release in the vicinity of cells electrophysiologically recorded with tungsten microelectrodes by cementing the electrodes together flush at their tips. Receptive field locations of cells near the graphite electrode were mapped by recording unit activity with the adjacent tungsten electrode. In vivo changes in CA release were assessed by post-in vivo calibration of each graphite recording electrode in standard solutions of norepinephrine and dopamine.

Exposure to bright room or strobe light following a 20 min period of dark adaptation caused a rapid increase (ca. 2-3 μM) in CA release in the visual cortex. The same effect was seen when a 20 deg diameter radial grating, previously shown to activate cortical neurons (McClurkin and Marrocco, J. Physiol. 348:135) was projected onto a tangent screen and rotated radially around the visual receptive field locations of the neurons recorded with the microelectrode. Returning the animal to the dark caused a decrease in CA release to dark adapted levels with a time course similar to the light-evoked increase.

The visual cortex receives diffuse noradrenergic input from the locus coeruleus, which has been suggested to trigger REM sleep. Light-induced changes in CA release were not simply a reflection of concomitant changes in behavioral arousal since no significant changes in EEG patterns, heart rate, CO_2, or rectal temperature were observed. Moreover, under identical conditions, CA release in the somatosensory cortex was unaffected by manipulation of the light level or by tactile stimulation of the skin surface. However, administration of the short acting barbiturate Suritol (10 mg/kg i.v.) decreased CA release in both brain regions.

Since there is little evidence for a CA containing neuronal input from the lateral geniculate, our results suggest the existence of a modality-specific, CA-mediated activation of visual cortical neurons not originating from the geniculo-cortical pathway. We conclude that the combined application of in vivo electrochemical and electrophysiological techniques will provide a useful tool in assessing the role of CA transmitter function in the primate cerebral cortex. Supported by NSF 82-07531, NS 13556, and MH 17148.

153.2 SENSITIZATION TO AMPHETAMINE IS ACCOMPANIED BY ENHANCED STRIATAL DOPAMINE RELEASE. E. Castañeda*, J.B. Becker, R. Wilcox* and T.E. Robinson (SPON: C.M. Butter). Department of Psychology and Neuroscience Laboratory Bldg., The University of Michigan, Ann Arbor, MI 48104-1687

The repeated intermittent administration of amphetamine (AMP) produces an enduring hypersensitivity ("sensitization") to its motor stimulant effects in nonhuman animals, and to its psychotomimetic effects in humans. It has been hypothesized that sensitization to AMP may be due to long-lasting changes in brain dopamine (DA) systems. In support of this, we previously reported that in rats AMP pretreatment in vivo produces an enduring enhancement in AMP-stimulated striatal DA release measured in vitro (Robinson & Becker, Eur. J. Pharmacol., 85:253, 1982). The present experiments were conducted to replicate and extend these findings.

Ovariectomized (OVX) female rats were used because: (1) females show more robust behavioral sensitization than males (Robinson, Psychopharmacol., 84:466, 1984); and (2) OVX females show less variation in DA activity than intact females. One week after OVX the animals received 6 daily injections of 3.0 mg/kg d-AMP or were left undisturbed. Seven days after the last injection they were decapitated. Each striatum was removed, diced into 1 mm^3 fragments and placed into superfusion chambers through which medium flowed at 100 μl/min (Becker et al, J. Neurosci. Meth., 11:19, 1984). Following a 1 hr equilibration period effluent samples were collected over 5 min intervals in chilled tubes containing 0.05 N $HClO_4$. Following the collection of 3 baseline samples DA release was stimulated by infusing medium containing 0.5 to 10 μM d-AMP for 2.5 or 5 min, and samples collected for an additional 5 intervals. The samples were later assayed for DA with HPLC-EC.

The addition of AMP to the medium stimulated endogenous DA release in all cases. However, striatal tissue from sensitized rats showed a significantly greater enhancement in DA release than tissue from control animals, when DA release was stimulated with 5 μM d-AMP over 5 min or 10 μM over 2.5 min. This effect was also obtained in a second independent experiment by R. Wilcox in which rats received 3.0 mg/kg AMP daily for 5 days, were withdrawn for 10 days, and DA release stimulated with 10 μM of AMP over 2.5 min. In contrast, low doses of AMP (0.5-2.75 μM over 5 min; 5.5 μM over 2.5 min) stimulated the same amount of DA release from sensitized and control tissue.

In summary, the repeated intermittent administration of AMP in vivo produces an enduring enhancement in AMP-stimulated endogenous DA release from striatal tissue in vitro. We suggest that this increased responsiveness of the striatum to AMP may be at least partially responsible for the long-lasting hypersensitivity to AMP seen in animals and humans previously exposed to AMP.

153.3 ENDURING ENHANCEMENT IN MESOCORTICAL DOPAMINE UTILIZATION IN AN ANIMAL MODEL OF AMPHETAMINE PSYCHOSIS. T.E. Robinson, J.B. Becker, C.J. Moore*, E. Castañeda* and G. Mittleman*. Department of Psychology and Neuroscience Laboratory, The University of Michigan, Ann Arbor, MI 48104-1687

In nonhuman animals the repeated intermittent administration of amphetamine (AMP) produces a progressive and enduring enhancement in many AMP-induced behaviors, and this "behavioral sensitization" is thought to be an animal analogue of AMP psychosis. There have therefore been many attempts to identify a neural correlate of behavioral sensitization, and most of these have focused on the nigrostriatal dopamine (DA) system. For example, we previously reported that AMP pretreatment produces an enduring enhancement in AMP-stimulated DA release from striatal tissue in vitro (Robinson & Becker, Eur. J. Pharmacol., 85:253, 1982). However, it is possible that AMP produces long-lasting changes in other mesotelencephalic DA systems as well.

To test this hypothesis we examined DA utilization in ovariectomized (OVX) female rats given 7-9 i.p. injections of 3.0 mg/kg of d-AMP or saline. The injections were given either daily or once every 3-4 days. OVX rats were used because they show, (1) more robust behavioral sensitization than males (Robinson, Psychopharm., 84:466, 1984) and (2) less variation in DA activity than intact cycling females. Eight to 10 days after the last injection of AMP or saline the rats received an i.p. injection of α-methyl-p-tyrosine (MPT; 200 mg/kg), and were killed by decapitation 30 min later. The medial frontal cortex, striatum and nucleus accumbens were assayed for DA with HPLC-EC, and the rate of decline in DA (relative to control animals that did not receive MPT) was used as an index of DA utilization. In addition, to assess the acute effects of stress on forebrain DA utilization some rats received 20 min of mild footshock prior to decapitation.

In two independent experiments, pretreatment with AMP produced an enduring (at least 10 days) enhancement in medial frontal cortex DA utilization (23-40%), but not in striatal or nucleus accumbens DA utilization. As reported previously, acute footshock stress also selectively enhanced mesocortical DA utilization.

These experiments show for the first time that the repeated intermittent exposure to AMP produces an enduring enhancement in mesocortical DA utilization. It is not clear why this method failed to reveal an effect on striatal DA utilization, because with other methods we have consistently found enhanced striatal DA utilization and release in sensitized animals. Nevertheless, the evidence reported here suggests that enhanced mesocortical DA activity may be involved in the behavioral changes associated with AMP sensitization in nonhuman animals, and the cognitive abnormalities (AMP psychosis) associated with stimulant drug abuse in humans.

153.4 **CHRONIC ANTIPSYCHOTIC DRUG EFFECTS ON DOPAMINE RELEASE IN VIVO: REGIONAL SPECIFICITY AND MECHANISMS OF ACTION.** C.D. Blaha and R.F. Lane, Inst. of Neuroscience, Univ. of Oregon, Eugene OR 97403

We have previously shown that repeated (21 day) treatment with typical, e.g. haloperidol (HAL), and atypical, e.g. clozapine (CLOZ) and thioridazine (THIO), neuroleptic drugs will decrease basal dopamine (DA) release and produce a tolerance to neuroleptic-induced release of DA differentially in rat striatum and nucleus accumbens (Fed.Proc. 1985 44:892). In order to investigate the possibility that the decreases in DA release may be partly due to neuroleptic-induced DA cell depolarization inactivation we examined the effects of apomorphine (APO) on DA release after repeated neuroleptic treatment. To examine the importance of the anticholinergic properties of the atypical neuroleptics, both basal and neuroleptic-induced DA release were also examined after repeated administration of HAL in combination with the anticholinergic trihexyphenidyl (THP, 1 mg/kg s.c.).

Chronoamperometric measurements using stearate modified electrodes (Blaha and Lane, Brain Res. Bull. 1983 10:861) implanted in both striatum and accumbens were performed in anesthetized rats. Administration of APO (5, 10, and 50 μg/kg i.v.) caused a dose-related increase in the release of DA in both brain regions when given 24 hr after repeated treatment with HAL. In contrast, APO increased DA release only in the accumbens after repeated CLOZ or THIO while decreasing release in the striatum (opposite effects of APO were observed after repeated treatment with the substituted benzamide metoclopramide). Repeated treatment with HAL in combination with THP completely and selectively prevented the decrease in basal and HAL-induced release of DA in striatum, normally seen after repeated treatment with HAL alone. Additionally, APO produced a typical dose-related decrease only in the striatum after administration of this drug combination.

Our results provide direct in vivo evidence that (1) the reduction in basal DA release, APO-induced increase in DA release, and tolerance to neuroleptic-induced release of DA after repeated treatment is predominantly due to the development of a state of tonic depolarization of DA neurons (2) the anticholinergic properties of atypical neuroleptics are partly responsible for their inability to alter DA release in the striatum after repeated administration and (3) suggests that the delayed onset of extrapyramidal dysfunctions and therapeutic effects during neuroleptic treatment are mediated, respectively, by selective inactivation of the nigrostriatal and mesolimbic DA systems. These results provide evidence that in vivo electrochemical methods employed to monitor changes in endogenous DA release during repeated neuroleptic treatment may serve as a model system in evaluating the therapeutic and neurological side effect potential of new pharmacological agents. Supported by USPHS Grants NS 13556 and MH 17148.

153.5 EFFECT OF CALCIUM CONCENTRATION ON THE MELATONIN-INDUCED INHIBITION OF ^{3}H-DOPAMINE RELEASE FROM RABBIT RETINA. Margarita L. Dubocovich and Julie G. Hensler, Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The hormone melatonin inhibits at picomolar concentrations the calcium-dependent release of ^{3}H-dopamine from the rabbit retina in vitro through activation of putative melatonin receptor sites (Dubocovich, Nature 306, 782, 1983; J. Pharmacol. Exp. Ther., in press). The aim of this study was to determine the involvement of calcium in the inhibition of dopamine release by melatonin at different frequencies of stimulation. Retinas from albino rabbits were labeled in vitro with ^{3}H-dopamine and superfused with Krebs solution. The release of tritium was elicited by field stimulation at either 1 Hz, 3 Hz or 6 Hz (360 pulses, 20 mA, 2 msec) twice within each experiment. The percent of total tissue radioactivity released above spontaneous levels during the first period of stimulation (S_1) for controls was: 2.09 + 0.23% (n=5) at 1 Hz, 2.63 + 0.29% (n=6) at 3 Hz, 2.71 + 0.4% (n=5) at 6 Hz in the presence of 1.3 mM calcium, and 0.73 + 0.08% (n=4) at 1 Hz, 0.85 + 0.14% (n=6) at 3 Hz, 1.37 + 0.13% (n=4) at 6 Hz in the presence of 0.65 mM calcium in the superfusion medium. When the calcium concentration in the Krebs' solution was 1.3 mM, melatonin (10 pM - 10 nM) inhibited in a concentration-dependent manner the release of ^{3}H-dopamine elicited by field stimulation at 1 Hz (IC_{50} = 50 pM), at 3 Hz (IC_{50} = 60 pM) and at 6 Hz (IC_{50} = 50 pM). The inhibitory effect of melatonin was potentiated when the calcium concentration was reduced in the superfusion medium to 0.65 mM. Under these conditions the IC_{50} for melatonin was 8 pM at 1 Hz, 13 pM at 3 Hz, and 16 pM at 6 Hz. When the D-2 dopamine autoreceptors were blocked by S-sulpiride (0.1 uM), in the presence of low calcium (0.65 mM), the potency of melatonin to inhibit dopamine release remained unchanged. Taken together these results suggest that regardless of the calcium concentration, the inhibitory effect of melatonin on dopamine release did not depend on the frequency of stimulation or on the activation of D-2 dopamine autoreceptors. The lack of effect of melatonin on the calcium-dependent release of dopamine from the rabbit striatum (Dubocovich, Nature 306, 782, 1983) would exclude an effect of melatonin on a presynaptic component directly involved with neurotransmitter release. We conclude that in the rabbit retina, as reported in the rat hypothalamus (Zisapel and Laudon, Brain Res., 272, 378, 1983), the potency of melatonin to inhibit dopamine release depends on the external concentration of calcium, suggesting an effect of this hormone through its receptor on the entry of calcium into the presynaptic nerve terminal. Supported by USPHS grant EY 04788 and MH 09215.

153.6 PROLACTIN (PRL) AUGMENTS DOPAMINE (DA) RELEASE FROM MEDIAN EMINENCE (ME) SYNAPTOSOMES BY A MECHANISM INDEPENDENT OF EXTERNAL Ca^{2+}. K.A. Gregerson and M. Selmanoff. Dept. of Physiology, Univ. of Maryland, Sch. of Medicine, Baltimore, MD 21201

We have previously reported that depolarization-induced release of preaccumulated ^{3}H-DA from ME synaptosomes prepared from male rats is enhanced following in vivo treatment with ovine PRL (Soc Neurosci Abstr 9:319). In the present studies, we explored the mechanism of action of this PRL-induced effect on DA release from ME nerve terminals. Synaptosomes were prepared from intact male rats treated with ovine PRL for 48h (4mg/kg, sc, every 8h) and from vehicle-treated controls. Synaptosomes were loaded by incubation in 0.1uM ^{3}H-DA for 30 min at 30°C and release was subsequently measured over 1- to 20-second time intervals under basal and depolarizing conditions using a rapid filtration technique.

Confirming our previous findings, pretreatment with PRL selectively increased the fractional release of ^{3}H-DA from ME terminals during depolarization while basal (5mM K^+_o) efflux was unchanged from controls. The difference in evoked release between PRL-treated and control ME terminals occurred when depolarization was induced with either high K^+_o (75mM) or veratridine (75uM). This effect of hyperprolactinemia appeared to be specific to the tubero-infundibular dopaminergic (TIDA) terminals since PRL treatment did not alter fractional release of DA under basal or depolarizing conditions in synaptosome preparations of caudate nucleus, nucleus accumbens or olfactory tubercle. The PRL-induced enhancement of DA efflux from ME synaptosomes during depolarization was evident over a wide range of external Ca^{2+} concentrations (0.01-3.0mM), suggesting that this effect of PRL is on a release mechanism independent of Ca^{2+}_o influx. This hypothesis was supported by the observation that 20mM Ni^{2+} (a competitive Ca^{2+} channel blocker), while blocking the graded dose response of DA release to increasing [Ca^{2+}_o], did not block the PRL-induced elevation in evoked DA release from ME synaptosomes. Furthermore, the effect of PRL did not appear to be due to carrier-mediated efflux of DA since the elevation in evoked DA release from ME terminals following PRL treatment was neither blocked nor reduced by binding up the carrier with the potent blocker, nomifensine (25uM).

In summary, the PRL-induced augmentation of DA release is specific to the ME and is coupled to depolarization via a mechanism other than activation of Ca^{2+}_o influx or "reverse" carrier transport. Possibilities for PRL modulation of this signal transduction include mobilization of Ca^{2+} from intraterminal sources or effects on secretory granules of TIDA terminals.

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153.7 THE MECHANISM OF DOPAMINERGIC ACTIVATION INDUCED BY A NEW PSYCHOSTIMULANT PIPERAZINE DERIVATIVE. F. Battaini, L. Lucchi*, S. Govoni, P.F. Spano and M. Trabucchi. Inst. Pharmacol and Pharmacognosy, University of Milan, 20129 - Milan and Chair of Toxicol., II[nd] Univ. of Rome, Italy.

Fipexide, a piperazine derivative, has in vivo psychostimulant properties; some of the behavioral, neurochemical and clinical effects of this drug are reminiscent of a dopaminergic activation. In particular, fipexide, acutely administered, strenghtens amphetamine induced behaviors, induces a decrease of striatal dihydroxyphenylacetic acid concentrations and inhibits the release of prolactin. On the other hand, fipexide has very weak or none direct displacing activity in in vitro binding studies using dopamine receptor tritiated ligands. On this line the present study investigates the effect of acute and chronic fipexide administration on both pre- and postsynaptic markers of dopaminergic activity. Male Sprague Dawley rats were used; fipexide was administered per os as a suspension with 2% starch. Dopamine uptake and release, met-enkephalin content, binding studies with the various ligands were performed according to published procedures. Fipexide acutely injected (30 mg/kg, 3 hrs) induces a significant decrease in dopamine uptake measured in striatal slices of treated animals. The drug has an inhibitory action also when directly added in vitro. In contrast no significant effect was observed on dopamine release after in vivo administration although a small increase in the amine release was noticed after in vitro addition of the drug to striatal slices. In line with the uptake data the acute administration of the drug induces a decrease in the Na^+-dependent binding of ^{3}H-Cocaine, a regulatory site located on dopaminergic terminals for a putative endogenous peptide active on dopamine uptake. After chronic treatment (20 mg/kg, twice a day for 15 days) tolerance developed to the effect of fipexide on dopamine uptake. On the other hand the ^{3}H-Cocaine binding is still reduced when killing the animals three hours after the last treatment. In addition, the drug, both acutely and chronically administered, induces a decrease of striatal met-enkephalin concentrations. Fipexide seems therefore to indirectly act at regulatory sites of dopaminergic presynaptic function. The drug may represent an useful tool for studying the mechanisms of dopaminergic activation.

153.8 DOPAMINE AUTORECEPTOR ACTIVITY OF CGS 15855A. B.S. Glaeser, J.C. Berry*, W.C. Boyar*, R.A. Lovell, A.Braunwalder*, P. Loo*, G. Stone*, H. Kalinsky*, A.J. Hutchison*. Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, N.J. 07901

CGS 15855A has a profile of a potent and selective dopamine autoreceptor agonist. Dopamine autoreceptor activity for this compound was demonstrated by in vitro and in vivo test systems. For in vitro studies CGS 15855A displaced the dopamine agonist ligand ^{3}H-ADTN with an IC_{50} of 2 x 10^{-8}M - a value equipotent to other compounds having dopamine agonist activity (e.g. apomorphine 2 x 10^{-8}M, NPNA 2 x 10^{-9}M, and 3-PPP 4 x 10^{-7}M). CGS 15855A was relatively weak as a dopamine receptor antagonist with an IC_{50} of 1 x 10^{-6}M in ^{3}H-spiroperidol binding and was inactive in other receptor binding assays-serotonin (5HT-1, 5HT-2) clonidine (α_2), prazosin (α_1), QNB (muscarininc cholinergic). In vivo biochemical tests complemented the in vitro receptor assays showing CGS 15855A to be a specific autoreceptor agonist. In the gamma-butyrolactone (GBL) model for dopamine autoreceptors, CGS 15855A had an ED_{50} of 0.16 mg/kg ip and 0.39 mg/kg po, which was relatively more potent than apomorphine (ED_{50} = 0.56 mg/kg ip), TL-99 (ED_{50} = 1.60 mg/kg ip) and 3-PPP (ED_{50} = 2.11 mg/kg ip). In DOPA accumulation studies, CGS 15855A significantly decreased the accumulation of DOPA in the striatum. CGS 15855A (1 mg/kg ip or 10 mg/kg po) had a short to moderate duration of action (30-60 min) on the reduction of the dopamine metabolites, DOPAC and HVA, in the striatum. CGS 15855A did not alter the striatal concentrations of dopamine, serotonin or 5-HIAA. Similar effects were observed for apomorphine (2.0 mg/kg ip), while an elevation of dopamine metabolites was observed for haloperidol. In other studies, CGS 15855A (2 mg/kg ip) decreased DOPAC and/or HVA in the olfactory tubercles, nucleus accumbens and striatum. In conclusion, CGS 15855A has the profile of a dopamine autoreceptor agonist as demonstrated by in vitro and in vivo biochemical studies.

153.9 DOPAMINE AUTORECEPTOR AGONISTS DECREASE DOPAMINE RELEASE IN MOUSE AND RAT. W. C. Boyar*, P. L. Wood, A. Hutchison and C. A. Altar. (SPON: R. Lovell). Neurosci. Research, Pharm. Div., CIBA-GEIGY Corp., Summit, NJ, 07901.

Administration of dopamine agonists decreases in vivo dopamine (DA) synthesis and release (W. Kehr et al, J. Pharm. Pharmacol. 24:24,744, 1972). Static levels of the O-methylated DA catabolite, 3-methoxytyramine (3-MT), which reflect DA release (A. Groppetti et al, Life Sci. 23:1763, 1978), are also decreased by a DA agonist, apomorphine (B. H. C. Westerink et al, J. Neurochem. 38:680, 1982). We determined effects of DA autoreceptor agonists, including a new agonist, CGS 15855A, on levels of 3-MT, DOPAC, HVA, and DA in the olfactory tubercle (OT) or caudate-putamen (CP) of mice with GC/MF using selected ion monitoring (P. L. Wood, Biomed. Mass Spec. 9:302, 1982).

CGS 15855A (4 mg/kg i.p.) suppressed dopamine release in the mouse or rat 0.5 hr post-injection (*p < 0.05; ** p < 0.01 versus vehicle; n = 6-9/group):

		CAUDATE-PUTAMEN CONC. (pmol/mg protein)			
		3-MT	DOPAC	HVA	DA
Mouse	Vehicle	7.5	35	58	651
	CGS 15855A	3.4**	22*	41*	808
Rat	Vehicle	2.7	85	83	456
	CGS 15855A	1.4**	59**	52**	593

TL-99, apomorphine, N-propylnorapomorphine and CGS 15855A decreased 3-MT, DOPAC, and HVA in accord with their rank-order potency in the GBL model for DA autoreceptors. In contrast, the D_2 antagonists haloperidol or chlorpromazine markedly increased 3-MT in the mouse. The mouse was used in all subsequent studies. CGS 15855A produced a dose-related decrease in 3-MT to 35% of control values in the OT and CP whereas DOPAC or HVA were decreased to a lesser extent. The (+) but not (-) isomer decreased 3-MT, DOPAC, and HVA for up to 1 hr post-injection. Tolerance to the dopamine release-suppressing effects of multiple CGS 15855A injections did not develop during 3 hr, whereas striatal DA increased following repeated injections.

We conclude that (1) the mouse CP and OT contain dopamine autoreceptors; (2) 3-MT (DA release) is suppressed more than DOPAC or HVA (DA turnover) by dopamine autoreceptor agonists, and (3) CGS 15855A decreases DA release without the acute development of tolerance.

153.10 DIRECT EFFECTS OF PROGESTERONE INFUSION UPON IN VITRO AMPHETAMINE STIMULATED DOPAMINE RELEASE FROM THE CORPUS STRIATUM OF FEMALE RATS. D.E.Dluzen* (SPON: V.D.Ramirez). Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We examined the effects of differing modes of direct infusions and doses of progesterone (P) upon in vitro amphetamine (AMPH) evoked dopamine (DA) release from superfused corpus striatal (CS) fragments from ovariectomized-estrogen primed female rats. Rats received four daily injections of estradiol benzoate (5µg/rat). On the fifth day, rats were decapitated, the CS removed and superfused in a modified Krebs-Ringer phosphate buffer medium (pH 7.4, containing .1% albumin, .18% glucose, .1% ascorbic acid and 3.5 x 10^{-4}M pargyline) at a flow rate of 30µl/min. Perfusate samples were collected at 10 min intervals for 24 intervals, acidified to a final concentration of .1N $HClO_4$ and DA determined with a radioenzymatic assay. At interval five, P, diluted in medium, was infused directly (either in short infusions - four discrete 10 min infusions each seaprated by 20 min or in long infusions - a continuous 40 min infusion) and at interval 19, AMPH (10^{-5}M) was infused for 10 min. The short infusion mode of P at 2 ng/ml resulted in a significant increase in AMPH-stimulated DA release as revealed in the area under the curve compared to control superfusions ($\bar{X}$±SE=2619±457 vs 912±105 pg/50 min, N=4) or to those receiving lower (.2 ng/ml) or higher (50 ng/ml) doses of P. When P was administered in long infusions, with identical total amounts, no significant increase in the AMPH-stimulated DA response was observed at any of the three doses tested and the higher doses actively inhibited this response (642±151 and 619 pg/50 min for the 2 and 50 ng/ml doses, N=4 and 2, respectively). We also used a progesterone metabolite-5αDHP (N=4), a synthetic progestin -R5020 (N=4), cholesterol (N=4) and estradiol (N=4) in short infusions at 2 ng/ml. The 5αDHP was also somewhat effective in enhancing the AMPH-stimulated DA response (1728±499 pg/50 min), however responses to the remaining substances were lower and did not differ from controls (1362±331,1092±452 and 908±257 pg/50 min for R5020, cholesterol and estradiol, respectively). The post-superfusion DA concentrations of CS receiving short infusions of 2 ng/ml P (1.17±.27 ng/mg) were significantly greater than controls (.71±.1 ng/mg). Long P infusions significantly lower tissue DA concentrations. Values for 5αDHP and R5020 were significantly higher, while those for cholesterol and estradiol were significantly lower. None of these compounds altered spontaneous DA release. These results demonstrate that a short infusion of 2 ng/ml P to CS tissue from ovariectomized-estrogen primed female rats can augment the in vitro AMPH-stimulated DA release and post-superfusion DA concentrations while a long infusion at this dose actively inhibited these paramters, indicating differential effects of P on CS tissue.

STRUCTURE AND FUNCTION: CORTICAL AND SUBCORTICAL ORGANIZATION II

154.1 DEVELOPMENT OF ACETYLCHOLINESTERASE ACTIVITY IN LAYER IV OF RAT VISUAL CORTEX: COMPARISON OF EFFECTS OF NEONATAL MONOCULAR AND BINOCULAR ENUCLEATION. Richard T. Robertson and Alberto A. Tijerina*. Department of Anatomy, College of Medicine, University of California, Irvine, CA 92717.

The presence of a transient pattern of acetylcholinesterase (AChE) activity appears to be characteristic of some developing thalamocortical systems, including the geniculocortical system of the rat. The appearance of the AChE histochemical reaction product suggests that the AChE is found in terminals of geniculocortical axons. The purpose of the present study was to determine the effects of neonatal monocular or binocular enucleation on the development of cortical patterns of AChE.

Subjects were laboratory born Long-Evans or Sprague-Dawley rats of 0-18 post-natal days (DPN) of age. Animals were anesthetized by hypothermia on 0 DPN and one or both eyes removed surgically. Animals were sacrificed by aldehyde perfusion on 6-18 DPN. AChE activity was detected histochemically in series of 50 um frozen sections by a modified version of the method of Koelle. Non-specific cholinesterase activity was inhibited by 10^{-4}M iso-OMPA. Adjacent sections were processed for a Nissl stain.

AChE activity appears in normal animals as a fine fiber-like plexus in layer IV of cortical area 17 at about 7 DPN. AChE reaction product reaches peak intensity in animals sacrificed at 10-12 DPN and declines until adult levels are reached by 21 DPN. The areal and laminar extent of the AChE plexus corresponds precisely to the terminal field of geniculocortical projections.

Neonatal monocular enucleation results in a marked loss of AChE in the medial sector of layer IV of cortical area 17 contralateral to the enucleated orbit, without detectable loss of AChE in the lateral sector of the contralateral hemisphere or in the hemisphere ipsilateral to the enucleated orbit. The cortical region sustaining the loss of AChE corresponds to the monocular segment of area 17. Neonatal binocular enucleation results in a complete absence of the band of AChE normally found in layer IV of area 17. The loss of AChE occurs in both hemispheres. The neonatal enucleation does not affect the transient bands of AChE in somatosensory or auditory cortex.

These data indicate the transient patterns of AChE activity normally found in developing rat visual cortex are dependent on normal afferent innervation and/or stimulation.

154.2 SYNAPTOGENESIS IN THE PREFRONTAL CORTEX: QUANTITATIVE EM ANALYSIS IN PRE- AND POSTNATAL RHESUS MONKEYS. J.-P. Bourgeois, P.S. Goldman-Rakic and P. Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

In order to relate neural maturation to the development of cognitive functions mediated by the prefrontal association cortex, synaptogenesis in the upper bank of the principal sulcus (Walker's area 46) was analyzed by electron microscopy (EM) in 18 rhesus monkeys ranging from the 47th embryonic day (E47) to 20 years of age. Two to four EM probes consisting of 100-120 consecutive electron micrographs were taken across the full cortical thickness in each specimen. The number of synapses per 100um^2 of neuropil and their classification into symmetric or asymmetric types and their localization on dendritic spines or dendritic shafts was determined in each probe.

Analysis of the E47 embryo failed to reveal any synaptic contacts within the prospective prefrontal cortex. By E78, synapses are present in the marginal zone (prospective layer I) and also in the subplate zone below the cortical plate. During the next fetal month synaptogenesis proceeds more rapidly in layer I than in the cortical plate itself. During the remaining two months of gestation, synaptogenesis increases exponentially in all cortical layers, and by birth (E165) the density of synapses is equivalent to that in the adult prefrontal cortex (15/100um^2). However, synaptogenesis continues at a high rate during infancy, attaining a peak of about 25/100um^2 between birth and the third postnatal month. Thereafter, synaptic density decreases slowly until puberty (3-5 years) when it again reaches the adult level of 15/100um^2. The changes in synaptic densities are predominately associated with the production and elimination of synapses situated on dendritic spines which form 60-70% of synapses at all ages. Synapses on dendritic shafts (30-40%) and cell somas (<1%) are less involved in age-related changes. The proportion of asymmetric to symmetric synapses also shows a transient increase, rising from about 4:1 at E89 and birth, to 7:1 at the peak, before decreasing back to 4:1 at maturity. The time of onset and the rate of synaptogenesis in the prefrontal association cortex are similar to that recorded in the primary visual cortex of the same animals (Bourgeois and Rakic, Neurosci. Abst. '83). In contrast, synaptic density during the stage of transient overproduction is lower in the prefrontal than in the visual cortex. Furthermore, unlike in the visual cortex, the excess of synapses within the prefrontal cortex is most pronounced in the supragranular (I-IV) layers, less in layer V, and absent in layer VI. Studies are in progress to examine how the period of elimination of excess synaptic contacts in the principal sulcus relates to the emergence and development of cognitive functions that depend on this area in the rhesus monkey (Diamond and Goldman-Rakic, Neurosci. Abst. '85). Supported by the U.S. Public Health Service.

154.3 A COMPUTER SYSTEM FOR REGIONAL VOLUME DETERMINATION OF PRIMATE AND HUMAN CEREBRAL CORTEX. W.K. Smith, M.C. de Lacoste, D.S. Schlusselberg, B.G. Culter* and D.J. Woodward. Dept. Cell Biology, Univ. Texas Health Science Ctr., Dallas, TX, 75235.

One aim in quantitative neuroanatomy is to determine regional volumes of brain structures from serial sections. A major problem is the number of sections to be processed. Representative data sets include 2000 fetal brain sections from the Yakovlev collection, 1500 adult sections from the UTHSCD Willed Body Program and 7000 primate sections from Dr. Stephan's collection at the Max Planck Inst. Brain Res. (Frankfurt). Efficient information handling strategies have been developed as part of the Computer Aided Reconstruction Package (CARP) to acquire perimeters, create solid-body models, calculate quantitative measures and display graphical data in three dimensions.

Perimeters are obtained in one of two ways: 1) contours are manually traced from photographic projections onto a graphics tablet attached to a time-sharing computer or 2) contours are semi-automatically collected from video digitized sections stored in a frame buffer using a high-speed graphics processor. After transformation into a biological coordinate system, the entire set of contours from a single brain is stored in one file. Tablet-based menus and terminal keystroke sequences are optimized for user interaction. Hardcopy output on standard pen plotters and electrostatic plotters is available.

CARP executes an interpreted command language which allows user specification of subsequent data processing. Arithmetic, database manipulation, graphical display and flow control operations are its commands. Three forms of output are provided: 1) graphical display of original and modeled data, 2) printed summaries of all quantitative information and 3) card image output formats suitable for input to SPSS-X and other statistical packages. This flexible command language has a major impact on user time required to process large amounts of data.

Quantitative values are obtained for each region. The region delimiters employed neocortical, allocortical and subcortical landmarks such as the rostrum of the corpus callosum, hippocampus and calcarine sulcus. The contour data facilitates generation of solid-body models of the regional volumes. Errors and artifacts not observed in the statistical analysis can be visualized in a solid surface display. Graphical imaging of modeled data allows the use of dividing planes and other methods for specifying regional boundaries. Supported by NSF BNS8316764 (MC de L) and the Biological Humanics Foundation (DJW).

154.4 FERRITIN LOCALIZATION IN RAT BRAIN BY IMMUNOCYTOCHEMISTRY. T.L. Martin*, R.C. Switzer, III, J. Joshi*†, and A. Zimmerman (SPON: N. Greenberg). University of Tennessee, Depts. of Medical Biology and †Biochemistry, Knoxville, TN 37920.

The presence of appreciable amounts of iron in the brain has long been known and is easily demonstrated histologically with the Perls method. Since the presence of iron in a cell triggers the synthesis of ferritin, an iron "storage" protein, the distribution of ferritin in brain might be expected to follow that of iron.

To determine the locations of ferritin in rat brain, freeze-cut sections were treated with immune serum containing antibody against rat ferritin. The antibody was raised in rabbits by injecting them with ferritin obtained from rat liver and spleen. The presence of antiferritin in the immune serum was checked by standard immunodiffusion. After application of the primary antibody, the sections were treated with the HRP-avidin-biotin (Vectastain) procedure to visualize the sites of antibody binding.

The regions of brain displaying ferritin-like immunoreactivity (herein, "ferritin staining") in general matched the description of iron containing areas in rat brain (Hill & Switzer, *Neurosci.* 11: 595, 1984). However, in those areas with positive immunoreactivity for ferritin, it was the neuron cell bodies and dendrites that were stained. This is in contrast to sections treated with the Perls method in which the glial cells are predominantly stained for iron and only a few neuronal populations are stained. In the neuronal populations with iron-positive cell bodies, i.e., central nucleus of amygdala, supraoptic nucleus, bed nucleus of stria terminalis, ferritin stained cell bodies were also found.

In the areas especially rich in iron, globus pallidus and substantia nigra, ferritin staining of neurons was particularly intense. The most striking pattern, however, was seen in layer 5 of cortex, where not only did neuron cell bodies stain but also the entire apical dendrite. In cingulate and entorhinal cortex, stained neurons were found in layers 2-5. The cerebellar purkinje cell bodies and dendrites were also stained except for those in the paraflocculus.

In our frozen sections, few glia stained for ferritin, but in preliminary results with vibratome sections, staining of glia was prominent in addition to neuronal staining. This suggests that freezing the tissue affects the state of ferritin in glia cells.

It is enticing to speculate on the presence of ferritin in neurons in which iron is not histologically demonstrable. Perhaps it is a strategy for those neurons in an iron-rich matrix to provide a means of preventing iron from participating in reactions that generate cytotoxic free radicals. This work was supported by the Robert H. Cole Neuroscience Foundation.

154.5 Neurochemical markers for subsets of inhibitory neurons in cerebral cortex: is neuropeptide Y a marker for a small subset of basket cells? A.J. Hodgson and J. Oliver. Centre for Neuroscience, Depts Immunology, Dept of Medicine. Flinders Univ. med. Sch. Flinders med. Centre Bedford Park, S. Australia 5042.

Inhibitory neurons in cerebral cortex have been divided into different subsets on the basis of their morphological criteria The majority of inhibitory neurons are thought to be GABA-ergic and can be identified by the Immunocytochemical localisation of markers glutamic acid decarboxylase (GAD) or the neurotransmitter GABA itself. Recently it has been shown that neurons containing GABA or GAD also contain neuropeptides and these delineats subsets of neurons:e.g. Somatostatin and cholecystokinin mark subsets that are distinct whereas somatostatin and neuropeptide Y (NPY) overlap in a high proportion of cells To further characterise these subset of cells we used a newly characterised antiserum to NPY. Rat brains were fixed by perfusion either with formaldehyde (4%), glutaraldehyde (0.1%) and picric acid (0.2%) or with glutaraldehyde (2%) Vibrotome sections were treated to enhance the penetration of antibodies (Llewelyn-Smith et al J. Histochem Cytochem, in press (1985) by incubation in 50% ethanol (3x30 min) and cyanoborohydride (0.1%,30min) NPY like immunoreactivity was detected by using an antiserum raised in sheep (Oliver.J. and Blessing W.W. unpublished), using the avidin-biotin technique. After reaction in diaminobenzidine the sections fixed in 1% osmium tetroxide then 2% aqueous uranyl acetate and flat embedded in TAAB resin on glass slides. Selected regions were re-embedded and examined in the electron-microscope. Certain sections were stained either as semithin sections on slides or as ultrahtin sections on grids with antisera to GABA using immunogold. The NPY antiserum stained a rich plexus of immunoreactive fibres and terminals as well as cell bodies and dendrites. most of these cell soma were small with patchy staining of the perikaryon. In the light microscope a small but significant proportion of the terminals appeared to form pericellular baskets consisting of a few fibres which had 3-10 boutons in apposition with the cell soma and what appeared to be proximal dendrites. In some cases the cell contacted also stained for NPY. In the electron microscope it was confirmed that the boutons that contacted the soma made synaptic contact with a Gray type II synapse. The same cell was contacted by numerous non-immunoreactive boutons. It was also possible to show that some of the cells contacted contained GABA. These results indicate that some NPY cells make contact with cell somata and proximal dendrites and on this basis appear to be similar to cells classically described as basket cells. From this study it is impossible to determine the cells of origin of the NPY terminals.

154.6 AN IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE DOPAMINERGIC (DA), NORADRENERGIC (NA), AND SEROTONERGIC (5HT) INNERVATION OF PRIMATE PREFRONTAL AND TEMPORAL CORTICAL REGIONS. D.A. Lewis*, M.J. Campbell, S.L. Foote, M. Goldstein and J.H. Morrison (SPON: E.L.F. Battenberg). Scripps Clinic and Res. Fdn., La Jolla, CA 92037 and N.Y.U. Med. Ctr., N.Y., N.Y.

The expansion and differentiation of the prefrontal (PFC) and temporal cortical (TC) regions is a distinguishing feature of primate brain. Using antisera against tyrosine hydroxylase and dopamine-B-hydroxylase, which visualize DA and NA fibers respectively, and anti-5HT, we characterized the innervation patterns of these three monoamines in PFC and TC of cynomolgus and squirrel monkey. Marked species differences in innervation patterns were observed. For example, relative to the supragranular layers, 5HT innervation in the infragranular layers of PFC was more dense in cynomolgus than squirrel monkey. Our findings in cynomolgus can be summarized as follows: 1) All three monoamine systems exhibited regional heterogeneity in fiber density. In PFC, DA fibers were most dense in area 9 and there was a medial to lateral decrease in fiber density on the dorsal surface. In TC, there was a superior to inferior increase in DA fiber density. NA innervation of PFC and TC was sparse compared to DA. The density of NA fibers increased from the rostral to caudal portions of PFC. 5HT innervation was generally denser in TC than in PFC. 2) Each monoamine system had distinctive laminar patterns of staining. In area 9 there was a dense arborization of DA fibers in layers I-III and decreased staining in the infragranular layers, whereas in the more sparsely innervated dorsolateral areas of PFC, DA fibers were more dense in the infragranular layers than in layer III. NA fibers were generally most dense in the infragranular layers of PFC. 3) There was a striking complementarity of the different monoamine laminar patterns. In TC, 5HT innervation was most dense in layer IV, whereas both DA and NA fibers were least dense in layer IV. There was also a complementary relationship between DA and NA fibers in layer I. DA fibers were dense in deep I and superficial II; in contrast, NA fibers were sparse in these layers and dense in superficial I. 4) Distinctive morphologic specializations, such as the 5HT "baskets" described by Tork and Mulligan (Neurosci. Abs., 1984), were seen. In summary, we used antisera which visualize DA, NA and 5HT axons to demonstrate that the monoamines have regional and laminar innervation patterns which are heterogeneous, distinctive and, in some cases, complementary or species-specific. These findings suggest specific differences in the functional roles of the monoamine systems in primate neocortex. Supported by K1-00519, AG0513, MacArthur Fdn., Sam and Rose Stein Charitable Trust, AA 07456-01.

154.7 NORADRENERGIC AND SEROTONERGIC INNERVATION OF CORTICAL, THALAMIC, AND TECTAL VISUAL STRUCTURES IN OLD AND NEW WORLD MONKEYS. Stephen L. Foote and John H. Morrison. Department of Psychiatry, University of California, La Jolla, CA 92093 and Department of Basic and Clinical Research, Research Institute of the Scripps Clinic, La Jolla, CA.

Antisera directed against human dopamine-beta-hydroxylase and against serotonin were used to characterize the noradrenergic (NA) and serotonergic (5-HT) innervation of several cortical and subcortical visual areas in squirrel monkey and cynomolgus monkey. Few species differences were observed for either monoamine. Cortical areas 17 and 18, as well as visual areas in the temporal and parietal lobe were found to exhibit regional specialization of both 5-HT and NA innervation. At the border between areas 17 and 18, the laminar innervation patterns characteristic of NA fibers in area 17 shift such that layer IV of area 18 contains more fibers than layer IV of area 17 ,and the overall density of fibers in area 18 is higher. For 5-HT, the laminated patterns characteristic of area 17 become less striking in area 18, and the overall density of fibers decreases. Visual areas of inferotemporal cortex were found to exhibit sparse NA fibers and very dense 5-HT fibers. Area 7 of the parietal lobule was more densely innervated by NA fibers, and less densely innervated by 5-HT fibers, than any other visual cortical region examined. Visual thalamic nuclei exhibited even greater regional differences in NA innervation. The lateral geniculate was found to be virtually devoid of NA fibers, while the pulvinar-lateral posterior complex was densely innervated. The density of 5-HT fibers was more uniform across these structures; all exhibited a moderate to high density of immunoreactive fibers. The superficial layers of the superior colliculus were found to be densely innervated by NA fibers, whereas 5-HT fibers were most dense in the intermediate layers. These observations suggest that functionally related visual regions share common and distinguishable densities of NA innervation. Specifically, tecto-pulvinar-juxtastriate structures are more densely innervated than geniculo-striate and inferotemporal structures, suggesting that, within the visual system, NA fibers preferentially innervate regions involved in spatial analysis and visuomotor response rather than those involved in feature extraction and pattern analysis. This is especially interesting given the proposed involvement of the locus coeruleus/NA system in cortical sensory processing and attentional mechanisms. The 5-HT innervation of visual areas was generally of high density and exhibited less specialization than was evident for NA innervation. Supported by NS21384 (SLF), AG0513 (JHM), and a grant from the MacArthur Foundation.

154.8 EFFECTS OF REVERSIBLE COLD LESIONS IN PRESTRIATE CORTEX ON PERFORMANCE OF VISUAL TASKS. Carol L. Martin-Elkins* and James A. Horel (SPON: J. Robson). Department of Anatomy and Cell Biology, SUNY, Upstate Medical Center, Syracuse, NY 13210.

It is well known that the inferotemporal cortex plays an important role in visual learning and retention. The principle visual input to this cortex is from a fairly circumscribed part of the prestriate area (Desimone & Gross). The particular role of this portion of the prestriate cortex in these processes has not been determined. In this experiment we used reversible cryogenic lesions to study the possibility that this prestriate input to inferotemporal cortex may participate in visual learning or retention.

Loops of stainless-steel tubing were shaped to fit over the preoccipital and fusiform gyri in the lateral and ventral aspects of prestriate cortex bilaterally. During experimental trials, cooled methanol was pumped through the cryodes which were maintained at -5°C. The cold produces a functional suppression of the area underlying the cryodes. During this suppression, the animals were tested on acquisition and retention of visual discriminations and performance on a delayed match-to-sample (DMS) task. The visual discrimination required the animal to respond to only one member of a stimulus pair in order to receive a juice reward. The stimuli consisted of photographs of monkey faces, objects or black-white patterns. The DMS task required the animal to respond to a sample photograph of an object and then, after a delay of several seconds up to several minutes, to a photograph of the same object now paired with another non-match object. Monkeys were tested with delays of 10 seconds and 45 seconds between the sample and match while cooling for two 20-trial blocks interspersed with control blocks. Also, a 6-minute delay was used in which cooling was administered either during presentation of the sample when the information is stored or the match when it is retrieved, or throughout the entire trial including both sample and match.

Cooling the lateral and ventral surface of prestriate cortex resulted in deficits in retention of black-white patterns and monkey faces, but not in retention of objects on visual discrimination tasks. However, acquisition did not appear to be affected. A more interesting finding was that while cooling throughout the entire trial, DMS performance at delays of 10 and 45 sec. was not impaired, but it was severely disrupted when the delay was increased to 6 min. An impairment also appeared when the cooling was limited to the match portion on 6 min. trials. There was a weaker effect when cold was applied only during sample. These results suggest that the prestriate cortex may be involved in long-term retention of visual information, but is not as important in short-term retention or learning. Supported by NINCDS grant NS 1829-03.

154.9 LAMINAR AND TANGENTIAL VARIATION IN THE MORPHOLOGY AND DISTRIBUTION OF GABA-CONTAINING NEURONS IN RHESUS MONKEY PREFRONTAL CORTEX. M.L. Schwartz, D.S. Zheng and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale University, School of Medicine, New Haven, CT 06510.

The distribution and morphology of GABAergic neurons in sensory and motor cortices suggest that this transmitter is restricted to local circuit neurons and is uneven in its radial distribution. In the present study we examined the organization and morphology of GABA-containing neurons in primate frontal association cortex to determine whether areal differences in the localization of this transmitter may provide information on the functional differences between cortical regions. Neurons in area 46 were labeled immunocytochemically for GABA (Immunonuclear Corp.) or for the synthetic enzyme GAD (Oertel et al., Neurosci. 6: 2715, 1981) and their distributions were mapped. GABA and GAD positive neurons are found in all cortical layers. Unlike the sensory areas of the monkey in which the highest concentration of GABA-containing neurons is in layer IV, in prefrontal cortex these cells are most numerous in layer II and superficial layer III. In addition, GABA-positive neurons exhibit a disjunctive tangential distribution: regions containing a high density of labeled neurons occur in 100-150 um wide zones that are separated by regions of lower density ranging from 100-300 um in width. In contrast to columns of cortico-cortical neurons, the tangential distribution of GABA neurons does not exhibit a regularly repeating periodicity. A more detailed examination of this distribution in serial sections is in progress to determine whether the GABA containing cells line up in stripes or are clustered into blobs. The size and morphology of GABA positive neurons show laminar variations. Stellate or multipolar cells with an average diameter of approximately 10 um are the predominant cell type in all cortical layers. Other cell types include bitufted and fusiform cells (10 um) that are prominent in large numbers in layers III, V and VI, and a small population of basket cells (12-18 um) found in layers III and V.

These results reveal that GABA-positive cells in prefrontal cortex are distributed in a specific laminar and tangential pattern. The laminar pattern is clearly different from that of several sensory cortices, but we do not yet know whether sensory regions exhibit a disjunctive tangential distribution. In prefrontal cortex, the tangential variations may be relevant to the modular patterns of afferent and efferent connections and functional activity of this area. It is likely that a similar relationship exists in many other regions of the cortex.

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154.10 CONTINUITY BETWEEN VENTRAL PALLIDUM AND OLFACTORY TUBERCLE ILLUSTRATED BY PROMINENT PATTERNS OF GLUCOSE UTILIZATION DURING CHEMICALLY-INDUCED SEIZURES. L. Churchill, T. Pazdernik*, R. Cross*, M. Giesler*, F. Samson and S. Nelson. R.L. Smith Res. Center, Univ. of Kansas Med. Center, KC, KS 66103.

Changes in 2-deoxyglucose uptake during chemically-induced seizures provides clues to the anatomical regions functionally related in seizure pathways. Basal forebrain activation assessed by the 2-deoxyglucose method has been compared during seizures induced by bicuculline (0.6 mg/kg, i.v.), penicillin G (500 units, intrahippocampal), soman (0.1 mg/kg, s.c.) and kainic acid (12 mg/kg, i.p.). During seizures induced by bicuculline, penicillin G and kainic acid, the pattern of prominent increase in glucose use illustrates a connection between ventral pallidum and olfactory tubercle in agreement with anatomical connections between these brain areas demonstrated by the distribution of ferric iron content and acetylcholinesterase-positive neurons (Switzer, R.C., III, Hill, J. & Heimer, L., Neurosci., 7:1891-1904, 1982) and enkephalin-positive immunoreactivity (Haber, S.N. & Nauta, W.J.H., Neurosci., 9: 245-260, 1983). The connection is illustrated here by a bridge of increased glucose use between the dome-shaped ventral pallidum and the olfactory tubercle during bicuculline- and penicillin-induced seizures and by many multiple bridges in kainic acid-induced seizures. In bicuculline- and penicillin-induced seizures, the bridge is parallel to the fundus striatum connecting the ventrolateral edges of the ventral pallidum and olfactory tubercle. In kainic acid-induced seizures the many bridges lay parallel to each other and are perpendicular to the ventral border of the pallidum and dorsal to the olfactory tubercle. On the other hand with soman, activation is observed in these areas but the bridge between olfactory tubercle and ventral pallidum is not as prominent. Seizures induced by all 4 convulsants increased glucose use more rostrally to form a continuous, wide band from olfactory tubercle to anterior commissure. This band includes ventromedial nucleus accumbens and ventral pallidum. With soman and kainic acid the continuous band of increased glucose utilization continues through bed nucleus of stria terminalis and lateral septum. The prominence of the glucose use pattern in ventral pallidum and olfactory tubercle during repetitive, generalized motor seizures induced by a variety of different agents suggests a functional role of these brain regions in seizure pathways. Supported in part by U.S.Army DAMD17-83-C-3242.

154.11 IBOTENIC ACID LESIONS OF MEDIAL SEPTUM ABOLISH ATROPINE-SENSITIVE HIPPOCAMPAL RSA BUT SPARE ATROPINE-RESISTANT RSA. D. J. Stewart and C. H. Vanderwolf. Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada, N6A 5C2.

Previous experiments have suggested that hippocampal rhythmical slow activity (RSA) can be produced by activity in either of two ascending pathways. Activity in a serotonergic pathway (Vanderwolf and Baker, this meeting) may be responsible for the atropine resistant RSA seen during Type 1 behavior (e.g., walking, rearing, struggling during handling) and muscular twitches during rapid eye movement (REM) sleep. Activity in neurons in the medial septum and diagonal band may provide a cholinergic input during both Type 1 and Type 2 behavior (e.g., waking immobility, face washing, tremor) and REM sleep. This input may produce the atropine-sensitive RSA seen in the intertwitch intervals of REM sleep and also appears to be responsible for all the RSA present in urethane anesthetized animals. The role of the septal nuclei in atropine-sensitive (presumably cholinergic) RSA was studied in rats that received injections of ibotenic acid (5, 10 or 20 µg, dissolved in Locke's solution) in the medial septal region. Hippocampal RSA was quantified by passage through a band-pass filter (6-12 Hz for undrugged and atropine conditions, 4-10 Hz for urethane anesthetized conditions) followed by rectification and integration. Following at least two weeks recovery, all lesioned rats displayed RSA during Type 1 behavior both before and after atropine (50 mg/kg, i.p.). Following urethane anesthesia (1.0-2.0 g/kg, i.p.) RSA could be elicited by a tail pinch in the control group (vehicle alone) and the group receiving 5 µg of ibotenic acid but not in the groups receiving 10 or 20 µg. Analysis of REM sleep periods in the 20 µg treated group suggested that the RSA normally associated with the intertwitch intervals was also abolished, but the RSA associated with muscular twitches was preserved. Muscular activity during REM sleep was defined by EMG recording, a movement sensor and visual observation. Histological analysis revealed a dose related cell loss in the septohippocampal nucleus and the dorsal part of the lateral septal nucleus. Slight cell loss was also observed in the most dorsal part of the medial septal nucleus in some rats. Acetylcholinesterase staining of hippocampal sections appeared normal in all lesioned groups. Control injections produced virtually no damage. These results suggest that atropine sensitive (presumably cholinergic) RSA can be selectively disrupted by lesions which disrupt septal circuitry.

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154.12 SLEEP-RELATED DISCHARGE OF BASAL FOREBRAIN NEURONS WHICH RESPOND TO CORTICAL ELECTRICAL STIMULATION. R. Szymusiak and D. McGinty. Neurophysiol. Res. (151A3), V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. Psychology, U.C.L.A., Los Angeles, CA 90024.

We have previously examined basal forebrain (BF) neuronal discharge in cats during sleep and waking using chronic microwire techniques. Fifty percent of the neurons studied had a "waking-active" discharge pattern, i.e., discharge rates during waking were much greater than those during nonREM sleep. Another 20% were classified as "sleep-active" neurons (sleeping rates >> waking rates). Sleep-active neurons were confined to the ventral BF and were distributed like cholinergic neurons. The remaining 30% of the cells were categorized as "state-indifferent" (waking rates ≃ sleeping rates). We have now examined the responses of these different BF cell types to electrical stimulation of various cortical and limbic system sites.

Three adult cats were prepared for chronic sleep recordings and with bundles of microwires (32µm tip diameters) aimed at various BF sites (diagonal bands of Broca, substantia innominata, and ventral globus pallidus). Stimulating electrodes were placed in sensorimotor cortex, orbitofrontal cortex, rostral cingulate cortex, and the fornix. Sleep-waking discharge patterns were determined for all cells which responded to stimulation.

A total of 20 BF neurons were influenced by stimulation; 16 were orthodromically activated and 4 were antidromically driven. Orthodromic responses typically consisted of a brief excitation followed by a much longer period of inhibition. Latencies ranged from 4 to 20 msec. Nine orthodromic responses were obtained from orbitofrontal cortex, 2 from cingulate cortex and 5 from the fornix. Of these 16 cells, 8 were state-indifferent, 5 were sleep-active, and only 3 were waking-active.

Of the 4 antidromically driven cells, 3 were activated from orbitofrontal cortex and 1 from the fornix. Latencies ranged from 5-18 msec. Three of the 4 cells were state-indifferent and the remaining cell was sleep-active.

These preliminary results indicate that the most common BF cell type (i.e., those with waking-active discharge profiles) are least likely to be influenced by electrical stimulation of the cortical and limbic system sites examined. Thus, sleep-waking discharge profiles may be correlated with the anatomical connectivity of BF neurons.

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154.13 STUDIES ON ELECTRICAL RHYTHMS BETWEEN CEREBRAL CORTEX, SEPTAL AND LIMBIC AREAS. R.J. Morgan, G.T. Schneider*, C.C. Turbes, and T.N. Solie*. Dept. of Anatomy, Creighton Univ. Sch. of Med. Omaha, NE 68178

These studies are of the electrical activity of the extracellular space of the frontal, temporal and occipital cerebral cortex and the amygdala septal nucleus. These experiments show the interactions of the slow wave field potential of spatially distant neuron populations. Twelve cats are used in these studies. Chronic electrodes are surgically implanted in the cerebral and limbic brain areas previously mentioned. The analog signals are recorded on FM tape and analog to digitally converted and processed with a Varian V-72 minicomputer. Initial studies utilized coherence, partial coherence and cross phase spectral analysis. The same analog data is stored on a hard disc to facilitate comparison studies between brain regions.

These studies on coherence showed the percent coherence of the signals from two brain areas. Since many brain areas are synaptically interconnected, partial coherence analysis is used to remove a possible third signal from the other two signals. The synaptic interaction of a third signal from a third brain area is indicated when there is a decrease in coherence between the two signals in ordinary coherence when the third signal is removed in the partialization process. In some cases, removal of a noise term from one of the signals of the ordinary coherence pair of signals results in an increase in coherence following partialization. The changes in coherence varies with different frequencies following the partial coherence analyses. There are changes in the cross phase spectrum following partialization process. There are changes in phase evident at different frequencies and show increase or decrease in degrees of positive and negative phase. In some cases there is a complete phase reversal following removal of the third signal. These changes reflect the possible interaction of two or more synaptically interconnected brain areas. Certain brain rhythms (i.e., 40 Hz rhythm) show a complex interaction involving certain cortical and subcortical regions when partial coherence is used.

154.14

WITHDRAWN

154.15 ELECTRICAL ACTIVITY OF THE CINGULATE CORTEX IN THE RAT: RELATIONS TO BEHAVIOR AND CHOLINERGIC INPUTS. L.S. Leung, J.G.G. Borst* and D.F. MacFabe*, Dept. of Psychology, Univ. Western Ontario, London, Ontario, Canada N6A 5C2.

The gross electrical activity of the cingulate cortex was studied in freely moving rats with chronically implanted electrodes. During waking immobility and slow-wave sleep, cingulate EEG showed a large amplitude irregular slow activity (<30 Hz, ISA), containing high amplitude, sharp events of about 20 msec duration (EEG spikes). During active movements (e.g.,walking and rearing), the EEG spikes were suppressed and theta rhythm and fast waves (30-100 Hz) predominated.

The cingulate EEG spikes appear to be behaviorally modulated by a cholinergic input. The low spike rate during walking (as compared to waking immobility) is increased by atropine sulfate (10-50 mg/kg i.p.) but not by atropine methyl nitrate. EEG spikes during immobility was suppressed by pilocarpine (25 mg/kg i.p.). Pilocarpine also enhanced the cingulate and hippocampal theta rhythms during immobility. Spectral analysis indicate that after atropine, ISA was increased and fast waves were decreased, but the absolute power of the theta rhythm in the cingulate remained unchanged.

Electrolytic lesion of the medial septal area (which usually spared the horizontal limb of the diagonal band) decreased the acetylcholinesterase (ACHE) staining in layers I,II,III and V of the cingulate and abolished the normal relation of cingulate EEG spikes with behavior, i.e. spikes appeared both during immobility and active movements. Medial septal lesions also paled the ACHE staining of the hippocampus and abolished the hippocampal theta rhythm. However, in some cases, the cingulate theta persisted or even increased after hippocampal theta was abolished, suggesting that theta rhythm can be generated intrinsically by the cingulate cortex, and is not due to hippocampal volume conduction. Substantia innominata lesion with kainic acid, whether unilateral or bilateral, did not affect the relation of cingulate spikes with behavior, even though ACHE stain in the cingulate layer IV and in the neocortex was paler than normal.

In conclusion, cingulate EEG spikes and EEG appear to be suppressed by a pathway which involves muscarinic cholinergic synapses and passes through the medial septum. Cingulate theta rhythm is probably driven by neurons in the diagonal band and medial septum which are partly distinct from those driving the hippocampal theta rhythm. (Supported by Canadian MRC and NSERC grants to L.S. Leung).

154.16 EVOKED POTENTIAL AND CURRENT SOURCE DENSITY (CSD) PROFILES IN RODENT NEOCORTEX IN VITRO. N.L.Chiaia, L.J.Cauller*and T.J.Teyler. Neurobiology Prog., NE Ohio Univ. Col. of Med., Rootstown, OH 44272.

In vitro brain slice preparations offer promise for investigations of information flow and microcircuitry within the CNS. Reduced slice preparations of several structures, including hippocampus, thalamus, striatum and amygdala, have already been reported and data from these investigations has advanced our understanding of the organization and path of information flow through these structures. The present investigation examines depth profiles of extracellular evoked protentials in slices taken from rodent visual and somatosensory cortex to identify prototypical response properties.

Coronal sections, 400 um thick, were prepared from the visual and somatosensory cortex of syrian hamsters in the manner described previously by Shaw and Teyler (Br.Res.,243:34 1982). To ensure stability, slices were incubated for a period of 1 hr following dissection before recording began. The tissue was stimulated via a 75 um concentric electrode placed at the white/gray matter boundry with single, .1ms, biphasic pulses, 20-30V. Evoked potentials were recorded via NaCl-filled micropipettes in a trajectory orthogonal to the surface.

In most cases, records were taken at distances of 300, 600 and 900 um from the white/gray border. Typical responses consisted of a triphasic waveform whose initial component was a pronounced negativity which occured approx. 1.5 ms post stimulus. This was followed by a positive inflection (peak latency 3.2 ms) and, finally, a second negative component (peak latency 5.0 ms). All three response components were observed at each of the three depths sampled. The latter two components diminished as recording approached the cortical surface. These late components could be abolished by incubation of the slice in a low calcium media, while the early component was found to be calcium insensitive.

In some cases, CSD profiles were constructed from averaged evoked potentials taken at 50 um steps from surface to depth. This analysis revealed the presence of two concurrent sinks at supra- and infra-granular layers which corresponded with maximal multiple unit activty. These layers were separated by a prominent current source through layer IV.

154.17 FIELD POTENTIAL AND CURRENT SOURCE DENSITY (CSD) PROFILES OF INTACT AND IN VITRO VISUAL CORTEX IN RATS. G.Vaknin, L.J.Cauller*and T.J.Teyler. Neurobiology Program, NE Ohio Univ.Col.of Med., Rootstown, OH 44272.

One-dimensional CSD analysis was applied to field potential depth profiles recorded perpendicular to the surface through visual cortex of rats. Strobe light visual-evoked profiles of the intact preparation are compared to profiles evoked by white matter stimulation of cortical slices. Both intact and in vitro recordings were from the same point on cortex, presumably area 17.

Coronal slices of cortex were prepared and maintained in the usual way (Shaw and Teyler,Br. Res., 243:35, 1982). The earliest (1-2 msec) response to white matter stimulation was an abrupt current sink in deep layers with a concurrent sink in adjacent superficial depths. This response probably represents the synchronous antidromic activation of pyramidal cells in layers V and VI. The antidromic dipole was immediately followed by a longer duration, reversed dipole with its source at the cell body depth and its sink above. The antidromic dipole consistently appeared to move upward from the cell body layer (0.2 m/s).

No sinks or sources were observed through the central 300-400 um region of the cortical slice. Somewhat later than the antidromic response (2-3 ms) was a massive current sink in upper cortical levels that lasted 10-40 ms.

The profile along a surface-to-depth trajectory that was recorded lateral to the point of stimulation revealed a pattern of sinks and sources that was virtually identical to the on-line profile described above. The off-line profile differed only in amplitude.

The CSD profile of the visual-evoked response in anesthetized rats resembled the pattern of activation observed in the slice but developed slower and lasted longer. The earliest response (35 ms) was a deep sink, superficial source dipole which migrated upward through the central layers (0.02 m/s). As in the slice, this initial response was shortly followed by a massive current sink in upper levels.

Although the ascending sink phenomenon was evident using the nearest neighbor method of CSD differentiation, the second-nearest neighbor method enhanced the appearance of sink movement.

154.18 THE NEURAL BASIS OF EVOKED FIELD POTENTIALS STUDIED BY CURRENT SOURCE DENSITY (CSD) ANALYSIS OF THE HIPPOCAMPAL SLICE AS A SIMPLE MODEL SYSTEM. L.J.Cauller*, N.Chiaia and T.J.Teyler, (SPON:T.Voneida) Neurobiology Prog., NE Ohio Col.of Med., Rootstown, OH 44272.

The well studied and relatively simple cytoarchitecture of hippocampus provides a model system in which to determine prototypical CSD profiles in relation to known sink/source generators. In vitro slices of rat hippocampus were prepared in the usual way (Teyler, Br.Res.Bull.,5:391, 1980). We sampled the profile of field potentials at 100 um steps through area CA1 along a trajectory perpendicular to the pyramidal cell body layer. CSD profiles were computed from profiles constructed from averages of these field potentials.

Separate profiles were evoked by: (i) antidromic stimulation of the alvear tract; (ii) orthodromic stimulation of stratum oriens and; (iii) orthodromic stimulation of discrete Schaffer collateral bundles through stratum radiatum.

The characteristic population spike evoked by antidromic activation of pyramidal cell bodies was generated by an abrupt current sink that was restricted to stratum pyramidale. The concurrent source was distributed over the most proximal apical dendrites and throughout statum oriens. The profile of the antidromic population spike was prototypical for all spikes including those evoked by orthodromic activation.

Discrete synaptic activation of apical dendrites via Shaffer collateral stimulation produced a large, but shallow current sink in stratum radiatum. This sink was surrounded by concurrent sources which extended both distally and proximally away from the activated sink. Sources extended to the distal extremities of the dendrites without decrement while the profile of sources along the apical shaft decayed exponentially with distance from the activated sink (population length constant: 100-150 um). Even though this somatopetal EPSP source usually decayed to extinction 200 um below the cell layer, apical EPSPs were always associated with large somatic current sources.

Synaptic activation of basilar dendrites via oriens stimulation evoked a massive sink throughout stratum oriens with a concurrent source that was restricted to the cell body layer.

155.1 ANGULAR DEPENDENCE OF THRESHOLDS FOR ELECTRIC FIELD EFFECTS ON FIRING RATES OF APLYSIA PACEMAKER CELLS. A.R. Sheppard, S.M. Bawin, M. Burton-Sagan* and W.R. Adey. VA Medical Center, and Loma Linda University, Loma Linda, CA 92357.

In order to gauge the importance of cell orientation and geometry in the effects of extracellular electric fields on neuronal excitability, Aplysia abdominal ganglia were exposed in vitro to alternating electric fields of 2 to 40 mV/cm rms over a range of angles defined by the field direction and the rostral-caudal axis of the ganglion. Alternating current at the natural firing frequency (ω1-3 Hz) was passed between orthogonal pairs of Ag-AgCl electrodes and adjusted to yield a vector sum that varied in 30° steps over a range of 180°. Intracellular records of cell firing were computer-analyzed for the temporal correspondence of action potentials with the phase of the sinusoidal waveform. The smallest detectable influence ("low threshold") and the level for strong synchronization ("high threshold") were found by statistical tests on the distribution of phase angles obtained from tests over all ranges of orientation angle and field magnitude. All cells showed significant angular dependence for both the "low" and "high threshold", often over the full 20:1 range. At optimal angles the "low and "high thresholds" were often as low as 2 and 20 mV/cm, respectively. Maximum and minimum sensitivities were frequently at right angles, but in several instances changes of 30° gave ten-fold changes in threshold, and multiple extremes of sensitivity sometimes occurred in one cell. For the RUQ "white" cells, maximum sensitivity was coincident with the rostral-caudal axis. Conductance of soma membrane is numerically dominant in these cells, but the data are consistent with effects at trigger zones in the axon (aligned along the axis). Cell L8 and others of the LLQ had more variable angular dependences, including maximum sensitivities perpendicular to the axis. Overall, the diversity of cell positions within the ganglion and cell morphology may account for great variations in angular responses. In low Ca++ solutions (1 vs 12 mM) field sensitivity was markedly greater but with the same angular dependence, suggesting these effects have little to do with synaptic coupling but depend on membrane status. (Research supported by the U.S. Department of Energy and Southern California Edison Company.)

155.2 **INTERNEURONAL AND INTERGLIAL GAP JUNCTIONS IN THE STOMATOGASTRIC GANGLION OF THE ROCK CRAB, CANCER BOREALIS.** **D.H. Hall, E. Marder, and M.V.L. Bennett, Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 01461, and Dept. Biology, Brandeis University, Waltham, MA 02254.**

Neurons of the crab stomatogatric ganglion (STG) which are connected by both electrical and chemical synapses were studied by thin section and freeze fracture electron microscopy. Although electrotonic coupling is common in invertebrate ganglia, gap junctions (gjs), the presumed morphological substrate, have often been difficult to demonstrate (e.g. King, J. Neurocytol. 5: 207-266, 1976). In crab STG, neurons contact each other via small gjs between secondary or tertiary neurites. The neuronal somata and primary neurites are sheathed by glial wrappings, so that primary neurites have only limited contact with other neuronal processes. Finer neuritic processes are segregated into glomeruli with little glial interposition where opportunities for synaptic contact are greater. Chemical synapses are common within glomeruli and are characterized by accumulations of vesicles, small pre-synaptic tufts, and post-synaptic densities. Terminals can be classified by their vesicle contents, small or large diameter clear vesicles. These vesicle classes may correspond to different transmitters used by intrinsic inhibitory neurons as in the lobster STG (King, op. cit.). Processes containing large dense core vesicles are also observed, perhaps deriving from extrinsic modulatory neurons.

Glial cells also form large gjs (often 1 micron in diameter) between their somata and between their processes in the outer glial sheath of the ganglion. Large desmosomes are also found between glia. Freeze fracture confirms an exceptionally high density of large gjs in the outer glial sheath. The individual particles (avg. 12.5 nm) are loosely organized. Most particles cleave with the P-face, but 10-30% are found with the E-face. This predominantly P-face association is unusual for arthropods.

As yet there is little freeze fracture evidence for gjs between neurons, due to difficulty in identifying neuronal membranes. The small size of neuronal gjs found thus far in the crab STG is typical of many invertebrate ganglia where there is electrotonic coupling (as shown in the mollusc, Navanax, Hall et al., J. Neurocytol. 12: 831-846, 1983), and the data from the crab STG provides further comparative data suggesting that electronic coupling in these invertebrate systems is mediated by the usual structure.

155.3 INTRACELLULAR CESIUM FLUORIDE LINEARIZES THE MEMBRANE PROPERTIES OF MAMMALIAN NEURONS FACILITATING THE EXPLORATION OF SYNAPTIC INPUTS. A.R. Kay*, R. Miles* R.K.S. Wong (SPON: C.R. Fourtner) Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Electrotonic theory provides a means for estimating the distance of a chemical synapse from the soma; however, this body of theory is largely invalidated by the presence of channels that are activated in the subthreshold range of potentials (Llinas & Sugimori, Soc. Neurosci. Abstr., 10:193.7 (84); Masukawa & Prince, J. Neurosci., 4:217 (84); Stafstrom et al., J. Neurophysiol. 53:153 (85)), uncertainty as to the distribution of channels mitigate against a predictive nonlinear theory. Instead of applying restrictive theories we have attempted to linearize the membrane by suppressing voltage-dependant conductances using intracellular agents.

When recording intracellularly with microelectrodes it is possible to eliminate most of the outward current by the iontophoretic introduction of cesium, while most of the inward current borne by sodium can be annuled by the injection of local anaesthetic agents. Here we show that intracellular fluoride can be used to eliminate most of the calcium conductance.

Neurons were isolated from adult guinea pig hippocampi using trypsin digestion. The dynamics of inward current activation were studied in these neurons using the whole-cell patch clamp recording technique. When CsF was used as the predominant intracellular electrolyte, the noninactivating calcium channel, which activates at about -40 mV, was blocked by fluoride as has been previously reported in snail neurons by Kostyuk (Nature, 257:691 (75)), however, a low-threshold, inactivating calcium conductance, which activates between -70 and -60 mV was not affected by fluoride ions. This channel is similar in its kinetics to that described by Carbone & Lux in chick dorsal root ganglion cells (Biophys. J., 46:413 (84)).

In experiments on guinea pig hippocampal slices, recordings were made from the CA3 pyramidal cells with electrodes filled with fluoride (0.1 - 0.5 M) and EGTA (10 mM). Broadened, presumed Ca-dependent, action potentials were selectively suppressed after 5-10m recording with KF filled electrodes. The occurrence of both spontaneous and evoked synaptic potentials was maintained. With CsF filled electrodes membrane potential could be varied by intracellular current injection over a wide range to levels more positive than the epsp reversal. In contrast to our experience with electrodes containing Cs Acetate, slow large amplitude membrane potential shifts did not occur at potentials between -50 and -10 mV. Instead the membrane appeared to behave linearly at potentials above -50 mV.
(Supported by NIH grant NS 18464).

155.4 DEVELOPMENT OF MINI-NETWORKS ON MULTIMICROELECTRODE PLATES: MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL ANALYSES. M. H. Hightower*, L. E. Czisny* and G. W. Gross, (SPON: J. Hines). Dept of Biology, The Texas Woman's University, Denton, TX 76204.

We are using glass multielectrode plates having 36 photoetched, transparent indium tin oxide conductors confined to a 1 mm x 0.5 mm recording area to monitor spontaneous activity from mouse spinal monolayer cultures. In order to limit the neuronal culture to the approximate size of the recording matrix, we have developed methods to generate adhesion islands of 1 to 2 mm diameters centered on the matrix and isolated from larger feeder areas that function to condition the medium (Hightower et al., Soc. Neurosci. Abstr. 9: 239, 1983). Under optimal conditions, these islands contain only a few hundred cells and provide spatially defined, simplified, and spontaneously active networks. Classification of cells according to size and morphology has so far revealed no distribution difference between the centered mini-network and large, peripheral feeder areas. Young cultures (1 to 2 weeks after seeding) usually exhibit random, uncoordinated activity that can be observed on many electrodes. Older cultures (3-6 wks) reveal primarily multisite, coordinated bursting that is often rhythmic. Cell counts after post-recording histology with Nissl or combined Bodian-Nissl stains (Hightower and Gross, Stain Technol., 1985, in press) suggest that a minimum neuronal density of approximately 100 neurons/mm^2 is necessary to ensure spontaneous, coordinated bursting. This required density reflects the random neuron adhesion relative to the fixed electrode matrix and probably also the "critical mass" of network components necessary to generate coordinated activity. In addition, 10 mM $MgCl_2$ has always terminated random spiking as well as coordinated bursting. This implies that chemical synapses are involved in the generation of the observed spontaneous activity. These requirements for a minimum cell density and for functional synapses support contentions that the observed activity is a network phenomenon. SEM analyses of specific cell-to-electrode coupling and pharmacological manipulation of synaptic activity are in progress.

This work was supported by a Texas Woman's University Institutional Grant.

155.5 DIFFERENCES IN THE PASSIVE MEMBRANE PROPERTIES OF HIPPOCAMPAL CA1 CELLS IN AGED AND YOUNG RATS A. D'Aguanno*, L. Takeuchi*, M.F. Davies, B.L. Bardakjian*, T.J. Blaxter* and P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital; Institute of Biomedical Engineering, Dept.'s of Physiology, Medicine, and Electrical Engineering, University of Toronto; Addiction Research Foundation, Clinical Institute; Ontario, Canada.

Aged rats (28-30 months) showed different passive membrane properties than young rats (6-8 months). Intracellular recordings were obtained from CA1 cells in hippocampal slices of Fischer 344 rats using 3M KAc or KCl electrodes. Results obtained from peeling analyses of the voltage decay produced by short hyperpolarizing constant current pulses (0.5 ms, 5 nA), showed that the aged animals had a significantly shorter (U-Test, $p<0.05$) neuronal time constant ($\bar{\tau}_0$=19.6 ms, n=14) than the young animals ($\bar{\tau}_0$=27.6 ms, n=14).

Préliminary spectral analysis using the Fast Fourier Transform supported the conclusions derived from the peeling analyses. In a resistive-capacitive network, the product of the time constant and the radian pole frequency is unity. Neuronal time constants derived from spectral analysis of voltage decay for aged neurons were significantly shorter (U-Test, $p<0.05$) ($\bar{\tau}_0$=19.4 ms, n=5) than the corresponding time constant of young neurons ($\bar{\tau}_0$=32.3 ms, n=5).

The input resistances derived from the peeling analyses were lower but not significant in aged versus young neurons ($\bar{R}_{in}$ aged=44.3 MΩ, $\bar{R}_{in}$ young=57.3 MΩ). Preliminary analyses of neuronal input capacitance (C_s) estimated from the coefficients and time constants of the exponential voltage decay, suggest that C_s in aged animals ($\bar{C}_s$=136 pF, n=6) is significantly smaller (U-Test $p<0.05$) than in young animals ($\bar{C}_s$=350 pF, n=6).

These data show that :

1) spectral and peeling analyses of voltage decay produced by short hyperpolarizing current pulses yield similar results.

2) neuronal time constant and input capacitance of hippocampal CA1 cells seem to decrease with age.

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155.6 MORPHOLOGY AND ELECTROPHYSIOLOGICAL PROPERTIES OF RETICULARIS THALAMI NEURONS IN CAT. M. Deschênes, A. Madariaga* and C. Mulle*. Lab. of Neurophysiology, Sch. of Med., Laval Univ. Québec, Canada, G1K 7P4.

Reticularis thalami neurons (RE neurons) were identified morphologically and their electrophysiological properties were studied in cat under barbiturate anesthesia. Intracellular horseradish peroxidase injections showed that RE neurons possessed very long dendrites bearing numerous filopodia-like appendages and that their axon was directed toward main thalamic nuclei. As a rule, small axonal branches were also emitted within the RE nucleus itself. At rest, the membrane potential of RE neurons displayed two types of oscillations: a slow 0.1-0.2 Hz oscillation and fast 7-12 Hz oscillations occuring on the positive phase of the former. Episodes of spindle (7-12 Hz) waves lasted for 2-3 seconds and were characterized by rhythmic depolarizations and burst discharges. Intracellular current pulses injections revealed the presence in RE neurons of a large somatic persistent Na^+ current. After blockage of this current by QX314, rhythmic low-threshold depolarizing responses occured at the break of hyperpolarizing current pulses. These responses lasted for 70 msec and required large somatic hyperpolarizations (up to 30 mV) to be de-inactivated. It was then concluded that they represented low-threshold Ca^{2+} responses of dendritic origin. In QX314 injected cells, selective components of oscillations were abolished, among them, the positive phase of the slow oscillation and late depolarizing humps that followed burst discharges within spindle sequences. However, the rhythmic occurence of spindle episodes at 0.1-0.2 Hz were never affected by DC currents, QX314 or Cl^- injections, suggesting that oscillations within a particular RE neuron partly reflected the oscillatory behavior of a network of cells. On the basis of these electrophysiological results and on the basis of already-known morphological and neurochemical features of RE neurons a model is proposed to account for their rhythmic behavior.

155.7 PHARMACOLOGICAL AND KINETIC PROPERTIES OF OUTWARD CURRENTS OF HIPPOCAMPAL PYRAMIDAL CELLS. R.K.S. Wong, R. Numann* and A.R. Kay*, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Voltage-dependent outward currents in the hippocampal pyramidal cells have been examined using acutely dissociated cells. Pharmacological agents including tetraethylammonium chloride (TEA), 4-aminopyridine (4AP), and Ca^{++} channel blockers (Mn^{++} and Cd^{++}) were used to characterize the different components of the outward currents. Under voltage-clamp at a holding potential of -50 mV, depolarizing steps of 10 mV increments elicited sustained outward currents followed by tail currents which decayed at a time constant of about 80 ms. When Mn^{++} (2 mM) or Cd^{++} was added to the perfusate, the outward current activated upon depolarization was suppressed and the amplitude of the current was not maintained for the duration of the depolarization. The tail current under this condition decayed more rapidly and its amplitude shows a Nernstian dependency on extracellular K^+ concentration. The transient outward current observed in Mn^{++} or Cd^{++} inactivates in two phases during maintained depolarizations: an initial fast rate of decay with time constants of about 50 ms followed by a slower decay with time constants of about 300 ms (at 0 mV membrane potential). TEA at 10 mM selectively suppressed the slowly inactivating outward current. At higher concentrations (15 mM) the initial fast decaying outward currents were also suppressed. 4AP at 1 mM suppressed both components of the outward current. The time constant of decay of the fast component was shortened. The 4AP action on the fast inactivating components also showed interesting voltage dependency with the blocking effect greatly enhanced upon increasing durations of hyperpolarizing prepulses. The effect of 4AP resembles that reported for the molluscan neurons (Thompson, J. Gen. Physiol. 80:1) and is consistent with the interpretation that 4AP in part acts on the closed but not inactivated potassium channels. Additional experiments also show that when cesium fluoride was used in the recording electrode, the outward current component dependent on Ca^{++} is largely suppressed.

Recent studies suggest that the outward current in the hippocampal pyramidal cells can be modulated by neurotransmitters. Characterization of the outward currents may provide the necessary information for a more detailed understanding on the mode of action of these transmitters.

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155.8 SYNCHRONOUS CONDUCTION AMONG CLUSTERS OF PYRAMIDAL TRACT NEURONS. Antonio Canedo* and A. L. Towe. Dept. of Physiology and Biophysics, Univ. of Wash. Sch. of Med., Seattle, WA 98195.

The rules of impulse conduction on CNS fibers are usually taken to be the same as for PNS fibers. Each fiber is treated as though it were immersed in a large, external current-carrying medium, and hence conducts independently of its neighbors. Therefore, over a long conduction distance (e.g., cortex to spinal cord), any volley of activity evoked synchronously from the cell bodies must arrive desynchronized at its destination. However, there is increasing evidence that this might not always be true; central pathway fibers originating from a cluster of cell bodies may gather into bundles in such a manner that they all conduct at the same speed. This possibility has been tested by recording the antidromic responses of PT cells isolated several at a time from lateral pericruciate cortex of Domestic cats. Stimulating electrodes were placed on the medullary pyramid and dorsolateral funiculus at C4, and were placed stereotaxically into the cerebral peduncle, red nucleus, lateral hypothalamus, and medial thalamus. Bipolar needle electrodes were placed in the central footpad of each of the four paws for orthodromic activation of the cells (chloralose). It was thus possible to sort out the different PT cells by using spike collision tests along the main axon, between the axon and its collaterals, and between the axon and synaptically-evoked spikes. 'Large-seeing-distance' micropipettes were used to record the activity of several PT cells simultaneously.

Most antidromic 'unit' response obtained with these electrodes turned out on testing to consist of a stack of superimposed units. The size of the stack changed in discrete steps at specific, quite stable stimulus strengths. Collision testing showed each of these steps to reflect a discrete single unit with defineable properties. Although all units within a stack had the same antidromic latency (usually within 0.1 msec of each other), they did not all project to the same sites: some responded only to peduncle, some to both peduncle and pyramid, and some also to spinal cord. Furthermore, they did not all share the same collateral sites. Both fast and slow PT stacks were recorded, but all stacks that contained units responding only to peduncle were 'slow' stacks. Using a reference sample of 1,326 PT antidromic latencies recorded independently in the same cerebral region, it was possible to estimate the chance that any particular stack might be seen, under the assumptions of random dispersion of cells and independence of condutions. The highest probability stack we found had a chance of having been recorded randomly of 7.6×10^{-4}. Under the same rules, the chance of having obtained our overall experimental results was effectively zero ($p<10^{-240}$). There seems little doubt that the rules of CNS fiber conduction differ in important ways from those derived from study of peripheral nerves.

155.9 PROPERTIES OF CULTURED CEREBELLAR GRANULE CELLS MEASURED WITH WHOLE-CELL PATCH RECORDING AND IMAGING OF Ca^{2+} INDICATOR FLUORESCENCE. P. E. Hockberger and J. A. Connor. Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

In preparation for studies on the role of cyclic nucleotides in cerebellar granule cell function, we have begun to characterize the properties of these cells grown in vitro under completely defined conditions. Explants of P3 to P10 rat cerebellum were cultured at 37°C on polylysine-coated glass coverslips and fed 3X per week with DMEM plus defined additives as detailed elsewhere (Ahmed, Z. et al., J. Neurosci. 3:2448,1983). Granule-like cells were identified using several criteria including soma size and shape, dendritic morphology, birthdate, survival in 10^{-4}M kainic acid, and response to GABA iontophoresis (also see Messer, A., Brain Res. 130: 1,1977). Intracellular and voltage clamp data were obtained using the whole-cell patch recording technique.

Granule cells were identifiable after 2-3 days in culture and increased in number throughout the first week. During that time the cells exhibited progressive changes in several membrane properties. Membrane resistance dropped (10^{-4} -> 10^{-3} Ω cm^2), resting potential increased (-30 -> -50 mV), and voltage-dependent conductances increased in amplitude. Small action potentials could be elicited after 7-10 days, and by 18 days in vitro overshooting action potentials were present. The following currents were recorded from these cells after 2-3 days in culture: (1) transient, 4-AP-sensitive outward current; (2) delayed outward current; (3) transient, TTX-sensitive inward current; and (4) GABA-activated current. The latter had a reversal potential around -50 mV (KAc-filled electrode) and was blocked by (+)-bicuculline or picrotoxin.

CCD imaging of cells loaded with the Ca-indicator dye fura-2 demonstrated that granule cells raised their cytoplasmic Ca^{2+} levels when exposed to either high potassium (25 mM) or 10^{-5}M GABA. The GABA response persisted in the presence of 0.3 µM TTX, a concentration sufficient to eliminate the inward current of these cells. The source of the Ca^{2+} elevation is presently under investigation.

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155.10 ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN NEOCORTICAL EXPLANT CULTURES. M. Gutnick*, B. Wolfson* and F. Baldino,Jr. (Spon: G. Christoph) Central Research and Development Department, E. I. du Pont de Nemours and Co., Inc., Wilmington, DE 19898.

Previous studies have shown that although many of the membrane properties of neocortical neurons recorded in brain slices are similar to those recorded in vivo, there are some differences. The lack of spontaneous action potential firing in neocortical slice neurons may be an acute effect of axotomy, as in the case of acute undercut cortex recorded in situ. Similarly, the increased incidence of electrotonic coupling in neocortical slices has been attributed to the effects of partial dendrotomy of almost all neurons during the slicing procedure (Gutnick et al., Neuroscience, In Press). In the present study, we have examined the electrophysiological properties of neurons in organotypic explant cultures: an in vitro preparation that does not entail traumatic damage to neurons at the time of recording.

Explants of parietal cortex were prepared from newborn rats 3-5 weeks prior to experimentation and maintained in vitro on cover slips using tissue culture techniques which are standard in this laboratory (Baldino and Geller, J. Physiol. 327: 173-184, 1982). Intracellular recordings were made using micropipettes filled with 4M potassium acetate, and placed visually with the aid of an inverted phase-contrast microscope. For recording, cultures were maintained at 36 ± 0.5 °C in a chamber that was continuously perfused with balanced salt solution.

In general, properties of membrane potential and resistance, current-voltage relationships, synaptic potential generation and evoked spike firing in cultured cells were very similar to those previously reported in slices (Conners et al., J. Neurophysiol. 48: 1302-1320, 1982). However, unlike slice neurons, all neurons in culture spontaneously fired action potentials at rates between 0.5 and 5 Hz. Intracellular injection of lucifer yellow CH revealed stellate and pyramidal shaped neurons with long, branching, spiny dendrites. Following exposure to bicuculline (5-50 µM), cultures displayed paroxysmal burst discharges which occured spontaneously and could be triggered by electrical stimulation. Characteristics of the intracellularly recorded paroxysmal response were essentially the same as those previously reported in neocortical slices (Gutnick et al., J. Neurophysiol. 48: 1321-1335, 1982).

These data demonstrate that the properties of neurons in neocortical explant cultures are organotypic, and that the intrinsic circuitry of the culture is sufficient for generation of spontaneous and evoked epileptic discharges.

155.11 THE INVOLVEMENT OF CHLORIDE CONDUCTANCES IN THE LOW CALCIUM FIELD BURSTS OF RAT HIPPOCAMPAL SLICES MAINTAINED "IN VITRO". N. Agopyan* and M.Avoli.(SPON:P.Gloor). Montreal Neurological Inst. Dept.Neurol.& Neurosurg.,McGill Univ.,Montrêal,PQ, H3A 2B4, Can.

Rhythmic and synchronous bursts are recorded in the CA1 subfield of hippocampal slices maintained "in vitro" and bathed in Ringer containing low [Ca^{++}] (0.2mM), high [Mg^{++}] (4mM) (Jefferys and Haas, Nature 300:448,1982). Synaptic transmission and thus synaptic currents are theoretically blocked in this type of medium suggesting that: (i) neuronal synchronization during the bursts depends upon field effects (Taylor and Dudek, Science 218:810,1982); (ii) occurrence and duration of the bursts result mainly from a balance between inward Na^+ currents and repolarizing mechanisms such as non-synaptic K^+ conductances and an energy dependent pump. However, some role in this type of activity might still be played by residual Cl^- GABAergic phenomena as well as non-synaptic Cl^- currents. In these experiments we studied in slices of the rat hippocampus the effects induced by manipulation of Cl^- conductances on spontaneous (the frequency of occurrence being typical for each individual slice) and alveus-induced low Ca^{++} synchronous bursts. Both bursts were characterized in extracellular recordings in s. pyramidale by a negative long-lasting (3-10s) potential on top of which population spikes were generated. Addition of the disinhibitory drug bicuculline (20uM) into the Ringer increased the frequency of occurrence of spontaneous bursts and evoked a change in the morphology of both spontaneous and induced bursts which now consisted of two phases: a first one with decreased amplitude and increased frequency of the population spikes (first 1-2s) and a second one with increased amplitude of the population spikes. Modifying the low Ca^{++} high Mg^{++} Ringer by partially substituting Cl^- with acetate or proprionate (20mM) decreased the occurrece of spontaneous bursts while increasing the duration and amplitude of the negative slow potential and the number of associated population spikes. Cl^- substitution at higher concentrations (30-100mM) abolished the spontaneous bursts while the duration of the induced burst increased and spreading depression followed. Bicuculline applied to the Cl^- substituted medium (20mM) was capable of increasing the occurrence of the bursts and rendering these biphasic. These data show that Cl^- conductances play a role in modulating the excitability of hippocampal neurons in low Ca^{++}, high Mg^{++} Ringer. Also, bicuculline effects suggest that these currents are in part synaptic, although this drug might display some non-synaptic action as well(Heyer et al, Brain Res 232:41,1982).

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155.12 NEURONAL BURSTING IN HUMAN EPILEPTOGENIC NEOCORTEX MAINTAINED "IN VITRO". M.Avoli and A.Olivier*. Montreal Neurological Institute, McGill Univ., Montrêal, PQ, H3A 2B4, Canada.

In presence of convulsants, neurons in "in vivo" and "in vitro" preparations display large amplitude membrane depolarizations associated with high frequency bursts of action potentials (aps). This phenomenon is considered to represent the underlying cellular feature of the focal epileptiform interictal discharge expressed in the EEG as a spike, thus knowledge of its genesis is important for understanding the mechanisms of focal epileptogenesis. In the present experiments I studied some features of the bursting displayed by human neocortical neurons in "in vitro" slices obtained from areas (2nd temporal gyrus) removed for the relief of seizures resistant to pharmacological treatment. These areas were considered epileptogenic since they displayed clear "spiking" when studied with implanted preoperative EEG electrodes and intraoperative ECoG. The techniques used were similar to those described by Schwartzkroin et al (Ann Neurol 13:249,1983) but Ringer containing 3.25mM K^+ was used as control medium. Intracellular recordings (K-acetate microelectrodes) were performed in neurons (5th and 6th layers) which were stimulated with tungsten cathodes placed in the white matter or within the cortex.

Low intensity stimuli evoked EPSPs rarely followed by hyperpolarizing potentials even at depolarized resting membrane potential (Vm). With higher strength stimulation nearly 70% of the neurons responded with large amplitude (up to 40mV) long lasting (half-width: 40-90ms) depolarizing potentials which could be associated with a burst of aps (up to 5) and/or fast prepotentials. By depo- or hyperpolarizing the Vm these giant stimulus-induced responses were decreased or increased as expected for a synaptic phenomenon, but voltage dependent potentials could be disclosed mainly during the late part comprising the repolarizing component. A dramatic increase in this late part could be obtained by increasing $[K^+]_o$ (up to 8.25mM) or applying 4 aminopyridine (10-20uM) in the Ringer. These procedures could lead to the appearance of spontaneous large amplitude depolarization with bursts of aps which responded to changes in Vm as expected for synaptic potentials, namely: (i) their frequency of occurrence was not dependent upon Vm; (ii) they increased or decreased in amplitude when Vm was decreased or increased respectively.

These data demonstrate that spontaneous bursting in human epileptogenic neocortex maintained "in vitro" (i) is modulated by K^+ outward currents and (ii) is synaptic in origin. These conclusions do also apply to the bursts elicited by strong focal extracellular stimuli. Using this paradigm, however, voltage dependent potentials can be observed during the late portion of the stimulus-induced response.

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155.13 BURST GENERATION IN LATERAL HABENULAR NEURONS RECORDED IN VITRO K. S. Wilcox*, M. J. Gutnick* and G. R. Christoph* (Spon. J. E. Carnahan) Central Research and Development Department, E. I. du Pont de Nemours and Co. Inc., Wilmington, DE 19898

The lateral habenula nucleus (LHb) of the rat is a dorsal diencephalic structure which receives imput from limbic structures in the forebrain and projects to various midbrain areas. We have studied the electroresponsive properties of LHb neurons recorded intracellularly. Experiments were carried out in 400 µm coronal or saggital slices of the dorsal diencephalon prepared with a vibratome and maintained in vitro at 36°C. Most neurons in LHb exhibited two modes of spike-firing behavior. Depolarizing pulses applied from resting potential evoked normal repetitive firing with spike frequency a linear function of pulse amplitude. However, when depolarizing pulses were delivered from a background of current-induced hyperpolarization, they evoked large, all-or-none waves of depolarization, usually with superimposed bursts of fast spikes. Following a hyperpolarizing pulse these neurons generated an initial, brief (<50msec) burst which was followed by an afterhyperpolarization (50 - 150 msec) and a subsequent, prolonged (>500 msec) plateau depolarization that triggered repetitive spiking. All components of this response were sensitive to amplitude and duration of the hyperpolarizing pulse. Results of experiments with blockers of voltage-dependent Ca and Na currents indicated that the burst behavior released by hyperpolarization depends on Ca electrogenesis. The data suggest that in LHb neurons, as in neurons of some other diencephalic structures (cf. Jahnsen and Llinas, J. Physiol. 349: 205-226, 1984), patterns of spike firing are controlled, in part, by a low threshold, rapidly inactivating Ca conductance.

155.14 PHASIC MAGNOCELLULAR NEUROENDOCRINE CELLS CAN BE 'PACEMAKERS' OR 'FOLLOWERS'. R. David Andrew, Anatomy Dept., Queen's Univ., Kingston, Ont. Canada, K7L 3N6.

A burst of action potentials can arise in two distinct ways: as an intrinsic property of the recorded cell or as an emergent property of synaptic interactions. Intrinsic bursting is common in invertebrate neurons but has only recently been identified in magnocellular neuroendocrine cells (MNC's) of the hypothalamic slice (Andrew and Dudek, Science 221 1050, 1983). During steady depolarizing current injection, repetitive bursting may persist in tetrodotoxin which blocks spontaneous and evoked PSP's (Andrew, in preparation). This strongly supports the concept that endogenous 'pacemaker' neurons exist in mammalian CNS.

Recruitment of MNC's to a phasic burst pattern is associated with increased vasopressin secretion from neurohypophysial terminals. Apart from intrinsic mechanisms, we examined if cells might be driven by periodic synaptic input. Intracellular recordings from 5 of 23 phasic MNC's in supraoptic nucleus revealed a sinusoidal oscillation of the membrane potential, where each depolarizing phase could support a burst. The oscillation had a smooth trajectory and fixed period (range 6 to 18 s), similar to those waveforms generated presynaptically in certain motor and thalamic neurons. The following evidence suggests that this rhythmic oscillation has a synaptic origin independent of intrinsic mechanisms. 1) The oscillatory frequency was voltage-insensitive, i.e. periodicity was unaffected by steady current injection. 2) Oscillation amplitude and frequency was unaffected by spontaneous or evoked action potentials. 3) The oscillation could abate spontaneously, leaving intact the ability to fire an endogenous burst triggered with brief depolarizing current. 4) Perfusion with 10mM Mg^{2+}/.05mM Ca^{2+} immediately blocked both the oscillation and evoked PSP's (although it also simultaneously blocked intrinsic bursting in 10 of 10 phasic cells).

The rhythmic oscillation probably results from periodic synaptic input generated within the slice; its source is not yet identified. Presumably this input could promote and support phasic bursting in MNC's lacking a pacemaker ability, although the latter mechanism is more commonly observed in slices.

155.15 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF PREOPTIC ANTERIOR HYPOTHALAMIC NEURONS IN EXPLANT CULTURES. B. Wolfson*, M.J. Gutnick*, and F. Baldino, Jr., (Spon. L. Liu-Chen) Central Research and Development Department, E. I. duPont de Nemours and Co. Inc., Wilmington, DE. 19898.

The preoptic anterior hypothalamus (POAH) of the rat particpates in a variety of regulatory functions including thermoregulation, endocrine homeostasis and autonomic functons. This region has been shown to possess a rich neurochemical diversity and a complex synaptic organization. We have previously shown that explant cultures are valid models of in situ POAH because they are organotypic with regard to cellular morphology, peptide content and extracellular unit responses to putative neurotransmitters. In this study, we describe some intrinsic electrophysiological properties of POAH neurons maintained in explant culture.

Explants of the POAH were prepared from newborn rats and maintained in vitro with standard tissue culture techniques for 3-5 weeks prior to experimentation. Intracellular recordings were made using micropipettes filled with 4M potassium acetate, and placed visually with the aid of an inverted phase-contrast microscope. For recording, cultures were maintained at 36 ± 0.5 °C in a chamber that was continuously perfused with balanced salt solution containing 2.0 mM Ca^{++} and 1.5 mM Mg^{++}.

In general, cells were characterized by resting potentials between -56 and -80 mV, and high apparent input resistances of between 70 and 200 Megohm. Current-voltage relationships were usually linear negative to resting potential; some neurons showed anomalous inward rectification upon depolarization. Depolarizing current pulses evoked trains of action potentials during which rapid spike broadening and frequency adaptation occurred. Usually these trains of action potentials were followed by hyperpolarizing afterpotentials which could last up to several seconds. Most neurons were spontaneously active and displayed spontaneous postsynaptic potentials.

Intracellular injection of lucifer yellow CH revealed neurons that were 20-25 µm in diameter and possessed 2-4 primary dendrites, most of which were spiny and extended up to 400 µm from the soma. Often, injection of dye into one neuron resulted in the staining of 2-6 cells, raising the possibility of electrotonic coupling.

These data demonstrate that the properties of neurons in POAH explant cultures are similar to those reported for other hypothalamic cells. We conclude that explant cultures are amenable to intracellular investigation of neuronal function in the POAH, and will be useful for gaining insight into the mechanisms of neurotransmitter action.

156.1 A KINEMATIC EXAMINATION OF THE DEFENSE RESPONSE IN CRAYFISH. T.M. Keliy* (SPON:G. Pilar). Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT. 06268.

When a crayfish is confronted with a threatening visual stimulus, it reacts by rearing back and raising its claws (Glantz,1974, Wiersma,1952). The kinematics of this defense response were analyzed using an optical recording system that permitted unimpeded movement in 3 dimensions. LED's were glued to the dorsal thorax and coxa,merus,carpus, propus segments of the cheliped. Infrared light emitted from the 5 LED's was sampled at a rate of 50 Hz. by a position sensing photodiode and positional information was stored for later analysis by an LSI-11 microcomputer.

The presentation of a visual stimulus, a large black target moved rapidly towards the crayfish, resulted in the animal extending its anterior walking legs while raising and extending its chelae to assume a posture that was maintained for a variable length of time. This movement of the chelae was performed by elevating and rotating the merus at the thoracico-coxal and coxal-basal joints, and by extending the carpus at the merus-carpus (MC) joint. Movement was usually initiated at the proximal joints and was accompanied, after a short latency, by extension at the MC joint.

Although there was variation between animals, single animals tended to assume the defense posture by using a characteristic set of joint angles. Standard error of the mean for 95% confidence limits (SEM) in 4 different animals for elevation (SEM= 3^{o}, 2^{o}, 3^{o}, 2^{o}), rotation (SEM= 4^{o}, 4^{o}, 5^{o}, 3^{o}), and MC joint angle (SEM= 2^{o}, 2^{o}, 2^{o}, 1^{o}) indicated that these joint angles varied only within narrow limits. During movements other than the defense response, excursion at these joints was through a much greater range. Coefficients of variance (CV) for the 4 animals showed that during the defense response there was very little error in attaining the MC joint angle (CV= 5%,3%,4%,4%) and somewhat greater error in elevation (CV= 15%,10%,11%,19%) and rotation (CV= 12%,8%,11%,10%). These results suggest that joint angle is the parameter specified by the nervous system during this behavior.

156.2 *COORDINATION BETWEEN THORACIC OSCILLATORS FOR RHYTHMIC LIMB MOVEMENTS IN THE CRAYFISH PACIFASTACUS LENIUSCULUS.* *F. Clarac and K.T. Sillar*. Dept of Neurobiology, CNRS, Univ. of Bordeaux, 33120 - Arcachon, France & Dept of Physiology, Univ. of Bristol, Bristol BS1 5LS (U.K.).*

In intact crayfish during walking, coordination involves more-or-less antiphasic contractions of homologous limb muscles in adjacent leg. During 'waving' behaviour and after amputation of the distal limb, adjacent legs (or stumps) move in phase (Pasztor, V. & Clarac, F. J. exp. Biol., 102, 59-77, 1983). It is not known whether in phase activity results from proprioceptive input from the basal limb joints or from central coordinating pathways. To investigate this problem we have studied interganglionic coordination in semi-isolated and isolated preparations of the thoracic ganglia.

When isolated from the rest of the animal, each hemiganglion can produce patterns of rhythmic motor output which correspond to fictive forward locomotion (Skorupski, P ; Sillar, K.T. & Bush, B.M.H., Neurosci-Abst., 10, 627, 1984). During such rhythmic activity, adjacent hemiganglia display a preferred in phase pattern, while contralateral hemiganglia burst independently. Ipsilateral in phase coordination occurs in all sets of homologous motoneurones (MNs). Thus, remotor muscle MNs of the 4th ganglion (T4) discharge in phase with remotor MNs of the ipsilateral 3rd ganglion (T3) and in antiphase with promotor MNs of both T3 and T4. In non-rhythmic preparations, antidromic stimulation of the remotor muscle nerve can evoke rhythmic activity in the same ganglion and in the anterior (but not posterior) ganglion, suggesting that ipsi-segmental oscillators are coupled by ascending excitation. In rhythmic preparations the same stimulus can strengthen the degree of coupling between oscillators and reset their output.

Rhythmic output of one hemiganglion can be entrained by rhythmic proprioceptive input. Sinusoidal mechanical stimulation of a basal joint proprioceptive complex (comprising a chrodotonal organ and a muscle receptor of the thoracic coxal joint) will entrain oscillatory motor output at frequencies close to those occurring spontaneously. A similar stimulus also entrains the output of the next anterior ipsilateral oscillator.

In summary our results suggest that an ascending central pathway exists for in phase coordination of ipsilateral limb oscillators, and that proximal sensory structures, as well as some MNs, have access to that pathway. Future experiments will investigate the possibility that stimulation of more distal sensory structures monitoring loading of the limb can produce the alternating mode of coordination observed in intact walking crayfish.

156.3 CRAYFISH BACKWARDS-WALKING COMMAND NEURONS INHIBIT ELEMENTS OF THE TAILFLIP CIRCUITRY. Donald H. Edwards and Ted W. Simon, Department of Biology, Georgia State University, Atlanta, GA 30303.

Previous workers have shown that command neurons which mediate two different forms of escape tailflip inhibit each other and the abdominal postural motor system. We have found that command neurons that excite the abdominal postural motor system also inhibit elements of the escape tailflip circuitry.

We have demonstrated that backwards walking can be evoked by a pair of identified sensory afferents, the caudal photoreceptors (CPRs). These cells project into the rostral CNS, where they appear to excite a descending command pathway that can release backwards walking and cyclical abdominal flexion. CPR stimulation that evokes the abdominal flexion motor pattern also excites the Flexor Inhibitor (FI) motoneuron, which inhibits the abdominal fast flexor muscle that produces the tailflip. CPR stimulation that fails to evoke postural flexor activity also fails to excite FI.

Similar FI excitation is produced by direct stimulation of the command fiber pathway, which is a discrete group of fibers on the lateral ventral surface of the nerve cord. Repetitive stimulation of these fibers evokes bursting abdominal flexor activity and also reduces or eliminates the responses to sensory input of mechanosensory interneurons, the lateral giant command neuron, the fast flexor (FF) motoneurons and the extensor inhibitor (EI) motoneuron. The responses of the FF and EI motoneurons to direct lateral giant stimulation are unaffected by stimulation of the command fiber pathway. Our preliminary conclusion is that the inhibition is directed both at elements upstream from the motoneurons and, through excitation of the FIs, at the fast flexor muscle.

We would like to suggest that mutual inhibition among command systems may prove to be widespread. Such a system would serve to raise the threshold for incompatible behavior patterns when one pattern is released.

156.4 INTERNEURONAL COMMAND NETWORK CONTROLS PATTERN INITIATION IN THE CRAYFISH ABDOMEN. D. Moore* and J.L. Larimer. Dept. of Zoology, University of Texas, Austin, TX 78712.

During backwards terrestrial walking, the crayfish Procambarus clarkii exhibits a complex cyclical pattern of activity in its abdomen, characterized by alternating movements of the flexion and extension postural muscles (Kovac, M., J. Comp. Physiol., 95:61-78, 1974). Using extracellular and intracellular approaches, the neural elements "commanding" pattern generation were investigated.

Pattern generation in the cyclic postural system is partially an emergent property of a network of interganglionic pattern initiating interneurons and partially arises from the activation of discrete ganglionic oscillators. The cyclic pattern may be elicited in all of the abdominal ganglia by extracellular stimulation of any of the connectives, through which multiples of pattern initiating neurons project in parallel and in close association with one another. No single fiber traverses the entire length of the abdominal cord. The network is bilaterally symmetrical with strongest signal conduction in the rostral-to-caudal direction and weaker conduction in the caudal-to-rostral direction and laterally across ganglia. For normal patterned motor outputs to occur, each ganglion receives bilateral pattern initiating signals through the connectives; the signals are both descending and ascending. Removal of any of these input signals causes a deficit in the patterned outputs. Isolated ganglia are capable of oscillatory activity but at reduced strength. Unlike central pattern generators in many other systems, the cyclic postural behavior requires continuous stimulation in order to maintain rhythmicity.

Pattern initiating interneurons were characterized in a series of neuropilar impalements in ganglia 4, 5, and 6 of the isolated abdominal nerve cord using Lucifer yellow-filled microelectrodes. Intracellular stimulation of any one of several different morphological types evokes a cyclical pattern indistinguishable from that elicited extracellularly in the connectives. Hyperpolarization of the interneuron during pattern generation fails to affect the cyclical pattern, indicating redundancy of function. Suggestive of electrical coupling among pattern initiating elements, the interneurons typically show a burst of spikes of several different amplitudes that precede pattern generation as well as consistent dye-coupling among morphologically identifiable cells. Additional interneurons exist which, when depolarized, elicit a partial pattern. Hyperpolarization of these cells during pattern generation results in incomplete motor outputs.

Additional experiments demonstrated that pattern generation apparently occurs without the recruitment of typical flexion- and extension-producing interneurons which are the premotor elements controlling tonic postural behaviors.

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156.5 **SEGREGATION OF PATHWAYS OF SLOW AND FAST FLEXOR MOTOR NEURONS WITHIN THE ABDOMINAL GANGLIA OF CRAYFISH.** E. M. Leise and B. Mulloney. Dept. of Zool., Univ. of Calif., Davis, CA 95616.

Within the abdomen of a crayfish are two sets of flexor muscles that participate in different motor activities. The fast flexor muscles generate the power-stroke for rapid tailflips. The slow flexor muscles maintain bodily posture; they help regulate the position of the abdomen on a moment-to-moment basis. To determine which ganglionic structures are important to the control of these behaviors, we made a series of cobalt backfills and intracellular HRP fills of the motor neurons to these muscles. We then traced the routes of the filled cells through the various axonal tracts, commissures and neuropilar areas of the abdominal ganglia (Skinner, 1985, J. comp. Neurol., in press).

The fast flexor motor neurons exit the abdominal ganglia through the main branches of the paired third roots. The slow flexor motor neurons exit through the superficial branches of the same nerve roots. The fast flexor motor neurons branch extensively in the dorsal commissures, in the dorsal-most layer of tracts just below the giant fibers, and also around the giant fibers. However, they bypass most of the ganglionic core - especially the large ventral horseshoe neuropil (HN) and lateral neuropils (LNs). In contrast, the slow flexor motorneurons arborize in both the ventral and dorsal commissure layers and in three of the four layers of axonal tracts. They also branch in the arms of the HN and within the LNs, where swimmeret pattern generation is thought to occur.

Several conclusions can be drawn from these data: slow and fast flexor motor neurons are known to receive input from the dorsal cord axons. These synapses probably occur in the neuropil of the dorsal axonal tracts. That the slow flexor neurons branch in more tracts, commissures and neuropils than the fast flexor motor neurons suggests that the slow flexors are components of many behavioral activities and that the fast flexors are active components of relatively few motor programs.

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156.6 TACTILE STIMULATION OF THE SEXUALLY DIMORPHIC SWIMMERET AFFECTS THE ABDOMINAL POSTURAL PROGRAM IN THE LOBSTER, HOMARUS AMERICANUS. V.C. Kotak* and C.H. Page. Rutgers University, Bureau of Biological Research, Piscataway, NJ 08854.

The influence of mechanical stimulation of the swimmeret upon the abdominal tonic motor program was examined using a preparation with a second swimmeret remaining attached to an isolated abdominal nerve cord. Tactile stimulation was applied manually or by a probe fixed to a speaker cone. Extracellular recordings were made from the tonic flexor and extensor roots of the first 3 abdominal ganglia. Activity of the tonic flexor motor neurons (f1-f6) was analyzed based upon their relative spike amplitudes and firing patterns.

Strong stimulation--manual stroking of the swimmeret--produced vigorous extension responses in both sexes which included excitation of the peripheral inhibitor (f5), strong inhibition of all the flexor excitors (f1-f4 & f6) and a large increase in extensor activity. Preliminary intracellular recordings from flexor motorneurons confirm that f5 is excited and f3 inhibited following strong stimulation. Moderate stimulation (5g) in males excited f5 without changing flexor excitor activity. In 50% of the females, in addition to f5 excitation, there was significant inhibition of one or more of the small-medium (f2-f4) excitors. In the other females, f5 activity remained unaffected, while the firing frequency of f3 and f4 increased. The response latencies of the flexors to a moderate stimulus ranged from 75-100 msec. The first and third segments had latencies of 150-200 msec.

Although the sexual dimorphism of the first abdominal swimmerets has been described (Page 1985), the remaining four pairs of swimmerets also differ between the sexes. In the second segment, these differences include the presence of an accessory lobe on the endopodite of the male and differences in the structure and distribution of the cuticular sensilla. The accessory lobe is covered with many short "bristly spines". For the females long "smooth hairs" line the margin of both rami as well as forming 8-10 clusters on the coxa and proxial rami. Males completely lack smooth hairs. In both sexes "feathery hairs" line the entire margins of the rami. Stimulation of smooth hairs elicited an increase in f5 firing while the bristly spines in the male excited the flexor excitors. In contrast stimulation of the feathered hairs did not evoke any response in either sex.

156.7 EFFERENT NEURONS OF THE ABDOMINAL PLEOPODS IN THE LOBSTER, HOMARUS AMERICANUS: SEXUAL DIMORPHISM AND SEGMENTAL VARIATION. K. A. Killian*, C. H. Page and D. A. Cipolla* (SPON: R. Notvest). Bureau of Biological Research and Department of Biological Sciences, Rutgers University, Piscataway, NJ 08854

The efferents whose axons project in the first roots of the first through fifth abdominal ganglia were stained to examine segmental variation and sexual dimorphism in the innervation of the pleopod appendages. They were stained by backfilling the first root with an 80:20 mixture of 300 mM $NiCl_2$:$CoCl_2$. The somata are organized into two ipsilateral clusters, a large anterior cluster and a smaller posterior cluster. In addition two somata (one in the first ganglion) are located on the contralateral side of the ganglion. Because of the large number of somata stained in the second through fifth ganglia, the whole mounts were serial sectioned to count the number of dye-filled somata.

It has long been known that the first pair of abdominal appendages is sexually dimorphic in the lobster (Herrick, Bull. U. S. Bur. Fish., 29, 149-408, 1909). In the male the first pleopod forms an elongated stylet while it is a small uniramus appendage in the female. The second through fifth pairs of abdominal appendages form the swimmerets. Each swimmeret comprises a proximal coxal segment to which two leaf-like rami are attached--an endopodite and an exopodite. The second segment swimmerets are sexually dimorphic. In the male the endopodite has an accessory lobe that is absent in the female. In contrast to the more anterior segments, male and female swimmerets in the third through fifth segments have similar morphologies.

There is a correlation between pleopod structure and the number of efferents that innervate it. When compared to the swimmerets, the first segment pleopods are innervated by relatively few efferents. In 24 successful backfills of the first ganglion 27.3 (range = 23-31) somata were stained. In contrast almost four times as many efferents 109 (91-117) were stained in 48 backfills of the second through fifth ganglia.

The sexually dimorphic first and second pairs of pleopods contain about 10% more efferent somata in the female when compared with the male. For the male 25.8 (23-28) first segment and 100.5 (97-108) second segment efferents were stained while in the female 28.8 (26-31) first segment and 109.8 (106-113) second segment efferents were dye-filled. These differences are significant at the .01% level (Student t test). In contrast the numbers of efferents that were stained in male and female third, fourth and fifth ganglia did not differ statistically.

156.8 SIMULATION OF REGULATION OF DYNAMIC MUSCLE STIFFNESS IN A CRUSTACEAN SLOW MUSCLE. W. D. Chapple, Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268.

Experiments on the stretch evoked reflex contraction of the ventral superficial muscles of the abdomen of the hermit crab, Pagurus pollicarus, indicate that dynamic muscle stiffness is constant over a range of stretch velocities (Chapple 1985). Since there is no tonic component to the reflex, and its latency is long (greater than 100 ms), negative feedback cannot be responsible for this control. An alternative explanation is that this graded slow muscle integrates the velocity dependent reflex burst of the motoneurons. The time constant of the muscle was experimentally determined to be about 470 ms; the time constant of the excitatory junction potentials (ejps) was about 40 ms. A model of neuromuscular system and muscle mechanics was simulated on a computer. Ejps with equilibrium potentials of +6mV were generated at varying frequencies. The resulting membrane depolarization was used to produce a contractile force proportional to the depolarization up to a value of -30 mV, beyond which point the contractile force was constant. This contractile force was in parallel with a viscous element and in series with an elastic element. Constant muscle stiffness in this model is found only when the time constant of muscle activation is about ten times that of the ejp time constant. In addition, reflex stiffness is not critically dependent upon the velocity dependence of the reflex burst in the motoneuron. A high sensitivity of the reflex to stretch velocity does not appear to be necessary to insure regulation of dynamic muscle stiffness.

156.9 COXAL AND BASAL LEG MUSCLES OF THE CRAB CARCINUS ARE INNERVATED BY THE COMMON INHIBITOR. Stacia Moffett, Daniel P. Yox and Linda B. Kahan*. Dept. of Zoology, Wash. State Univ., Pullman, Washington, 99164.

In the crab Carcinus maenas, a neuron has been described which innervates the basi-ischial levators and depressor and the coxal remotors and promotor (Yox and Moffett, 1983, Soc. Neurosci. Abs. 9:382; Bevengut et al., 1983, J. Comp. Neurol. 221:185-198). Working with the muscles and nerves of the fifth pereiopods of Carcinus maenas, we have now extended the description of this neuron to show that it is an inhibitor and that it corresponds in its distal branching pattern to the common inhibitor. In extracellular recordings from branches of the leg nerves, this neuron has a small spike and demonstrates a correspondingly high threshold to electrical stimulation. Tension levels in the anterior levator muscle drop dramatically when this unit is active. When the anterior levator nerve is stimulated at 100 Hz in increasing voltage steps, the tension developed by the excitors is abolished when the threshold for this unit is reached. We have not detected IPSP's in the superficial fibers of the posterior head of the anterior levator muscle, and we conclude that the major action of this neuron on the anterior levator is presynaptic inhibition.

Both electrophysiological mapping of the axon branches and mapping with cobalt and nickel dyes indicate that this neuron supplies not only the coxal and basal muscles but also distal leg muscles such as the dactyl opener. This is the only neuron we have found that innervates the muscles proximal to the autotomy fracture plane and also projects to the more distal muscles. The soma of this neuron is located near the midline of the ganglion. Bilateral backfills of the nerves of the coxal or basal muscles revealed that the bilaterally paired somata of these neurons are contralateral to their axonal projections in at least some crabs. We believe that the neuron we have described in Carcinus is the common inhibitor and that it corresponds to the ventral midline neurons described by Young and Govind (1983, Brain Res. 280:251-262) in backfills of cheliped nerve roots in fiddler crabs and also to the dorsal inhibitors of the claw closer muscles that undergo dramatic changes in both position and size during claw reversal in snapping shrimps (Mellon, 1981, Trends in Neurosci. 4:245-248).
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156.10 LOCAL SPIKING INTERNEURONES AND THEIR ROLE IN THE CONTROL OF LEG MOVEMENTS OF THE LOCUST

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Spiking local interneurones provide the first integrative step in the processing of sensory information from the legs of a locust. Within a population of these interneurones that have cell bodies at the ventral midline of the thoracic ganglia, three classes are recognised. First, interneurones that receive inputs only from external mechanoreceptors. Second, interneurones with inputs only from external mechanoreceptors (proprioceptors), and third, interneurones with inputs from both internal and external mechanoreceptors. The first class receive direct inputs from afferents innervating hairs, and in turn make direct connections with some leg motor neurones. Their neuropilar branches are divided into two fields; one in a ventral region of neuropile to which the afferents project and possessing predominantly input synapses; the other in a dorsal region of neuropile to which the motor neurones project and possessing predominantly output synapses. Each interneurone is excited by a particular array of receptors on the leg that comprise its receptive field, so that the surface of the leg is mapped onto a population of these interneurones. The second and third classes show a spectrum of responses to movements of a single joint, that range from a maintained change in the frequency of their spikes for a particular position of the joint, to phasic changes to a movement of the joint in either direction. The inputs to these interneurones are derived from a variety of receptors at the joints, but for many of those monitoring the femoro-tibial joint, their responses can be mimicked by independent manipulation of the apodeme of a chordotonal organ.

The interneurones mediate local postural and compensatory reflexes of the leg, a role in which the organization of their sensory fields can most usefully be viewed. During voluntary movements of a leg the interneurones may signal the movement made, and contact with external objects or with other segments of the leg. They also respond in ways indicating that they are part of the neural machinery actually initiating the voluntary movement.

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156.11 INTERNEURONS IN THE FLIGHT SYSTEM OF THE CRICKET, TELEOGRYLLUS OCEANICUS. R. Meldrum Robertson, Department of Biology, McGill University, Montreal, P.Q., H3A 1B1, Canada.

In the locust some important flight interneurons are located in abdominal neuromeres and are serially repeated in at least 4 of the 6 segmental neuromeres known to contain flight neurons. It has been proposed that these features support the pleural appendage theory for the evolutionary origin of insect wings (Robertson et al., Science 217:177, 1982). Given that insect wings are thought to have evolved only once it might be possible to find homologies in flight interneurons and their organization in related species. One such species is Teleogryllus oceanicus and I have intracellularly stained neurons phasically active during an expression of the flight rhythm of this animal. Adult female T. oceanicus of mixed ages post terminal moult were used. A dorsal approach was made to the thoracic ganglia which were deafferented and stabilized on a stainless steel probe. Thoracic neurons were filled with Lucifer Yellow after recording their activity during flight sequences.

The flight rhythm was initiated by blowing air over the head or the cerci of the animal. When this stimulus released a short duration flight sequence (5-20s) the cycle frequency was around 15 Hz. In some preparations a similar stimulus triggered a flight rhythm which lasted indefinitely (over 5 mins) with a cycle frequency greater than 20 Hz and in the normal range of wingbeat frequency. The basic properties of flight neurons are similar to those of the locust. During short flight sequences motoneurons fire bursts of large amplitude spikes with an intraburst frequency less than 100 impulses/s (during longer sequences at a higher cycle frequency motoneurons tend to fire only once per cycle) whereas interneurons fire bursts of smaller amplitude spikes at an intraburst frequency greater than 200 impulses/s. Activity in flight neurons was driven by excitatory and inhibitory synaptic input which set up membrane potential oscillations 10-20 mV in amplitude. Different interneurons fired bursts either in phase with elevator or with depressor motoneuron activity. Although the structure of some cricket interneurons appeared dissimilar, some other interneurons bore a marked similarity to locust flight interneurons; specifically to pattern generator interneurons that exist as sets of serially repeated homologues. This similarity is, to date, suggestive only and it remains to be discovered how far the similarities between individual interneurons extend in terms of both their physiology and their morphology. On these discoveries depends conclusions concerning which features in the flight systems of the cricket and the locust are a result of their common evolutionary heritage.

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156.12 EFFECTS OF NEUROCHEMICAL AND NEUROANATOMICAL MUTANTS ON THE LANDING RESPONSE AND FLIGHT MUSCLE ACTIVITY OF *DROSOPHILA MELANOGASTER*. M Gorczyca and J C Hall. Brandeis University, Waltham, MA 02254

During tethered flight when an object approaches a fly from the front, a behavior termed the landing response is elicited. This consists of a down and outward movement of metathoracic and mesothoracic legs as well as a forward and upward extension of the prothoracic legs. If the approaching object has an upward component as well, the neural activity to the indirect flight muscles increases during and slightly after the approach and then briefly ceases. Normal activity returns shortly thereafter.

In mutant flies which were deficient for choline acetyltransferase (ChAT), the synthetic enzyme of ACh, both the landing and flight muscle responses disappeared. This occurred in Cha^{ts1} and Cha^{ts2}, the two temperature sensitive alleles studied and is correlated with the time dependent decrease in ChAT activity induced by pre-incubation at non-permissive temperature. A dopa decarboxylase temperature sensitive mutant (Ddc^{ts2}) was analyzed as well. It's responses were similar to that of wild-type.

A number of brain morphology mutants were also examined and two of these exhibited abnormal landing and flight muscle responses. The sol^{KS58} (*small optic lobes*) mutant responses were present but intermittent, and not as robust as in wild type. Other characteristics of the landing response have been reported to be aberrant in this variant. Visual stimuli which elicit strong responses in wild-type were ineffective in omb^{H31} (*optomotor blind*). In contrast, a stimulus that produces no response, or only a weak one, consistently gave strong landing responses.

The value of this simple assay system is to test the ever-increasing number of morphological and neurochemical mutants that exist for *Drosophila* and to do so in a way that reveals behavioral as well as physiological function. In this way the extent to which a particular genetic lesion disrupts the nervous system can be more fully determined.

156.13 GIANT VISUAL MOTION-SENSITIVE NEURONS ARE DIRECTLY CONNECTED TO NECK MUSCLE MOTORNEURONS IN FLIES: 1. STRUCTURAL ORGANIZATION. N.J.Strausfeld and J.J.Milde. EMBL, Meyerhofstraße 1, D-6900 Heidelberg, West Germany (FRG).

Flies move their heads in response to static or moving visual stimuli in a way that is reminiscent of optokinetic eye movements by crustacea and mammals (Land,M.S. In: Handbook of Psychobiology. Eds. M.Grazzaniga and C.Blakemore. Academic Press,49,1975). Two recent accounts describe the prominent role of eight giant vertical-motion-sensitive neurons (VS) of flies in connections between the brain and neck muscle motorneurons (Strausfeld,N.J. and Seyan,H.S.; Strausfeld,N.J. and Bassemir,U.K. Cell and Tiss. Res.,240,601 and 616,1985).

Using computer reconstructions of the head and body exoskeleton we have analysed the articulation between the head and prothorax and have determined how identified muscles insert into the rear head capsule, the articulating condyle and the prothoracic box. Reconstructions implicate certain of the 16 pairs of identified muscles in lateral, rotatory and vertical head movement.

By isolating each muscle, still attached to the CNS by its motorneuron axon, we have filled its motorneuron with cobalt ions. Two pairs of muscles, arranged so as to pull the head downwards, are each invested by a motorneuron whose dendrites in the brain are cobalt-coupled to two VS neurons viewing a frontal strip of the visual field. Another three pairs of muscles, arranged to pull the side of the head downwards (head rotation) are invested by three motorneurons that arise in the prothoracic ganglion where they are cobalt-coupled to a unique descending neuron originating in the brain. This cell (DNOVS 1) is, in turn, cobalt-coupled to six VS cells subtending the lateral part of the visual field. Other muscles are supplied by motorneurons whose dendrites interact with primary afferents from the organs of balance (halteres) or the wings. Certain other motorneurons interact with descending neurons that receive inputs from small-field visual neurons and horizontal-motion-sensitive neurons. These motorneurons invest oblique muscles that move the head around its vertical axis (side-to-side movement).

Cobalt coupling occurs between neurons that share gap junctions (Strausfeld,N.J. and Bassemir,U.K., J. Neurocytol.,12:971,1983). We conclude that control of head movement involves two sets of muscles directly connected to wide-field vertical-motion-sensitive neurons. Other muscles are separately invested by motorneurons that acquire their major inputs from halteres, wings and horizontal-motion-sensitive neurons.

156.14 GIANT VISUAL MOTION-SENSITIVE NEURONS ARE DIRECTLY CONNECTED TO NECK MUSCLE MOTORNEURONS IN FLIES: 2. PHYSIOLOGICAL ORGANIZATION. J.J.Milde and N.J.Strausfeld. EMBL, Meyerhofstraße 1, D-6900 Heidelberg, West Germany (FRG).

Visually induced optomotor responses of flies are supposed to be mediated by giant vertical- and horizontal-motion-sensitive neurons that originate in the lobula plate (Poggio, T. and Reichardt,W., Quart.Rev.Biophys.,9:377,1976). However, connexions between giant lobula plate cells and muscles involved in optomotor behaviour have not yet been established. We have recorded from motion-sensitive motorneurons in each of the four pairs of nerves that innervate head muscles. Each unit was most excited by a movement in a particular direction. Movement in the opposite direction elicited an inhibitory response or no response at all.

Response characteristics and directional selectivity of the motorneurons corresponded almost exactly with the well-documented properties of intracellularly recorded lobula plate giant neurons (Hausen,K., Biol.Cybern.,46:67,1982, Hengstenberg,R., J.Comp. Physiol.,149:179,1982, Eckert,H., J.Comp.Physiol., 149:195,1982). Moreover, presentation of stimuli to different parts of the visual field in combination with eye occlusions demonstrated the correspondence between the receptive fields of motorneurons and specific lobula plate giant neurons (VS and HS cells). For example, motorneurons supplying the cervical nerve (CN) responded maximally to downward movement of horizontal gratings in the frontal visual field as do the frontal-looking vertical cells (VS 2 and 3). Anatomical investigations reveal a direct connexion between VS 2,3 and the CN motorneurons (see N.J.Strausfeld and J.J.Milde, this issue). Stimulation by downward movement of horizontal gratings to the lateral visual field evoked best responses from motorneurons in the frontal nerve (FN). This result is in accordance with the response characteristic of VS 4-9 cells whose dendritic domains subtend the lateral retina. The connexion between VS 4-9 cells and the FN motorneurons has been confirmed anatomically (see N.J.Strausfeld and J.J.Milde). In the anterior dorsal nerve (ADN) and ventral cervical nerve (VCN) all recorded units preferred horizontal front-to-back movement in the ipsilateral visual field. None of these motorneurons are connected to VS cells. Rather, the response properties of ADN and VCN motorneurons suggest that they receive inputs from horizontal-motion-sensitive giant neurons. Our results are the first demonstration of direct connexions between so-called optomotor neurons and identified motor outputs. We suggest that giant visual neurons of the lobula plate directly control head attitude or head movement, or both.

156.15 MOTOR CORRELATES OF ASSOCIATIVE PHOTOTAXIC SUPPRESSION IN HERMISSENDA. W.G.Richards and J.Farley.Program in Neuroscience, Princeton University, Princeton, NJ 08544.

Light-rotation pairings result in increased latency and decreased velocity of light-evoked locomotor behavior in Hermissenda. Except for a single identified motoneuron (MN1, Goh & Alkon, J.Neurophys., 1984), little is known concerning the neural control of locomotion in this animal. We have examined motor correlates of phototaxic behavior and their modification by associative training using anatomical, lesion, and extracellular recording techniques. Bilateral lesions of either P1 or P2 (but not P3 & CP2) pedal nerves in untrained animals, which anatomical studies indicate innervate the pedal musculature, substantially reduced phototaxic behavior. Lesioned animals exhibited significantly longer light-evoked start and finish latencies and decreased locomotor velocities when compared to sham controls.

Extracellular suction electrode recordings from naive animals revealed net increases in total mulit-unit activity (MUA) of 10% during a 5-min light presentation for nerves P1, P2, P3, and CP2. A prominent component of nerve P2 activity patterns was synchronous, high frequency bursting in groups of cells evidenced as spindle-like peaks in PST histograms. Simultaneous recordings from pairs of nerves indicated that units within ipsilateral nerves P1 and P3 burst in phase with P2 spindle units, while spindle bursts in contralateral P2 nerves are typically out of phase. Spindle bursts (0.5-1/min frequency) were typically 3-15sec in duration in the dark adapted preparation. A 5-min presentation of light resulted in increases in frequency (42%) and duration (23%) of spindle bursts. Further increases in spindle frequency were noted during the initial 2-min period following light offset (+130% baseline rate; duration = +10%).

P2 nerve recordings were also obtained from behaviorally trained animals. Fifty paired or random light-rotation trials were administered on each of three days, and nerve recordings were made at 24 or 48 hour retention intervals, following behavioral tests. For random preparations (n=22), light resulted in increases in spindle burst frequency (+50%) and duration (+5%) comparable to those of untrained animals. Light offset responses were also similar (+55% freq.; +23% duration). By contrast, preparations from paired animals (n=22) exhibited light-induced reductions in spindle frequency (-8%) and duration (-6%). Spindle frequency increases following light offset were similar to those noted for random preparations (+54%), but the duration changes were absent (-3%). Thus, associative training resulted in a selective decrease in light-evoked spindle burst frequency and duration. These results suggest that associative training results in decreased visual excitation of pedal musculature.

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156.16 WHOLE-BODY WITHDRAWAL OF THE POND SNAIL LYMNAEA STAGNALIS. G.P. Ferguson and P.R. Benjamin*. School of Biology, University of Sussex, England.

The whole-body withdrawal response of Lymnaea is induced by photic (light-off) and tactile sensory stimuli, and consists of the retraction of the head-foot of the animal into its shell.

This behavior is mediated by two muscle systems, the columellar muscle (CM) and the dorsal longitudinal muscle (DLM). The CM is innervated by the columellar nerves and contracts to longitudinally shorten the ventral head-foot and pull the shell down over the animal's body. The DLM is innervated by the superior and inferior cervical nerves, the left parietal nerve and the external right parietal nerve. Contraction of the DLM longitudinally shortens the dorsal head-foot and is synchronous with CM contraction.

The CM and DLM are both innervated by an electrotonically coupled network of withdrawal response motoneurons. The somata of these cells are located in all nine ganglia of the CNS, but are especially concentrated in the A clusters of the cerebral ganglia. The CM is innervated by cells in the cerebral and pedal ganglia and the DLM by cells in the cerebral, pedal, pleural and left parietal ganglia. Intracellular iontophoresis of Lucifer Yellow confirmed that the withdrawal response motoneurons project into the nerves innervating the CM and DLM. Individual motoneurons have large, but discrete, motor fields which often overlap with those of other motoneurons. Simultaneous intracellular recording from withdrawal response motoneurons and individual muscle fibers showed that motoneuron action potentials evoke one-for-one, non-facilitating excitatory junction potentials within muscle fibers. No all-or-nothing action potentials were recorded from the CM or DLM, neither was there any evidence of inhibitory innervation.

The withdrawal response motoneurons are high threshold cells which show little spontaneous activity. Most cells on one side of the CNS are electrotonically coupled strongly to each other, but only weakly coupled to cells on the contralateral side of the CNS.

Photic (light-off) and tactile stimulation of the body wall of reduced preparations evokes excitatory postsynaptic potentials (EPSPs) within withdrawal response motoneurons. These EPSPs usually depolarize the cells to above threshold value for action potential initiation, so causing contraction of the CM and DLM. Together, the synchronous reception of sensory input and the electrotonic coupling between the withdrawal response motoneurons are responsible for producing and coordinating the behavioral response.

This work was supported by a SERC (UK) studentship to GPF.

156.17 ROLE OF THE PEDAL COMMISSURE IN THE COORDINATION OF NORMAL AND SEROTONIN-STIMULATED LOCOMOTION IN THE SNAIL MELAMPUS. Keith Snyder and Stacia Moffett, Department of Zoology, Washington State University, Pullman, WA 99164-4220

Locomotion in the pulmonate snail Melampus bidentatus has been characterized in intact and CNS-lesioned animals (Moffett, Biol. Bull. 157:306, 1979; Moffett and Snyder, Amer. Zool. 231:894, 1983). The latter study, a behavioral evaluation of snails recovering from operations, suggested that the bilaterally symmetrical coordination of the step cycle of pedal locomotion is mediated via either an intact cerebral commissure or an intact pedal commissure. However, recent evaluation of the effects of acute lesions in tethered, semi-intact snails using extracellular recording techniques contrasts with the behavioral observations. We found that transmission in the cerebral commissure does not suffice to coordinate movements on the right and left sides of the body after the pedal commissure has been cut. Transection of the pedal commissure uncouples the right and left pedal oscillators and typically changes the cycle rate of both.

Serotonin added to the bathing medium stimulates locomotion in Melampus as it does in Aplysia (Mackey and Carew, J. Neurosci. 3:1469, 1983). At concentrations below 5×10^{-6} M only sporatic pedal movements result. At 10^{-5} M, pedal waves that correspond to normal locomotion are reliably generated. Increases in serotonin concentration up to 10^{-3} M cause more exaggerated movements and the disappearance of some aspects of the normal behavior. The maximum rate for tethered locomotion is observed at 10^{-3} M serotonin and approaches the fastest locomotion seen in freely locomoting snails (18 step cycles per minute). Combining serotonin-stimulated locomotion with central lesions, we found that cutting the cerebro-pleural and cerebro-pedal connectives has no significant effect on pedal wave rate, although the rate almost doubles on the two sides when the pedal commissure is cut.

We conclude that locomotion is generated by right and left oscillators which control the corresponding sides of the foot. These are synchronized via connections in the pedal commissure. The rhythm of pedal movements generated by the individual oscillators is faster than the rhythm of the coupled oscillators.

156.18 RHYTHMIC MEMBRANE POTENTIAL OSCILLATIONS IN MOTORNEURONS OF ASCARIS: J.D. Angstadt* and A.O.W. Stretton (SPON: P. Smith). Neurosciences Training Program and Department of Zoology, University of Wisconsin, Madison WI 53706.

Our laboratory is investigating the neural basis of locomotion in the large parasitic nematode Ascaris. Intracellular recordings have revealed rhythmic oscillations in the membrane potential of Ascaris motorneurons. The presence of rhythmic activity is a common feature of many neurons involved in locomotor systems. We believe the observed oscillations may have an important role in the control of locomotion in Ascaris and have therefore begun to examine them in greater detail.

Rhythmic activity occurs most frequently in inhibitory motorneurons (type VI and DI) and consists of a slow depolarization followed by a faster depolarization-repolarization sequence. The activity has a peak-to-peak amplitude of 10-20 millivolts and a duration of several hundred milliseconds. The period of the rhythm ranges from 0.2 to 2 seconds. The phase of the rhythmic activity can be reset with intracellular or synaptic current, which suggests that the motorneurons are intimately involved in the generation of the oscillations and not simply following a rhythm imposed on them from another source. Phase response curves have been constructed for a variety of pulse durations and amplitudes and have been shown to predict the limits of entrainment of the rhythmic activity. The oscillations are blocked by removal of $CaCl_2$, Co^{++} substitution for Ca^{++}, or the addition of $CdCl_2$ to the saline solution. The loss of oscillatory activity in such salines can occur before the loss of neuromuscular transmission.

We believe these rhythmic oscillations are important for several reasons: (1) oscillations can be induced in quiescent inhibitory motorneurons by prolonged depolarization of presynaptic excitatory motorneurons; (2) synaptic input from excitatory motorneurons can produce changes in the frequency and amplitude of spontaneous oscillations; (3) oscillations in VI and DI motorneurons which innervate muscle on opposite sides of the worm (i.e. ventral vs. dorsal) maintain an antiphasic relationship. The above features are consistent with a model in which tonic drive to excitatory motorneurons results in the generation of oscillatory activity in inhibitory motorneurons. The activity in the inhibitors, and therefore in the dorsal and ventral muscle fields, is maintained in antiphase by the synaptic connections between the motorneurons.

PRESYNAPTIC MECHANISMS II

157.1 MEPP AMPLITUDE DISTRIBUTION IN FUNCTION OF THE RELEASE SITE POSITION ALONG THE FROG NEUROMUSCULAR JUNCTION. R. Robitaille*, G. Grenon* and J.P. Tremblay. Laboratory of Neurobiology, Laval University, Québec, Can G1J 5B3.

A new method, called the spatial decay method was used to evaluate 1) the position (X) along the frog neuromuscular junction (nmj) of the release site producing a MEPP and 2) this MEPP amplitude (Ar) before any spatial decay. The experiments were performed on the frog cutaneous pectoris muscle. The method requires simultaneous intracellular recording at both distal ends of a nmj. The electrodes were positioned using Nomarski optics. The assumptions that the MEPP amplitude is maximum in front of its release site and decays exponentially toward the recording electrodes placed distally permit the calculation of the position (X) of the release site producing each MEPP recorded intracellularly. The position (X) (i.e. the distance between electrode 1 and the release site of a given MEPP) is evaluated from the ratio of the MEPP amplitudes (A_1 and A_2) recorded respectively by electrodes 1 and 2, the distance (D) between these recording electrodes and the space constant λ of the muscle fibre.

$$X = (D - \lambda \ln (A_1/A_2))/2$$

By evaluating the release position of thousands of MEPPs recorded at a given nmj, we have already reported that the spontaneous release activity is not occurring uniformly along the frog nmj (Tremblay et al., Neur. Lett. 51, 247, 1984).

The spatial decay method also permits to evaluate the amplitude (Ar) of a MEPP before any spatial decay, i.e. the amplitude in front of its release site.

$$Ar = A_1/(e^{-X/\lambda})$$

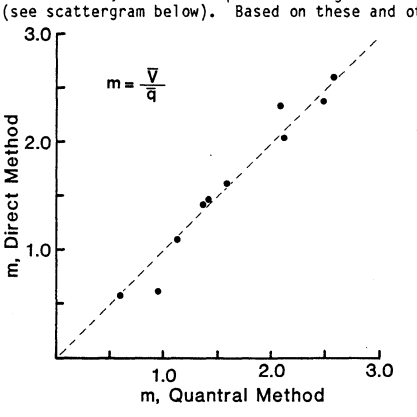
The MEPP amplitude corrected for spatial decay are not normally distributed (Tremblay et al., Brain Res. 328, 170, 1985). We have now studied the relationship between the position of the release site (X) and the corrected amplitude (Ar). Our results indicate that the few MEPPs produced at the distal ends of the nmj are small MEPPs. Large size MEPPs are present only in the central region of the nmj. This central region does however also contain small and medium size MEPPs. Variations of MEPP amplitude may be due to either fluctuation in the amount of neurotransmitter liberated or to variation in the receptor density on the postsynaptic membrane in front of the release site. Our results do not permit to select one of these mechanisms. It is however interesting to point out that the distal region of the nmj which has a low probability of releasing neurotransmitter to produce a MEPP has also a low probability of producing large MEPP, whereas the central region which has a high probability of producing a MEPP has also a high probability of producing a large MEPP. Are these two phenomena interrelated?

157.2 QUANTAL RELEASE PARAMETERS COMPARED AT THE CRAYFISH NEUROMUSCULAR JUNCTION USING A NOVEL AND CONVENTIONAL METHOD. C.L. Keenan, G. Barrionuevo, D. Baxter, T.H. Brown. Div. of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

The quantum hypothesis and the analytical techniques derived therefrom have proven historically to be valuable in understanding mechanisms responsible for changes in synaptic efficacy. We have been developing (see Barrionuevo, et al., Soc. Neurosci. Abstr., 11: in press) a novel method of quantal analysis, based on a cepstral-like algorithm, that does not depend on the questionable assumption (Brown, et al., PNAS, 73:2913, 1976) that the actual quantal release process is well-approximated by a simple Poisson or binomial probability function.

Here we test this method on experimental data obtained from the crayfish opener-excitor neuromuscular synapse, using intracellular recording procedures (Baxter, et al., PNAS: in press). The mean quantal content m and the mean quantal size q were determined using both the novel method (based on the quantrum) and the conventional direct method (based on the amplitudes of spontaneous miniature excitatory postsynaptic potentials). Each method was applied without knowledge of the outcome of the other method; then the results were compared.

The values of m and q determined by the novel (quantral) and conventional (direct) methods were in excellent agreement. In the 10 synapses studied, the coefficient of determination (r^2) exceeded 0.9, and the slope of the regression was close to unity (see scattergram below). Based on these and other (Barrionuevo, et al., Soc. Neurosci. Abstr., 11: in press) results, we conclude that the quantral method offers new opportunities to understand changes in synaptic microphysiology.



(Supported by NIH Grants NS21561, NS18295, AA06517, and a McKnight Foundation Scholars and Development Award).

157.3 SPONTANEOUS TRANSMITTER RELEASE FROM CHOLINERGIC RETINAL NEURONS IN CULTURE. N Stockbridge* & D.G. Puro (SPON: C.B. Smith), Laboratory of Vision Research, National Eye Institute, Bethesda, MD 20205.

Neurons dissociated from embryonic day 9 chickens were cocultured at a density of $10^6/cm^2$ on skeletal muscle from postnatal day 1 rat. Under these conditions cholinergic synapses form between retinal neurons and muscle cells within minutes and persist for several days (PNAS 74:4977, 1977). Such synapses reveal themselves in spontaneous postsynaptic potentials in electrical recordings from muscle fibers. In what sense are such synapses normal?

We have begun to investigate the electrophysiological properties of these synapses. To minimize cable effects on the amplitude and time course of these events, short fibers were selected for these studies and recordings were made from preparations cocultured for two to four hours to minimize the growth of processes.

Under these conditions, 10 - 50% of muscle cells are innervated and have events at rates from 1 to 10 per minute. These events are depolarizations ranging in amplitude from as small as can be detected above the noise (typically about 0.2 mV) to about 20 mV in the same muscle.

These events are distributed as would be expected for a random process; we find no evidence for temporal clustering.

The histogram of event amplitudes is not multimodal and shows no evidence that large events are multiples of small events. Small events are much more common than large events. If large events were composed of small events, it might be expected that large events would have slower rise times and slower decays due to asynchrony in release. We found no correlation between event amplitude and either of these parameters.

In order to diminish the probability that the events we observe are due to presynaptic electrical activity, we examined the effect of TTX on the event rate. We have also examined the effect of a calcium channel blocker alone and in concert with TTX. Neither 10 μM TTX nor 5 mM cadmium had any effect on the event rate or amplitude distribution.

We believe that transmitter release from recently formed synapses must be confined to one or a few closely spaced release sites and that the variation in amplitude of postsynaptic potentials results from variations in the amount of transmitter in individual 'quanta'.

157.4 DEVELOPMENT OF A MODEL SYSTEM TO STUDY ACETYLCHOLINE SECRETION. Joy A. Umbach and Cameron B. Gundersen. Dept. of Pharmacol. and Jerry Lewis Ctr., UCLA Sch. Med., Los Angeles, CA 90024.

The evoked release of acetylcholine (ACh) at vertebrate motor nerve terminals is dependent on calcium entry, but the events triggered by calcium are poorly understood. To approach this problem, we are examining whether components of the secretory machinery can be reconstituted in Xenopus oocytes. It has been established (Gundersen, et al. PNAS 82 608) that messenger RNA from the electric lobe of Torpedo induces these oocytes to produce functional choline acetyltransferase. This results in the synthesis of ACh in the mRNA-primed oocytes. We were curious whether these oocytes had also acquired a mechanism by which this ACh could be released in response to appropriate stimuli. Preliminary studies showed that the "leakage" of ACh (measured using a gas chromatographic-mass spectrometric assay), from oocytes containing 200-600pmole of ACh, was less than 0.5pmol-hr^{-1} (at 21°C in frog Ringer containing 0.1mM eserine or BW284-C51 as an anticholinesterase). By contrast, ACh efflux from frog sartorius muscle (with 40-60pmole of ACh) is about 2pmole-hr^{-1}. Thus, the background output of Ach from the mRNA-injected oocytes is relatively low. Several protocols (which evoke ACh release from motor nerve endings) were tested for their efficacy in inducing ACh release from these oocytes. Individual cells were bathed in Ringer solutions (with eserine, 0.1mM) containing either: high K^+, the calcium ionophore A-23187 (10μM), veratridine (25μM), ouabain (0.1mM), lanthanum (0.2mM) or a solution made hyper-osmotic by the addition of sucrose (to 50mM). None of these procedures enhanced the output of ACh from the oocytes. However, in parallel experiments we established that these treatments produced at least a temporary rise of ionized calcium in the cytoplasm of the oocyte (calcium-ion activity was monitored with a calcium ion-sensitive microelectrode). These data argue against the presence in these mRNA-injected oocytes of a calcium-activated process for the secretion of ACh. Paradoxically, when ACh-containing oocytes were bathed in a nominally calcium-free Ringer (no added calcium) ACh was released at a rate between 0.2 and 1.0pmole-min^{-1} per oocyte. This output of ACh in calcium-free Ringer was prevented by the addition of other divalent cations (Mg^{2+}, Ba^{2+}, Sr^{2+}) to the calcium-free Ringer; a result which suggests that the calcium-independent enhancement of ACh efflux is due to the removal of divalent cations which otherwise maintain a low permeability of the plasma membrane to ACh. In conclusion, our results indicate that mRNA from the electric lobe of Torpedo can give Xenopus oocytes the capacity to synthesize ACh, but it does not confer on them the ability to release this ACh in a fashion that mimics the release process at nerve endings in muscle or electric organ.

157.5 RELATIONSHIP BETWEEN EVOKED QUANTAL ACETYLCHOLINE RELEASE AND PRESYNAPTIC INTRAMEMBRANE PARTICLES. D. Muller*, M. Garcia-Segura*+ and Y. Dunant* (SPON : J.J. Dreifuss). Dept. of Pharmacology, CMU, 1211 Geneva 4, Switzerland, and +Inst. Cajal, 28006 Madrid, Spain.

A macro patch clamp technique (Dudel, J., Pflüger's Arch, 391:35, 1981) was used to analyse the evoked release of acetylcholine (ACh) at the Torpedo nerve-electroplaque junction. As at neuromuscular junctions, ACh release could be evoked in a quantal way with the amplitude of the first quantum corresponding to the amplitude of spontaneous miniature electroplaque currents. The amplitude distribution of evoked responses also closely followed a Poisson's law. A maximum rate of release has been estimated by electrodes of different sizes and was found to be of about 1.5 quanta/μm^2 of presynaptic innervating membrane.

When analysed by quick freezing techniques in similar physiological conditions (Dunant et al., C.R. Acad. Sc. Paris, 13:547, 1984), evoked ACh release could be correlated in its time course by a transient increase in the density of presynaptic intramembrane particles, but not with modifications in the number of endo-exocytotic images. The change in particle density could not be observed if calcium was omitted in the bathing solution, suggesting that it was not produced by the depolarization of the nerve endings. However, the maximum number of particles appearing simultaneously with the maximum rate of ACh release corresponded to approximately 400 particles/μm^2 of presynaptic innervating membrane. This does not allow a simple correlation between one such particle and a quantum of ACh or a molecule of ACh.

157.6 EFFECT OF CALCIUM ON SPONTANEOUS QUANTAL RELEASE IN FROG NEUROMUSCULAR JUNCTION. D. Kneifel* and M.I. Glavinovic (SPON: P. Braun), Departments of Research in Anaesthesia and Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

High concentrations of extracellular potassium are known to result in much increased frequency of miniature end-plate currents (MEPC-s). Morphological evidence shows that at high [K+]o (20 mM) quantal secretion occurs randomly, not only at but also in between the active zones (Ceccarelli, Grohovaz & Hurlbut, 1979, J. Cell Biol. 81, 163-177). Such changes in the spatial distribution of quantal release lead also to greater variability of MEPC amplitudes and faster clearance of ACh molecules in the synaptic cleft (Glavinovic, 1985, J. Physiol. 358, 85P). A high concentration of extracellular calcium counteracts the increase in frequency of MEPC's induced by high [K+]o but its mechanism is poorly understood. This study is an attempt to see how elevated [Ca2+]o affects other physiological changes induced by high [K+]o.

Experiments were done on the frog (Rana Pipiens) cutaneous pectoris preparation at room temperature (19-21°C) in physiological Ringer as well as in solution with elevated [K+]o (8 or 16 mM). Cholinesterase was either active or was inhibited by edrophonium chloride (20 μM; Roche).

The variability of MEPC amplitudes is decreased with high [Ca2+]o. The effect appears greater if extracellular K is high. Time constants of decay of MEPC-s are shortened with high [Ca2+]o if cholinesterase was active, indicating the action of [Ca2+]o on activated ionic channels, much in agreement with previous studies (Magleby & Weinstock, 1980, J. Physiol. 299, 203-218). MEPC-s are however usually marginally prolonged with high [Ca2+] if experiments are done in the presence of cholinesterase inhibitor showing that the clearance of ACh molecules in the synaptic cleft is slowed. High [Ca2+]o hence appears to counteract all major changes in spontaneous quantal secretion induced by high [K+]o (elevated frequency of miniature discharge, greater variability of MEPC amplitudes and faster ACh clearance).

Changes in spontaneous quantal discharge induced by high [Ca2+]o can be explained, assuming that at any potassium concentration [Ca2+]o enhances the miniature discharge at the active zones but depresses it in between. Alternatively it may be that [Ca2+] affects the frequency of MEPC-s, variability of their amplitudes and ACh clearance through different mechanisms.

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157.7 SYNAPTIC DEPRESSION IN FROG NEUROMUSCULAR JUNCTION. M.I. Glavinovic, Departments of Research in Anaesthesia and Physiology, McGill University, 3655 Drummond St, Montreal, Quebec, Canada H3G 1Y6.

According to currently held view, synaptic depression, observed during high frequency stimulation at high levels of quantal release, occurs because the number of quanta released is progressively diminished. However, evidence has been recently presented to suggested that newly formed acetylcholine (ACh) preferentially replenishes the store of quanta released by nerve stimulation, probably the immediately available store. In the absence of newly produced ACh, when ACh synthesis is impaired, nerve stimulation leads to release of partially filled quanta, although spontaneously released quanta appear almost normal (Glavinovic, 1985, J. Physiol. 361: 17P, 1985). This raises the possibility that even when ACh synthesis is not impaired the synaptic depression may be to a significant extent caused by a decrease in quantal size of nerve evoked quanta rather than by a lower quantal content.

Experiments were done on the frog (Rana Pipiens) 'cut' cutaneous pectoris preparation at room temperature (19-21^{o}C) using voltage clamp technique. Cholinesterase was inhibited by edrophonium chloride (20-25 μM; Roche).

As a result of prolonged high frequency stimulation (20 min; 5 Hz) end-plate current (EPC) amplitudes are diminished significantly (±SD) (42 ± 4%). The effect is predominantly presynaptic although a small decrease (8 ± 3%) of miniature end-plate current (MEPC) amplitudes is also observed. Shortening of EPC-s, which is also observed, is greater (31 ± 3%) than the shortening of MEPC-s (9 ± 2%) and results not from diminished spatial overlap of quantal events (that occurs because quantal content may be decreased) nor from lower 'lingering ACh' in the synaptic cleft, but from diminished quantal size of nerve evoked quanta. The contribution of such diminished quantal size to synaptic depression was estimated from the amplitude dependence of the time constants of decay of MEPC-s (Glavinovic, 1984, J. Physiol. 354, 43P) and known shortening of EPC-s that is of presynaptic origin and was found to be surprisingly high (67 + 13%). It appears that the synaptic depression occurs to a large extent because of decrease of quantal size of nerve evoked quanta.

Supported by MDA and MRC (Canada).

157.8 CHANGE IN PERITERMINAL IONIC ENVIRONMENT PRODUCED BY POLARIZING CURRENTS AND ITS EFFECT ON THE VOLTAGE DEPENDENCE OF AMPLITUDES AND TIME CONSTANTS OF DECAY OF END PLATE CURRENTS. R. Nissen* and M.I. Glavinovic (SPON: N. Lake), Depts. Anaesthesia Research & Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

Polarizing currents applied to change postsynaptic membrane potential are known to change periterminal ionic environment, change in potassium [K+] concentration being easiest to detect (Takeuchi & Takeuchi, 1961, J. Physiol. 155, 46). In this study such periterminal K+ concentration changes are estimated at different [K+]o and their effect on the voltage dependence of the amplitudes and the time constants of decay of end plate currents (EPC-s) examined.

Experiments were done on the frog (Rana pipiens) 'cut' cutaneous pectoris preparation. Membrane potentials were changed in a steplike manner from -50 mV to various levels but extreme being +50 or -150 mV. As a result of such membrane potential change the frequency of miniature end-plate currents (MEPC-s) changes slowly in solutions with high K+ concentrations. Periterminal K+ changes can be estimated from the change in frequency of miniature discharge. Absolute changes in periterminal K+ concentration are significant (~1 mM/25 mM) and are moreover only weakly dependent of [K+]o. This shows that the relative changes in periterminal K+ concentration are greater the lower [K+]o, and suggests that it may be increasingly less meaningful to examine the dependence of reversal potential on [K+]o if cleft K+ changes induced by polarizing currents are not taken into account.

Effect of such cleft K+ changes on the EPC vs Vm curve was also examined. As a result of a step like-change in membrane potential EPC amplitudes change rapidly. EPC vs Vm curve determined briefly after a membrane potential jump (20 - 500 msec) is only marginally different from that determined 1 min after such jump, even when extracellular K+ concentrations were elevated (~ 10 mM). The voltage dependence of the time constants of decay of EPC-s is found similarly independent of presumed periterminal K+ changes. These paradoxical findings can be explained by the fact that altering extracellular [K+] concentrations does not lead to any significant postsynaptic changes. Effect on nerve evoked quantal release is also small. Large increase in [K+]o (from 0.25 to 8 mM) leads to an increase in EPC amplitudes of only 25%.

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ACTION POTENTIALS AND ION CHANNELS III

158.1 POLYCLONAL ANTIBODIES WITH SPECIFICITY FOR VOLTAGE-SENSITIVE CALCIUM CHANNEL PROTEINS. Hemin Chin, Karl Krueger*, Troy Beeler*†, and Marshall Nirenberg. NHLBI, NIH, Bethesda, MD and †Uniformed Services University of Health Sciences, Bethesda, MD

Rat skeletal muscle transverse tubule (T-tubule) membrane preparations were obtained that contained 50 pmoles of 1,4-dihydropyridine calcium channel antagonist binding sites per mg protein (H. Chin and T. Beeler, Biophys. J. 47, 265, 1985). T-tubule membranes were solubilized with 1% CHAPS, incubated with [^{3}H]PN-200-110, a potent dihydropyridine antagonist, and specific [Glycoprotein•[^{3}H]PN-200-110] complexes were purified 20-fold by wheat germ agglutinin (WGA) agarose column chromatography. The glycoprotein complexes were denatured, reduced with DTT, and fractionated further by SDS-polyacrylamide gel electrophoresis (5-15% gradient). Strips of gel were excised that contained the α, β, or γ protein subunits of the Ca^{2+} channel (Mr = 135,000, 50,000, and 32,000, respectively (B. Curtis and W. Catterall, Biochem. 23, 2113, 1984). Rabbits were injected with particles of polyacrylamide gel containing α, β or γ subunits of the Ca^{2+} channel and were boosted later with injections of Ca^{2+} channel subunit proteins that were electrophoretically eluted from portions of the gel. Antisera were obtained from 4 rabbits, HK-2, HK-9, HK-10, and HK-16. Analysis of transblots of protein from rat T-tubule membranes fractionated by SDS-PAGE showed that HK-2 antibodies recognize both the reduced and nonreduced forms of the 32,000 M_r γ-subunit, HK-9 and HK-10 recognize both the reduced and nonreduced 50,000 M_r β -subunit; whereas, HK-16 antibodies bind only to the reduced form of the 135,000 M_r α-subunit.

Antibody specificity for Ca^{2+} channel proteins solublized from human brain crude P2 membrane preparations was examined. HK-2 antibodies recognized on transblots a 34,000 M_r band of protein, and HK-9 and HK-10 recognized a 50,000 Mr band; however, HK-16 antibodies did not recognize α-Ca^{2+} channel subunits of human brain.

IgG purified from the antisera by protein A-Sepharose column chromatography was coupled to CNBr-activated Sepharose. Fractionation of solubilized T-tubule membrane proteins on affinity columns containing the HK-9 or HK-10 IgG yielded a single protein with an M_r of 50,000, the expected value for the β-Ca^{2+} channel subunit from solubilized T-tubule membrane protein preparations. These results show that the antibodies can be used for the identification and purification of voltage-sensitive Ca^{2+} channel proteins from skeletal muscle and brain. The antibodies also should be useful as probes for detecting the expression in E. coli of cloned recombinant cDNA for Ca^{2+} channel proteins.

158.2 [^{3}H]METHOXYVERAPAMIL ([^{3}H]D-600) AND [^{3}H]DESMETHOXY-VERAPAMIL ([^{3}H]D-888) LABEL MULTIPLE RECEPTORS IN BRAIN AND SKELETAL MUSCLE. Ian J. Reynolds, Adele M. Snowman* and Solomon H. Snyder. The Johns Hopkins University School of Medicine, Department of Neuroscience, Baltimore, MD 21205.

Numerous studies have demonstrated the presence of two or three separate receptors for calcium antagonist drugs associated with the voltage sensitive calcium channel (VSCC). The use of radiolabeled dihydropyridines such as nitrendipine indicate the presence of a single population of sites for these drugs. Such studies also show that a separate site exists for verapamil-like drugs. In the present study we have employed [^{3}H]D-600 and D-888 to label the verapamil receptor in brain and skeletal muscle microsome preparations.

In rat cortical microsomes [^{3}H]D-888 labels two populations of receptors with affinities of about 1.2 and 64 nM, with receptor concentration of 3.9 and 20 pMol/mg protein respectively. Binding to these sites is inhibited by low concentrations of nitrendipine (0.1 - 1 nM). [^{3}H]D-600 labels a similar population of nitrendipine sensitive sites. However, nitrendipine only displaces 30-50°/° of bound D-600 and D-888. By contrast analogues such as verapamil, tiapamil, and diltiazem completely inhibit binding.

Kinetic studies in rabbit skeletal muscle microsomes also indicate the presence of two sites for these drugs. Dissociation of [^{3}H]D-888 initiated by 80-fold dilution gives a biphasic dissociation curve. The addition of unlabeled D-888 and D-600 to the diluting buffer results in an accelerated dissociation rate. This indicates that the multiple sites are able to interact. Interestingly, the addition of dihydropyridines at the initiation of dissociation cause a marked slowing of the dissociation rate.

These results show that there are similar multiple interacting sites for verapamil-like drugs in brain and skeletal muscle, and that the interactions between receptor sites on the VSCC are complex.

158.3 A PEPTIDE TOXIN FROM CONUS GEOGRAPHUS BLOCKS VOLTAGE-GATED CALCIUM CHANNELS. D. H. Feldman and D. Yoshikami. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

We previously reported (Kerr & Yoshikami, Nature 308:282-4, 1984) that omega-Conus geographus toxin (ω-CgTX), a 27-amino acid peptide purified from the venom of a marine snail (Olivera et al, Biochem 23:5087-90, 1984) irreversibly blocks evoked-release of transmitter in frog, and also attenuates the Ca^{2+} component of the action potential of chick dorsal root ganglion (DRG) neurons. Those results suggested that ω-CgTX interferes with Ca^{2+} influx into the presynaptic terminal, perhaps by blocking Ca^{2+} current or by augmenting K^+ current. We report here gigohm-seal whole-cell voltage clamp experiments which show that ω-CgTX specifically blocks voltage-gated Ca^{2+} channels.

Ca^{2+} currents in cultured, dissociated chick DRG neurons were pharmacologically isolated by using a bath containing 10mM Ca^{2+} and tetrodotoxin, and by recording with pipets containing CsCl and tetraethylammonium-Cl (TEA-Cl). ω-CgTX (5μM) was applied directly onto neurons by pressure ejection from a pipet.

Step depolarizations from -90 mV holding potentials elicited cobalt-blockable inward Ca^{2+} currents with 'transient' and 'prolonged' components (cf. Carbone & Lux, Nature 310:501-2, 1984; Nowycky et al, Biophys. J. 45:36a 1984). These currents were irreversibly blocked by ω-CgTX within a few seconds of its application.

In contrast to this striking effect on Ca^{2+} current, ω-CgTX had no discernible effect on K^+ currents (recorded with 10mM Co^{2+} instead of Ca^{2+} in the bath, and with KCl instead of CsCl and TEA-Cl in the recording pipet).

Thus, ω-CgTX rapidly, specifically, and irreversibly blocks neuronal Ca^{2+} currents. This action of ω-CgTX is sufficient to explain its inhibition of transmitter release. These findings indicate that ω-CgTX may be a useful tool to study neuronal and perhaps other Ca^{2+} channels.

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158.4 D890: A NOVEL INTRACELLULAR CALCIUM ANTAGONIST IN PYRAMIDAL CELLS OF THE GUINEA PIG NEOCORTEX? R.A. Deisz* and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

D890, a quarternary methyl derivative of the Ca^{++} channel blocker D600, was proposed to act as a specific Ca^{++} current antagonist when applied intracellularly. We have examined the effects of D890 on pyramidal cells of guinea pig neocortical slices maintained in vitro under standard conditions at 37°C. Intracellular electrodes were filled with a suspension of nominal 10 μM D890 in 4M KAc. Only neurons with a stable E_m of at least -68 mV, APs of 90-110 mV and R_m exceeding 40 MΩ were considered. Brief depolarizing current pulses (20-150 msec) after topical application of 40 mM TEA and 10^{-6} M TTX invariably gave rise to Ca^{++} spikes lasting up to about 200 msec. About 20 min. after impalement with D890 electrodes the amplitude and duration of Ca^{++} spikes progressively decreased. In one neuron, where TEA was applied 70 min. after impalement with a D890 electrode no decrease was observed, perhaps because D890 already reached its full, but incomplete effect. The threshold for eliciting Ca^{++} spikes gradually shifted to more positive potentials and in 2 out of 9 neurons, Ca^{++} spikes no longer could be obtained. Recording with D890 containing electrodes in normal solutions also caused after about 10 min., a frequency-dependent decline of Na^+ AP amplitudes, an effect not seen in control cells. Moderate depolarizations eliciting 1-10 APs at frequencies above 0.5 Hz progressively reduced the AP amplitude. The first AP of a train was attenuated by about 20%, the last one by up to 40%. Unlike the effects of local anesthetics, the depression of AP amplitudes produced by D890 was not reversed by hyperpolarization. At frequencies below 0.25 Hz the decrement was below 10% (n=8). The burst of several APs at the beginning of depolarizing current pulses, seen in some cells was enhanced by hyperpolarization and decreased by depolarization, suggesting that they were due to activation of a low voltage activated Ca^{++} current. These events were little affected by D890. The slow envelope of the epileptiform depolarization shifts (DSs) induced by bath application of 10^{-5} M bicuculline were virtually unaltered by D890 (n=3). At frequencies of DS stimulation above 0.5 Hz the initial fast Na^+ spikes were reduced to a similar extent as those evoked by direct depolarizing current pulses. The effects described above are probably not due to cell deterioration since AP amplitudes remained constant and frequency independent for more than two hours in control cells and cessation of stimulation for 1-2 min. in cells impaled with D890 electrodes transiently restored the Na^+ AP amplitude. Our results show that intracellular D890 affects Na^+ dependent as well as some Ca^{++}-dependent events. A tentative explanation may be the positive charge of D890 due to quarternarization, may give rise to additional effects.

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158.5 SOMATOSTATIN REDUCES VOLTAGE-DEPENDENT CALCIUM CURRENT IN AtT-20 MOUSE PITUITARY CELL LINE. Deborah L. Lewis and Forrest F. Weight. Lab of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Somatostatin inhibits the secretion of ACTH evoked by secretagogues including corticotropin releasing factor, vasoactive intestinal peptide, isoproterenol, and forskolin in mouse pituitary tumor AtT-20 cells. In recent studies on AtT-20 cells Luini et al. (in preparation) have found that: (i) somatostatin decreases basal cytosolic Ca^{2+} levels and inhibits the cytosolic Ca^{2+} rise evoked by the above secretagogues; (ii) nifedipine reduces basal and secretagogue stimulated cytosolic Ca^{2+} levels; (iii) the effects of somatostatin and nifedipine are not additive; and (iv) TEA does not block the effect of somatostatin on cytosolic Ca^{2+}. These results raise the possibility that somatostatin may effect Ca^{2+} conductance. In view of this possibility, we used the patch-clamp method to study the effect of somatostatin on the Ca^{2+} current in these cells.

AtT-20 cells were cultured in Dulbecco's Modified Eagle Medium containing 10% fetal calf serum at 37°C in a humidified 10% CO_2 atmosphere. After subculturing 8-10 days, membrane currents were recorded using the patch-clamp method in the whole cell voltage-clamp mode. Currents were recorded in an external solution containing (in mM): 150 TEA, 0.8 $MgCl_2$, 5.4 KCl, 10 $CaCl_2$, 10 HEPES (pH 7.4), 45 glucose, 10^{-6}M TTX, and 1mg/ml albumin with an osmolarity of 340mOsm. The solution in the patch pipette contained (in mM): 120 CsCl, 11 EGTA, 2 TEA, 2 $MgCl_2$, 10 HEPES (pH 7.4), 4 Mg_2ATP, 20 creatine phosphate, and 50 units/ml creatine kinase with an osmolarity of 318mOsm. All recording was at room temperature.

Cells were voltage-clamped at a holding potential of -80mV and stepped from -120 to +80mV using steps 100ms in duration. Cell input resistance, measured between -120 and -60mV, was 2.0-7.9 Gohms. An inward current activated rapidly at potentials positive to -40mV, peaked in 6-7ms, inactivated slowly, was inhibited by 2mM Co^{2+}, and was present in 10mM Ba^{2+}. I-V curves for this voltage-dependent Ca^{2+} current (I_{Ca}) were obtained by measuring I_{Ca} at peak amplitude. Somatostatin (10^{-8} to 10^{-6}M) was applied from a macropipette lowered into the external solution near the cell under study. Somatostatin decreased peak I_{Ca} by 30.1±6.7% (n=6) during voltage steps eliciting maximum current amplitude (+10-15mV). Within two min after beginning somatostatin washout, I_{Ca} returned to control level. Control solutions of peptide vehicle (acidified bovine serum) diluted with external solution (identical to the peptide dilutions) had no apparent effect on I_{Ca}. These results suggest that reduction of peak I_{Ca} by somatostatin may contribute to the somatostatin induced inhibition of ACTH secretion.

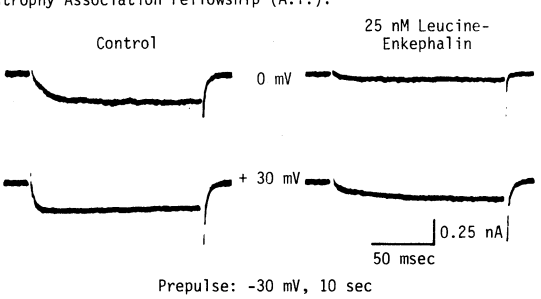
158.6 ENKEPHALIN AND SOMATOSTATIN BLOCK OF CALCIUM CHANNEL IN NEUROBLASTOMA CELLS. A. Tsunoo*, M. Yoshii* and T. Narahashi (SPON: C.A. Berry). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We have previously found that neuroblastoma cells (N1E-115) have two types of voltage-gated Ca channels: one generates a transient Ca current (type I) and the other generates a long-lasting Ca current (type II)(Tsunoo et al., Soc. Neurosci. Abstr. 10, 527, 1984, Tsunoo et al., Biophys. J. 47, 433a, 1985; Yoshii et al., Biophys. J. 47, 433a, 1985). We have now examined the action of neuropeptides in modulating Ca channels. Ca channel currents as carried by Ba^{2+}(50 mM) were recorded using the whole-cell variation of patch clamp technique.

Leucine-enkephalin (Leu-Ek) (25 nM) caused a 70-80% reversible decrease in the amplitude of type II current associated with a step depolarization to 0 mV from a 10 sec prepulse at -30 mV without markedly changing its time course. This time-independent block was not voltage-dependent. However, with depolarizing pulses to potentials more positive than +10 mV, the Leu-Ek block was relaxed with an exponential time course during the depolarizing pulse. Naloxone (1 μM) antagonized the inhibitory effect of 25 nM Leu-Ek. Leu-Ek did not affect the type I current even at a concentration of 0.5 μM. Somatostatin (50 nM) also blocked type II current by 75% in a time-independent manner. With depolarizations to potentials more positive than +10 mV, the block was also relaxed in a time-dependent manner. Somatostatin did not affect type I current even at a concentration of 0.5 μM.

The selective block of type II Ca channel by enkephalin and somatostatin may provide the basis for presynaptic inhibition of transmitter release by these peptides.

Supported by NIH grants NS14144 and RR05370 (M.Y.), and Muscular Dystrophy Association Fellowship (A.T.).



Prepulse: -30 mV, 10 sec

158.7 PHENYTOIN SUPPRESSES CALCIUM CHANNEL CURRENTS IN NEUROBLASTOMA CELLS. D. Twombly* and T. Narahashi (SPON: S.-C. Cheng). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

One of the suspected anticonvulsant modes of action of phenytoin (diphenylhydantoin, DPH) is its effect on calcium-dependent processes. Phenytoin inhibits post-tetanic potentiation in neuromuscular preparations, and suppresses calcium accumulation in synaptosomes and brain slices. The present study tested its ability to block calcium channels of N1E-115 neuroblastoma cells, neurons possessing both inactivating (type I) and non-inactivating (type II) calcium channels.

Voltage clamp experiments were performed on internally perfused neurons with the whole-cell/suction pipette technique. Barium (50 mM) was added to the external medium to serve as charge carrier through calcium channels. Phenytoin (100 uM) was bath applied at room temperature (23 C). Under control conditions, type I currents were activated at potentials positive to -40 mV, with maximum currents occurring at clamp steps to -10 mV or -20 mV. Time to peak of the type I currents ranged from 15 ms to 40 ms. Currents inactivated according to a single exponential time course (time constants: 30-55 ms). Type II currents, activated at potentials positive to 0 mV, displayed little or no inactivation during 200 ms test pulses. The amplitude of these currents was generally largest at potentials of +10 mV.

In the presence of phenytoin, peak type I currents were 35-80% of control amplitudes (62 +/- 17%, mean +/- s.d., n=9). This suppression of peak currents was not accompanied by changes in the kinetics of activation or inactivation. Holding potential significantly influenced drug potency; block of inward current was considerably greater at a holding potential of -60 mV than at -80 mV. At a given holding potential, however, the degree of block was comparable at all command potentials. These effects of phenytoin on type I currents were partially reversed during a ten minute washing period. In contrast to the inactivating currents, the non-inactivating currents were relatively insensitive to phenytoin. Neither the amplitudes nor kinetics of sustained, type II currents were substantially changed in the presence of phenytoin.

Calcium channels are considered important in regulating excitability of mildly depolarized cell membranes, and they are suspected to play a critical role in normal and pathological bursting activity. Through its effects on calcium channels, phenytoin could limit the rhythmic depolarization shifts associated with epileptogenesis, preventing the temporal and spatial propagation of seizure activity. (Supported by NIH grant NS14144).

158.8 NITRENDIPINE RESISTANT Ca^{++} CURRENTS AND VOLTAGE DEPENDENT INHIBITION OF NITRENDIPINE SENSITIVE Ca^{++} CURRENTS IN GH3 CELLS. Steven M. Simasko, Robert E. Oswald, and Gregory A. Weiland. Dept. of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Voltage sensitive Ca^{++} currents were examined in GH3 cells, a pituitary derived cell line, by use of the whole-cell voltage-clamp variation of the patch-clamp technique. The internal solution consisted of 154 mM CsCl, 2 mM $MgCl_2$, 2 mM ATP, 5 mM EGTA, and 10 mM HEPES (adjusted to pH 7.4 with NaOH). The bathing solution consisted of 120 mM tetraethylammonium, 1 mM MgCl, 19 mM glucose, 10 mM HEPES (adjusted to pH 7.4 with KOH), and either 25 mM $CaCl_2$ or 25 mM $BaCl_2$. These conditions isolated voltage-dependent Ca^{++} currents since there was no external Na^+ to flow through the voltage-sensitive Na^+ channel, and the internal Cs^+ and external tetraethylammonium blocked any current through K^+ channels. In most experiments external Ca^{++} was replaced by Ba^{++} because Ba^{++} resulted in larger current signals.

The effects of nitrendipine (NTD), a dihydropyridine Ca^{++} channel antagonist, were examined on the isolated Ca^{++} currents. NTD was applied via bath application after a successful whole-cell clamp had been achieved and allowed to stabilize. A dose-response relation was determined for NTD inhibition of Ca^{++} currents. When the cells were held at -80 mV before a test voltage of +10 mV was applied for 50 msec, NTD blocked the voltage-dependent current with an IC_{50} of 1 µM. However, the maximal block achieved was only 60-70% of the total Ca^{++} current. The effect was the same regardless of whether Ca^{++} or Ba^{++} was the current carrier. Inhibition of the current was found to be voltage dependent. When the cell was tested at a dose of 0.1 µM NTD and the holding potential was -80 mV virtually no blockade of current was observed with a test pulse to +10 mV. When the holding potential was -30 mV and the test potential +10 mV (this current was 30% of the current produced when holding at -80 mV), 0.1 uM NTD blocked 55% of the current.

The activation voltages of Ca^{++} currents when cells were held at -80 mV were examined in the presence and absence of 10 µM NTD. In the absence of NTD half-maximal activation was achieved at -20 mV. However, in the presence of 10 µM NTD, the half-maximal activation was shifted to -28 mV.

These data suggest that GH3 cells either possess two voltage dependent Ca^{++} channels, one sensitive to NTD and the other resistant to nitrendipine or that, rather than simply blocking a sub-population of NTD sensitive channels, NTD alters the open time and activation voltage of a single population of channels.

158.9 EFFECTS OF PYRETHROIDS AND VERATRIDINE ON TWO TYPES OF CA CHANNELS IN NEUROBLASTOMA CELLS. M. Yoshii*, A. Tsunoo* and T. Narahashi (SPON: E.M. Silinsky). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611

It has recently been shown that a variety of neuronal membranes has two types of voltage-sensitive Ca channels: one generates a transient Ca current (type I), and the other generates a long-lasting Ca current (type II). Using a whole-cell variation of patch clamp technique in neuroblastoma cells (N1E-115), we have shown that these two channel types are distinguishable physiologically and pharmacologically (Tsunoo et al., Soc. Neurosci. Abstr. 10, 527, 1984; Tsunoo et al., Biophys. J. 47, 433a, 1985; Yoshii et al., Biophys. J. 47, 433a, 1985). Tetramethrin, a synthetic pyrethroid, is a potent Na channel modulator, and was found to block type I Ca channel preferentially. In the present study, the effects of two types of pyrethroids and veratridine, a Na channel modulator, on Ca channels were compared.

Tetramethrin (50 µM), a type I pyrethroid without containing a cyano group at the α position, caused a progressive block of type I and type II Ca channel currents over a 10-15 min period. When a steady state was achieved, type I current was blocked by 75% while type II current was blocked only by 30%. The tetramethrin block of both channel types was time-dependent, being enhanced during a 400 msec depolarizing pulse. The time-dependent component of block was easily reversible after washing with drug-free solution, while the time-independent component or resting block persisted for at least 40 min after washing. Deltamethrin and fenvalerate, type II pyrethroids containing a cyano group at the α position, are also potent Na channel modulators. Unlike tetramethrin, deltamethrin and fenvalerate (10 µM) had no effect on either type of Ca channel currents during 30 min of exposure. Veratridine (100 µM) did not alter type I current but decreased type II current by 40-50%. However, in veratridine, the reduced type II current was followed by a prolonged inward tail current upon repolarization. This suggests an increase in outward current rather than a decrease in type II current.

The results indicate that type I pyrethroids are Ca channel blockers as well as Na channel modulators. The two components of Ca channel block, a time-dependent reversible block and a time-independent irreversible block, suggest two separate sites of action of tetramethrin in Ca channels. However, type II pyrethroids modify Na channel only. This difference in action between type I pyrethroids and α-cyano type II pyrethroids may be partially responsible for the different symptoms of poisoning in animals.

Supported by NIH grants NS14143, NS14144, HL32577 (M.Y.), RR05370 (M.Y.), and Muscular Dystrophy Association Fellowship (A.T.).

158.10 BARBITURATE INHIBITION OF DIVALENT CATION POTENTIALS IN NOCICEPTIVE LEECH NEURONS. A.L. Kleinhaus and J. Johansen, Dept. Neurology Yale U. Sch. Med. New Haven, Ct. 06510

We have previously reported that barbiturates prolong the action potential of Retzius and nociceptive leech neurons, presumably by interfering with Ca-entry necessary for activation of repolarization mechanisms in these cells (Kleinhaus and Prichard, 1977;1979).

To verify this hypothesis directly we examined the actions of methohexital (MTX), pentobarbital (PTB) and phenobarbital (PNB) on divalent cation potentials evoked in medial (Nm) and lateral (Nl) nociceptive cells in Na-free, tetraethylammonium chloride (TEA) containing Ringer. The maximal rate of depolarization (Vmax) amplitude, and duration of the long-lasting (sec) regenerative potentials depended on $\{Ca\}_o$ and $\{Sr\}_o$; they were reversibly blocked by Mn and Co and were resistant to 50 uM tetrodotoxin, a concentration which abolished the Na-dependent potential. In both types of N cells, the three barbiturates reversibly and in a dose-dependent manner reduced the duration and Vmax of Ca- or Sr-dependent action potentials. The apparent K_I's for inhibition by the three drugs were estimated from the data points which could be fitted with reverse Langmuir adsorption isotherms. The order of potency of the barbiturates for inhibition of Vmax of the Sr-dependent action potentials was: MTV> PTB> PNB, the respective apparent K_I's being MTX: 560 uM, PTB: 800 uM and PNB: 3 mM. The inhibition of Vmax of the divalent cation potential occurred at concentrations which had little (PTB and PNB) or no (MTX) effect on the Vmax of the Na-dependent action potential recorded in the same cell-type in normal Ringer.

The barbiturates produced only minimal (<4mV) changes in resting membrane potential and conductance in Na-free, TEA-Ringer at the highest concentrations used in this study.

In contrast, in Na containing Ringer, MTX, PTB, and PNB depolarized the Nl cells whereas only PBN depolarized the Nm cell, the two other drugs having no effect. The drug-induced depolarization was accompanied by a large reduction in membrane input resistance. These effects may be related to the known excitatory properties of barbiturates which in these cells seem to be Na-dependent.

The results strongly suggest that barbiturates block Ca-entry through voltage-gated channels in identified leech neurons as they do in mammalian nerve terminal preparations and mammalian neurons in culture. Because leech neurons are easily identified and can be maintained in culture, the preparation is eminently suitable for the study of drug actions on Ca-permeability.

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158.11 LACK OF VOLTAGE AND FREQUENCY-DEPENDENCE OF ETHANOL BLOCK OF CALCIUM CURRENT IN APLYSIA NEURONS. P. Camacho-Nasi and S.N. Treistman. Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

Among a variety of ionic currents that we have studied (I_{Na}, I_K, I_A) in isolated neurons of the marine mollusc, Aplysia californica, the calcium current (IC_a) is the most sensitive to ethanol (EtOH). At physiologically relevant concentrations (50mM) of EtOH, the amplitude of IC_a is reduced by 20% (Biophys. J.,47:435a,1985). Voltage clamp experiments show that the depression of current induced by EtOH is not accompanied by a shift along the voltage axis of IC_a activation, making it unlikely that the mechanism of blockade occurs by a surface charge screening effect. The efficacy of some anesthetics has been shown to be dependent on the prior activity of the channels which they interact with. We have done double pulse and pulse train experiments to determine whether the blockade of IC_a by EtOH is voltage and/or frequency-dependent. Either Ca^{++} or Ba^{++} served as charge carrier. Isolation of IC_a from competing sodium and potassium currents (Na replaced on equimolar basis with TMA; 140 mM TEA; 5 mM 4-AP) was successful within the range of voltages used in the present experiments and was confirmed by the absence of contaminating currents when Ca-channel blockers were added to the perfusion medium at the end of each experiment. In the control condition, the amplitude of IC_a during a test pulse was decreased by 70% when preceded by 10 pulses given at a frequency of one every 25 msec. EtOH did not change the amount of IC_a depression during these repetitive depolarizations (EtOH did not induce "use-dependent" block). Similarly, in double-pulse experiments in which a pre-pulse was made progressively more depolarized EtOH did not enhance the blockade of IC_a during a subsequent test pulse (EtOH did not induce voltage-dependent block). These results suggest that EtOH does not block the calcium channel by occlusion in a manner similar to that of some local anesthetics. (Supported by NIAAA grant No. AA05542).

158.12 Ca^{2+}-MEDIATED INACTIVATION OF Ca^{2+} SPIKES IN HIPPOCAMPAL NEURONS. T.A. Pitler* and P.W. Landfield (Spon: D.J. Goode), Dept. of Physiol. & Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27103.

In several invertebrate systems, Ca^{2+} currents have been shown to diminish following membrane depolarization, or other manipulations which increase the internal Ca^{2+} concentration ($[Ca^{2+}]i$) (cf. Eckert and Tillotson, J. Physiol., 1981). In addition, we recently found that some Ca^{2+}-dependent processes (e.g., Ca^{2+}-dependent K^+ conductance) also exhibit Ca^{2+}-dependent inactivation in mammalian hippocampal neurons (Pitler and Landfield, Soc. Neurosci. Abst., 1984). As yet, however, Ca^{2+}-mediated inactivation of Ca^{2+} currents in central vertebrate neurons has not been clearly demonstrated.

Using intracellular recording techniques in the rat hippocampal slice, we examined Ca^{2+} spikes in cesium-loaded, tetrodotoxin-treated pyramidal cells, in order to determine whether Ca^{2+} influx is modified by procedures that increase $[Ca^{2+}]i$. Control values for the Ca^{2+} spike (induced by 70 ms intracellular depolarizing pulses) were obtained and intracellular Ca^{2+} was then elevated by 4 Hz repetitive activation of the Ca^{2+} spike or by depolarizing pulses of varying durations (2, 5, and 10 sec.). In all experiments, membrane resistance and membrane potential were monitored to determine whether cesium-loading had completely blocked Ca^{2+}-dependent K^+ conductance, since such conductance can confound Ca^{2+} spike measures.

During brief repetitive activation or following long depolarizing pulses, the latency from the onset of the depolarization to the peak of the Ca^{2+} spike increased, and the amplitude and duration of the Ca^{2+} potential decreased. The magnitude of these changes was dependent on the degree of previous depolarization. Spike recovery was generally complete within 1-5 min following activation.

To ensure that the observed effects were mediated by an increase in $[Ca^{2+}]i$, the Ca^{2+}-chelator EGTA was leaked into the cell through the recording electrode. This procedure significantly reduced inactivation of the Ca^{2+} spike during repetitive activation.

Thus, these results indicate that Ca^{2+}-mediated inactivation of Ca^{2+} conductance is present in mammalian brain neurons, as well as in invertebrate cells, and may be a widespread regulatory mechanism for limiting deleterious elevations of intracellular Ca^{2+}. (Supported by AG 04207 and AG 04542).

158.13 EFFECTS OF DIHYDROPYRIDINE AGONISTS AND ANTAGONISTS ON Ca^{++} UPTAKE AND NEUROTRANSMITTER RELEASE BY RAT BRAIN SYNAPTOSOMES. T.J. Turner* and S.M. Goldin(Spon.D.Lester), Dept.of Pharmacology, Harvard Medical School, Boston, MA 02115.

The voltage-sensitive Ca^{++} channel is responsible for the depolarization-dependent entry of Ca^{++} into the nerve terminal. The resulting increase in intracellular $[Ca^{++}]$ is thought to initiate neurosecretion. We have determined that Ca^{++} channels are present in rat brain synaptosomes, and that they are sensitive to dihydropyridine Ca^{++} channel antagonists such as nitrendipine [NTP] and nifedipine [NFP](J. Neurosci. 5, 841-849 (1985)). In order to further characterize neuronal Ca^{++} channels, we have measured the effect of NTP and NFP on the depolarization-dependent release of ^{3}H-norepinephrine (NE) from preloaded synaptosomes, and have examined the effect of the dihydropyridine agonist Bay k8644 on depolarization-dependent Ca^{++} uptake and NE release by rat brain synaptosomes.

In order to study depolarization-dependent release of NE, we developed a superfusion apparatus that allows the superfusate to be switched from normal 5mM K^+ to 57.5 mM K^+. This increase in $[K^+]$ results in a transient, 5-10 fold increase in the rate of NE release. The release of NE during the first 10-20 sec. of depolarization is attenuated 40-60 % by including 1 uM NFP in the superfusate. However, at later times (20-60 sec.) NFP has no effect on the rate of NE release, suggesting that this secretion is mediated by Ca^{++} entry via potential-sensitive pathways other than the Ca^{++} channel, possibly the electrogenic Na/Ca exchange pathway. A dose-response relationship was obtained for the inhibition of NE release by NTP and NFP. The IC50 values of 75 and 90 nM, respectively, are approximately 50% greater than the values obtained previously for inhibition of depolarization-dependent Ca^{++} uptake into synaptosomes (56 and 63 nM).

The Ca^{++} channel agonist Bay k8644 was found to potentiate the ability of K+ to stimulate the uptake of Ca^{++} into synaptosomes. An increase in the depolarization-dependent Ca^{++} uptake was observed at all K+ concentrations above 5 mM, both in the presence and absence of external Na^+. This increase was blocked by NFP, indicating that the effect of Bay k8644 was mediated by Ca^{++} channels. However, Bay k8644 did not significantly alter the rate of depolarization-dependent NE release from synaptosomes at any level of depolarization tested.

The conclusion drawn from these studies is that the neuronal Ca^{++} channel has biochemical and pharmacological properties similar to those observed in other excitable tissues such as heart and smooth muscle. Synaptosomes present a viable system for the study of neuronal Ca^{++} channels.

158.14 DIHYDROPYRIDINE EFFECTS ON MAMMALIAN NEURONAL VOLTAGE-SENSITIVE CALCIUM CHANNELS. M.Jia* and M.J. Litzinger (SPON: P. Loh) Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD. 20205

The dihydropyridines are believed to act on voltage-sensitive calcium channels as agonists or antagonists of calcium current based on their chemical structures. Previous microelectrode recordings in dorsal root ganglion neurons (DRG) taken from 12-14 day old fetal mice spinal cords and co-cultured with spinal cord neurons showed only mild inhibition of the calcium spike or dV/dt (30% of control) at concentrations of 1 μM nitrendipine (NTP), (Litzinger, et al., submitted,1985). Previous binding studies in these cultures showed a high affinity site (Kd = 1.1 nM) and a low affinity site (Kd=50nM) in 21 day old cells (Litzinger and Brenneman, BBRC 127, 112,1985). No correlation between NTP binding and electrophysiological recordings have been found thus far in our culture system.

In these experiments, two electrode voltage-clamp technique was used to study the effect of NTP and Bay K 8644 (Bay K) on calcium currents measured in DRG neurons. Recording solutions contained (mM):50 NaCl, 5 KCl, 5 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 100 TEA-Br, 1 4-AP, 1 μM TTX and 10 HEPES adjusted pH to 7.35; sucrose was added to adjust the osmolarity to 325 m OSM. Experiments were performed routinely at 22°C. Electrodes (50-70 MΩ) contained 1M Cs_2SO_4, a potassium channel blocker. Perfusion pipettes had tip diameters of 5-10 μM and were positioned 30-50 μM from the cell. The membrane potential was held at -50 mV and 30 ms voltage jumps were used to activate calcium currents; at -10 mV the transient calcium current (T current) reached maximal amplitude while at 0 mV or +10 mV the slow noninactivating calcium current (L current) was maximal as others have described. Bay K at 1 μM consistently increased L current, while at 10 nM this effect was not observed in all cells. The peak transient current was also increased, but we could not determine whether this was due to an increase in the T current, or only to the increase in L current. NTP at 1 μM and 10 nM showed no difference from ethanol, the drug vehicle, in its effect on either component of the calcium current.

Our results suggest that Bay K is a potential calcium agonist in mammalian neurons, and that NTP is not effective as an antagonist of either voltage-sensitive calcium mechanism.

158.15 CALCIUM CHANNELS IN SARCOPLASMIC RETICULUM (SR) MEMBRANES ARE ACTIVATED BY CAFFEINE. Benjamin A. Suarez-Isla, Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, Maryland 20205.

Calcium channels have been detected in SR membranes from rabbit skeletal muscle (Orozco, Suarez-Isla, Froehlich and Heller, Biophys. J., 47, 57a, 1985) assembled at the tip of patch pipets with the double-dip method (Suarez-Isla et al., Biochemistry, 22, 2319, 1983). In 200 mM symmetric CaCl2 the single channel conductance was 5.6 ± 0.5 pS (mean ± S.E.M., n=8) and linear between +50 and +200 mV (positive inside the pipet). The conductance vs. Ca concentration relationship obtained between 50 to 200 mM CaCl2 (symmetric) showed saturation and could be fitted to a hyperbolic function giving a maximal single channel conductance of 7.9 pS and a $K_{0.5}$ of 83 mM Ca. Extrapolation to 5 mM (maximal estimated intraluminal concentration) gave a single channel conductance of 0.5 pS.

Barium could substitute for Ca as a current carrying species and the single channel conductance was 1.3 times larger than the conductance for Ca.

The channels appeared in bursts during application of steady polarizations of the patch pipet (+50 to +200 mV, positive inside). Bursts of activity lasted several seconds (mean 6.6 s) and were followed by longer silent periods of up to a minute. Bursts were prominent at all voltages studied and mean burst length did not show a significant voltage dependence in the few patches where possibly only one channel was present. During a burst, the channel fluctuated rapidly between open and closed states, showing rapid closures of 0.5 to 10 ms duration(mean = 1.8 ms).

However, addition of millimolar concentration of caffeine, a drug that produces a strong Ca release from the SR in both intact muscle and SR vesicles, produced a dramatic increase in mean burst length. In the absence of ATP and/or Mg, 1.6 mM caffeine increased burst length to minutes with concurrent appearance of several current levels, without change in single channel conductance. Activation took place within seconds but it decreased thereafter, even during sustained application of the drug. In contrast, ryanodine (1 mM) caused a transient inhibition of the Ca channel.

These results suggests that this Ca conductance may be associated with the postulated Ca release channel of sarcoplasmic reticulum.

158.16 VOLTAGE-CLAMP ANALYSIS OF INWARD CALCIUM CURRENT IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. K.L. Zbicz and F.F. Weight. Laboratory of Preclinical Studies, DICBR, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

An inward Ca^{2+} current has been previously observed in CA3 hippocampal pyramidal neurons (Johnston, D. et al. Nature 286:391, 1980; Brown, D.A. and Griffith, W.H. J. Physiol. 337:303, 1983). This current was described as having a slow activation time course and no observable inactivation with maintained depolarization. We have further studied inward currents in hippocampal CA3 pyramidal neurons using the single microelectrode voltage-clamp (SEC) technique (Wilson, W.A. and Goldner, M.A. J. Neurobiol. 4:411, 1975). Thin slices (400-450u) of guinea pig hippocampus were held submersed in a transverse flow recording chamber and neurons in region CA3 were impaled with a single microelectrode (15-25 Mohm) containing 3M CsCl. Diffusion of Cs^+ into the neurons produced a large reduction in outward K^+ currents, revealing a depolarization activated inward current. This current could be reduced by the application of Co^{2+}, Mn^{2+}, or Ca^{2+}-free solutions, and is therefore presumed to be mediated by Ca^{2+}. In Cs^+ loaded neurons the inward current activated rapidly (<30 msec) and gradually decayed with maintained depolarization. This decline in inward current was incomplete, however, and the recorded current often remained net inward during a depolarization lasting several seconds. The decline in inward current was also observed when tetraethylammonium chloride (124 mM) was substituted for NaCl or Ba^{2+} was substituted for Ca^{2+} in the superfusing solution. In addition, the inward current that was activated during step depolarizations decreased as the holding potential was made more positive.

The inward Ca^{2+} current that was activated by a depolarizing step decayed with a complex time course upon return to a more hyperpolarized potential. This decay had both rapid and slow components. The rapid component was too fast to be accurately followed by the SEC. The slow component was observed as an inward tail current, the decay of which could be described by a single exponential function. The time constant for decay of this slow component was dependent upon the potential of the neuron, decreasing at more hyperpolarized potentials.

The data presented suggests that the inward Ca^{2+} current rapidly activates and that a fraction of this current inactivates with maintained depolarization. This inactivation may not be dependent on Ca^{2+} since it occurred when Ba^{2+} was substituted for Ca^{2+} in the artificial CSF used. The complex deactivation time course for the inward current suggests the possibility that two or more populations of Ca^{2+} channels are present in these cells.

158.17 CALCIUM CURRENTS IN SKELETAL MUSCLE OF THE RAT. I. Uribe* and R. F. Valdiosera* (SPON: J. E. Villarreal) Depts. of Physiology and Pharmacology. CINVESTAV-IPN, Apdo. Postal 14-740, México 07000,D.F.

Recently, Ca currents have been recorded from a fast twitch skeletal muscle of the rat (omohyoid), using the three-microelectrode voltage clamp technique (donaldson, P.L. and Beam, K.G. J. Gen. Physiol. 82: 449, 1983). Ca currents were preceded and followed by outward currents that remained even in the presence of K channel blockers. Although the early outward current seems to be carried by delayed rectifier channels not completely blocked by the TEA present in the bath, the late outward current seems to be less satisfactorily explained. For this reason, we have measured Ca currents of the omohyoid with the vaseline gap voltage clamp since this technique allows the control of the internal ionic composition. Recordings were made at room temperature (22-24°C) in an external solution that contained in mM: TMA or $TEACH_3SO_3$ 135, $CaCH_3SO_3$ 2 or 10, MOPS 5, glucose 12, and TTX 1 µM. Fibers ends were cut in intracellular medium containing TMA-aspartate 125, $(TMA)_2$EGTA 20 and MOPS 5, pH was adjusted to 7.35 in both solutions. This concentration of EGTA did not block contraction completely specially at strong depolarizations when Ca concentrations was high. We tried higher concentrations of EGTA (25-30 mM) but the fiber did not last long at this concentrations. Contraction was blocked when the internal solution contained 0.125 mM citrate in addition to the 20 mM EGTA. Citrate probably reduces Ca release from the sarcoplasmic reticulum since less EGTA is needed to block contraction when the external Ca is low (2 mM). Under these conditions, Ca currents are recorded with similar kinetics to the ones reported previously but without any noticeably outward currents. When Cs is substituted for TMA in the internal solution, inward currents are followed by a late outward current, these late outward currents are prominent when the external Ca concentration is low (2 mM) and are reversibly reduced or abolished by raising the Ca concentration to 10 mM. Both currents, are blocked by Cd (2 mM). On the other hand replacing Na for TEA in the external solution a second peak of inward current is recorded whose magnitude may be greater than the first, is more prominent in low Ca (2 mM) and is also reduced or abolished when Ca is raised to 10 mM. Again, both currents are blocked by 2 mM-Cd. Our results could be explained by recent findings showing that in frog muscle, Ca channel becomes permeant to monovalent cations in the absence of extracellular Ca (Almers, W., E.W. McCleskey and P. T. Palade, J. Physiol. 353: 565, 1984). If this were true in rat muscle, then Ca depletion in the tubular system could drop the Ca concentration to values low enough to activate non-selective conductance. A more systematic study in media of various pCa is needed.

This work was partially supported by CONACyT.

158.18 CALCIUM CURRENTS IN THE SECRETORY NEURON SOMA OF THE X-ORGAN OF THE CRAYFISH. U. García*, C. Onetti* and R.F. Valdiosera*. (SPON: P. Huizar). Depts. of Physiology and Pharmacology, CINVESTAV, Apdo. Postal 14-740, 07000 México, D.F. and CUIB, Universidad de Colima, Apdo. Postal 199, 28000, Colima, Colima, México.

Calcium currents in the soma of X-organ cells of the crayfish (Procambarus clarkii) were recorded at room temperature (22°C) with the whole cell clamp method. The cell bodies were exposed by gentle dissection of the connective tissue covering the X-organ and their axons were cut as close as possible to the cell bodies. The preparation was then perfused with van Harreveld's solution during 45-60 min. For recording, the bathing solution contained in mM: 195 choline-Cl, 20 $CaCl_2$, 2 $MgCl_2$2, 10 MOPS-CsOH and the pipette solution contained in mM: 217 CsCl, 2 $MgCl_2$, 0.2 $CaCl_2$, 5 Cs_2 EGTA and 10 MOPS-CsOH. The pH was 7.35 in both solutions. Cells chosen for recording were 20-30 micron diameter and no enzimatic treatment was necessary .

Depolarizing pulses every five second from a holding potential of -90 mV elicit inward currents starting at -30 mV that reach a maximum peak value of -40 µA/cm^2 at about 10 mV in 6 msec and inactivates with a relatively fast time course. The steady state inactivation curve of this current has a midpoint around -45 mV. These currents have the expected properties of a calcium current, they are stable for about 15 minutes, are not affected by 1 µM of TTX and are blocked by 2 mM Cd. No currents were recorded in axotomized neurons (n = 10) when Na (220 mM) and Cd (2 mM) were present in the bathing solution. In intact cells, however, uncontrolled inward currents were observed, that disappeared when TTX (1 µM) was added to the bath, These Na dependent currents seem to originate in the axon, since they are not present in axotomized neurons.

Our results suggest that the action potential in the soma of these neurons is calcium dependent and the sodium dependence observed in earlier studies with microelectrodes (Iwasaki, S. and Satow, Y. J. Gen. Physiol., 57: 216, 1971) was probably caused by sodium currents in the axon. More experiments are needed to settle this point.

159.1 INTRACELLULAR ZN, CU, AND FE IN CULTURED ASTROGLIA; LEAD-INDUCED CHANGES WITH AND WITHOUT THIAMIN. E. Tiffany-Castiglioni*, J. Zmudzki*, T.E. Rowles*, J.-N. Wu*, and G.R. Bratton* (SPON: A.J. Castiglioni, Jr.). Dept. of Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

The effects of lead acetate on intracellular concentrations of zinc, copper, and iron were measured in cultured astroglia by atomic absorption spectroscopy. Results were correlated with lead uptake and viability. Our findings were as follows: first, astrocytes cultured for 10-15 days from newborn rats were resistant to lead toxicity (10^{-7} to 10^{-4} M lead acetate), as measured by trypan blue dye exclusion. However, the astrocytes took up and concentrated lead from the culture medium. For example, cultures treated with 10^{-5} M lead acetate had an intracellular lead concentration 55x that of the medium after 3 days. Intracellular Cu concentrations (µg/2 x 10^6 cells) were 4x that of controls in cultures given 10^{-4} M lead acetate for three days and harvested 2 weeks later. No consistent change was seen in Zn and Fe concentrations. Increased intracellular Cu is a hypothesized mechanism of lead toxicity (Niklowitz, Adv. Neurotoxicol., 1980). Though cell death was not increased by a 3-day exposure to lead, proliferation was significantly inhibited after two weeks by either 10^{-6} or 10^{-4} M lead acetate. Simultaneous administration of thiamin (10^{-4} M), which is used experimentally to treat lead intoxication, did not prevent either the intracellular accumulation of Cu or the loss of proliferation. We previously reported a similar lack of protection for SY5Y human neuroblastoma cells. These findings suggest that the mechanism by which thiamin protects the CNS from lead intoxication does not involve direct contact with target neural cells. The mechanism may reside in other processes, such as digestive or renal absorption.
Second, we found that oligodendrocyte cultures prepared from neonatal rat brain were much more sensitive to lead-induced loss of viability (permeability to trypan blue) than either astrocytes or meningeal fibroblasts at lead acetate concentrations of 10^{-5} to 10^{-3} M. This finding suggests that the hypomyelination observed in the CNS of lead-intoxicated young animals is, at least in part, a direct effect of lead upon the oligodendrocytes.

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159.2 LEAD INTOXICATION IN THE DEVELOPING RAT CNS: BLOOD AND BRAIN CONCENTRATIONS. E.P. Finnerty, W.A. Anderson, and P. Kunkler. Univ. of Osteopathic Med. & Hlth. Sci., Des Moines, IA and Indiana Univ. Sch. of Med., Terre Haute Center for Med. Education, Terre Haute, IN.

Lead has been noted as being toxic to a number of organ and metabolic systems, though the developing CNS appears to be especially vulnerable. These observations represent a portion of an on going investigation of the toxic manifestations of lead ingestion in a rat model.

Animals were divided into 5 groups by litter. The control group received sodium acetate while each of the other groups received 2, 3, 4, or 5 doses of lead acetate (600 mg/kg/day) beginning on day 0 postnatally. The lead or sodium acetate was administered via gastric intubation. Animals were sacrificed on day 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, or 21 following the lead dosing. In each sacrifice group half the animals were selected for morphologic study while the other half were selected for blood and tissue lead analysis. Specimens obtained for lead analysis included: blood, kidney, liver, GI tract, brainstem, cerebellum, midbrain, and forebrain including cerebral cortex.

The lead concentration of the blood and other tissues was determined by standard methods using atomic absorption spectroscopy (Hitachi Model 170-70). This method allowed an analytical sensitivity of 15 µg/L and a percision of 8-15%.

Control animals were found to have blood lead concentrations of < 15 µg/L while the immediate post dose blood lead ranged from 10 (2 dose) to 16 (5 dose) mg/L. Thus demonstrating that gastric intubation is an effective means of administering lead and that there is a significant GI absorption in the rat pup. The elimination of lead from the blood displayed an apparent first-order elimination rate with the disappearance for all groups being parallel and requiring 15-17 days for 95% elimination, though at 21 days significant amounts of lead were detectable.

Hemoglobin concentrations were decreased in a dose-dependent fashion from days 1-14. Both body and brain weights of the lead-treated rats displayed a slower gain over the period than the controls.

This study demonstrates the effectiveness of the method using various doses for producing a model of lead intoxication in immature rat pups. This model is being used for further investigations of lead toxicity in the developing CNS.

We gratefully acknowledge the assistance of Dr. R. Serfass of Iowa St. Univ. for his technical expertise in the lead assays.

159.3 THE EFFECTS OF VARYING DOSES OF LEAD INITIATED AT DIFFERENT POSTNATAL INTERVALS. P.E. Kunkler*, W.J. Anderson, G.W. Patrick. Indiana Univ. Sch. Med., Terre Haute and Ft. Wayne Ctrs. for Med. Educ., Terre Haute.

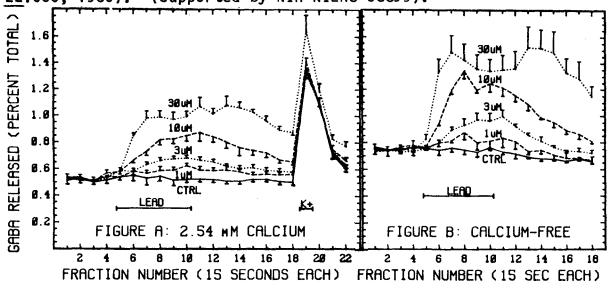
The brains of neonatal rats chronically exposed to lead, display vascular hemorrhages and stunted neuronal differentiation. The purpose of this study was to determine the effects of varying doses of lead acetate upon neuronal and vascular development when administration began at birth, 10 days of age, and 20 days of age. Rat pups from pregnant Long-Evans rats were given 600 mg lead acetate/kg body weight every 24 hours beginning at birth, at 10 days, and at 20 days of age. Cumulative doses of from one dose (600 mg/kg) through six doses (3600 mg/kg) were administered via stomach intubation. The pups were sacrificed every 24 hours after administration of the last dose. Five fixed sacrifice intervals were kept for comparison among all dose levels and different administration periods. These were at 10 days, 21 days, 25 days, 30 days, and 50 days. Animals receiving lead acetate beginning at birth displayed vascular hemorrhages throughout the brain, being most prominent in the cerebellum. A minimum of two doses were required to produce vascular effects. No significant body weight changes were found except in one lead treatment where there was a significant increase. Brain weight changes were significantly greater in all dose treatments. Blood lead concentrations were greatest 48 hours after the last dose and dropped ten-fold by 10 days of age. No significant body or brain weight changes occurred in any treatment group when treatment began at 10 or 20 days of age. When treatment began at 10 days of age, chromalytic neurons were present in large numbers in the nucleus gracilis and cuneatus and in smaller amounts in the dorsal horns of the spinal cord and the spinal trigeminal nucleus of 5 and 6 dose treatments. No hemorrhages nor obvious nerve degeneration was found. When treatment began at 20 days of age, no chromatolysis was found in sensory related neurons but peripheral degeneration was found in the sciatic nerve indicating a polyneuropathy was present. This study indicates that lead neurotoxicity may present different neurological deficits in the rat when exposed at different postnatal intervals.

159.4 RELATIONSHIP BETWEEN LOCOMOTOR ACTIVITY RESPONSE AND STRIATAL TYROSINE HYDROXYLASE ACTIVITY IN LEAD-EXPOSED RATS. PM McGinnis.* (SPON: GP Cooper). Dept. Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Childhood susceptibility to the adverse neurological and/or psychological effects associated with exposure to relatively low levels of lead may be mediated by a derangement in CNS synaptic function. The purpose of this study was to relate locomotor behavioral activity to alterations in dopamine neurochemistry (striatal tyrosine hydroxylase activity, TOH) in male rats exposed chronically to lead in a model simulating childhood lead exposure. Suckling pups were exposed to lead-contaminated milk during lactation then weaned onto 0.2% lead acetate in their drinking water. Control pups were exposed to non-contaminated milk during lactation then weaned onto distilled water. Body burden of lead was assessed on days 21 and 95 by analysis of lead concentration and erthyrocyte protoporphyrin. Blood lead was significantly elevated ($p<.0001$) on both days (day 21: 1.5 vs. 54.3 ug/dl; day 95: 2.8 vs. 84.8 ug/dl) in lead-treated rats relative to controls. Erthyrocyte protoporphyrin was also significantly increased ($p<.0001$) in the lead treatment group (day 21: 42.2 vs. 49.3; day 95: 53.0 vs. 84.7). At 91-95 days of age, locomotor activity was measured under red light in photocell activity chambers. The number of photocell breaks was measured at 5 minute intervals. d-Amphetamine sulfate (1mg/kg, sc) was administered after 30 minutes of habituation in the test chamber and then activity monitored for an additional 90 minutes. There was no difference in rate of habituation to the test environment between treatment groups. A significantly greater ($p<.05$) increase in activity 10 minutes following d-amphetamine was seen in the lead-treated rats. Animals in both treatment groups were chosen for subsequent quantitation of TOH activity by Z score criteria based on their activity at this 10 minute interval. The criteria produced 3 groups: low responders, normal responders and high activity responders to d-amphetamine. Animals were sacrificed at day 96-101 for determination of striatal TOH activity by accumulation of 3,4-dihydroxyphenylalanine (DOPA) following inhibition of aromatic-L-amino acid decarboxylase with NSD-1015 (75 mg/kg, ip). HPLC with electrochemical detection was adapted for measurement of DOPA. The use of various doses of α-methyl-p-tyrosine in conjunction with NSD-1015 showed the analytical methodology could discriminate a 22.6% decrease in TOH activity. There was no alteration in striatal TOH activity as measured by DOPA accumulation in adult male rats with a moderately raised body burden of lead due to chronic low level lead exposure since birth. There was no correlation between an individual's TOH activity and locomotor activity response to d-amphetamine nor to his lead body burden. These data suggest the CNS insult due to lead is not mediated by an alteration in striatal TOH activity. (Supported by ES-07073, & U.C. Graduate Studies & Research Office).

159.5 IN VITRO LEAD-INDUCED GABA RELEASE FROM SUPERFUSED RAT CORTICAL SYNAPTOSOMES. D.J. Minnema* and I.A. Michaelson. Dept. Environ. Hlth., U. Cinti. Coll. Med., Cincinnati, OH 45267

Previous studies have reported that inorganic lead (Pb) in vitro does not alter GABA release from rat brain synaptosomes (Silbergeld et al., J. Neurochem. 34:1712, 1980; Ramsey et al., Brain Res., 187:383, 1980). In contrast, the present study demonstrates that Pb (1-30 uM) added in vitro induces ^{3}H-GABA release from preloaded cortical synaptosomes in a dose-dependent manner. Purified synaptosomes (240 ug protein) were resuspended in physiological HEPES buffer and loaded with ^{3}H-GABA (250 nCi, 37°C, 30 min, under 95% O_2/5% CO_2) before being layered onto a 0.65 um pore-size cellulose acetate filter. The synaptosomal bed was superfused at 2 ml/min with HEPES buffer (37°C). After a 10 min washout, superfusate fractions were collected at 15 sec intervals. The first 4 fractions (F1-F4) served as a baseline to which the effects of Pb could be compared. Pb-acetate (1,3, 10 & 30 uM) or Na-acetate (20 uM) was added to the superfusing buffer during F5-F10; followed by normal HEPES buffer (F11-F18); followed by a 1 sec exposure to 61 mM KCl-buffer at the start of F19; then switched back to normal buffer for F20-F22. The amount of ^{3}H (dpms) in each fraction and that remaining on the filter was quantified by LSC spectroscopy. The Pb-induced GABA release was more pronounced in the absence (Figure B) than in the presence (Figure A) of calcium (2.54 mM) in the superfusing buffer. Similar effects of in vitro Pb on dopamine release (this lab) and ACh release (Suszkiw et al., Brain Res. 323:31, 1984) from rat brain synaptosomes have recently been observed. The present finding that Pb in vitro induces GABA release does not support the view that altered GABA release following chronic, neonatal Pb exposure can be explained solely by some factor other than Pb (i.e. elevated Δ-aminolevulinic acid) (Silbergeld & Lamon, J. Occup. Med., 22:680, 1980). (Supported by NIH-NIEHS-03399).



159.6 ZINC-LEAD INTERRELATIONSHIPS IN DEVELOPING RAT BRAIN. E.J. Kasarskis and T.M. Forrester* Depts. Neurology and Toxicology, VA and University of Kentucky Medical Centers, Lexington KY 40536.

Previous investigators have suggested that hippocampal Zn metabolism may be perturbed during postnatal Pb intoxication and may underlie the morphological and behavioral disturbances seen in this condition. Although this hypothesis is attractive and supported by the initial studies of Sato et al. (Soc Neurosci Abstr 9:936,1983), the regional susceptibility and dose-response relationships have not been established.

Female Sprague-Dawley rats (275-300g) were maintained on commercial rat chow and deionized water throughout gestation. After parturition and reduction of litters to 6, developing pups were exposed to Pb indirectly, by administering Pb acetate solutions (0%-4.5%) to groups of dams as their sole fluid source. Growth- and food-restricted groups were included to control for non-specific effects of inanition in the higher Pb groups. Pups were sacrificed at 21 days of age by barbiturate overdose. For each litter, individual brains were removed, dissected into 7 regions which were pooled, digested in nitric acid, and analyzed for Zn and Pb by atomic absorption spectrophotometry. With increasing Pb doses, Pb accumulated in all brain regions; the greatest relative enrichment (20-50 fold) was in brainstem and striatum. Despite near-lethal Pb intoxication and high regional Pb levels, the concentration of Zn was relatively unaffected (2%-15% decrease) in brainstem, midbrain/hypothalamus, striatum, and frontal and occipital cortex. In contrast, the Zn concentration in whole hippocampus and eye was reduced by 50-60% in the highest Pb group when compared to controls.

These data demonstrate marked regional differences in the magnitude of Pb/Zn interaction in brain and suggest that specific Zn pools may be preferentially affected by Pb exposure during CNS development. Greater differences may be detected by Zn analysis of individual hippocampal subfields and may disclose a specific antagonism of the mossy fiber-associated Zn pool by Pb. (Supported by the VA Research Service and the Muscular Dystrophy Association).

159.7 AREA SPECIFIC EFFECT OF LEAD ON ^{3}H-NITRENDIPINE BINDING IN RAT BRAIN. S.Govoni,R.A.Rius*,F.Battaini and M.Trabucchi.Inst. Pharmacol. and Pharmacognosy, Univ. of Milan and Chair of Toxicol., Univ. of Rome, Italy.

Lead (Pb) has been shown to interfere with Calcium (Ca) metabolism in brain (Silbergeld,E.K.,Life Sci.,20-:309,1977). The availability of Ca antagonists offers a biochemical probe for the study of the mechanisms regulating Ca transport in different experimental conditions. The present study investigates the binding of ^{3}H-Nitrendipine (^{3}H-ND) to synaptic membranes prepared from different brain areas after in vitro and in vivo Pb exposure. Females S.D. rats were exposed from day 16 of pregnancy to Pb acetate in the drinking water (2.5 mg/ml). The offspring received the same drinking solution which had been supplied to their mothers until they were killed (at 2-3 months of age). ^{3}H-ND binding was performed according to Gould et al. (P.N.A.S.,79:3656,1982). The in vivo exposure to Pb produced an increase in ^{3}H-ND binding to membranes prepared from striatum (ST; +48%) but not from hippocampus (HI). The increase in ^{3}H-ND binding in ST was not observed in EDTA-EGTA washed membranes, indicating that the effect was due to the persistence of Pb in the tissue. In order to further investigate the mechanisms leading to the different effect of Pb on ^{3}H-ND binding in ST and in HI, the in vitro action of Pb on ^{3}H-ND binding to membranes prepared from different brain areas was studied. Pb stimulated ^{3}H-ND binding in ST and cortex (CX) but not in HI. In addition, in hippocampus Pb inhibited the stimulation of the binding elicited by 200 uM Ca. The area-selective actions of Pb on ^{3}H-ND binding in brain are in line with previous data indicating that Pb displays regional selectivity in its effects on neurotransmitter metabolism (Govoni et al.,Toxicology,12:343,1979). More in general a differential sensitivity of Ca channels to ions of toxicological interest may explain the selective neurotoxic effects of some metal ions in different brain areas.

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159.8 EFFECTS OF ALUMINUM ON NEUROTRANSMITTERS AND THE cAMP SYSTEM IN RAT BRAIN. S.A. Khatib, D.W. Allman* and P.A. Shea. Depts. of Biochemistry and Psychiatry, Indiana University School of Medicine, Indianapolis, IN 46223.

Aluminum (Al) has been implicated as the etiological agent in several clinical disorders including dialysis encephalopathy and osteomalacia. In animal studies, toxic amounts of Al caused liver and kidney necrosis, brain atrophy and death. These effects were dependent upon the form and dose of the administered Al. The mechanism(s) of Al toxicity in the CNS is still not known. However, in vitro studies have demonstrated an inhibitory effect of Al on neurotransmitter uptake by synaptosomes and Al was shown to interact with brain calmodulin.

To further investigate the effects of Al on the CNS, 3 groups of rats (7/group) were fed a normal diet for 4 weeks with Al (as $Al_2(SO_4)_3$) added to their drinking water as follows: control group (I) 0% Al (w/w), Al-group (II) 0.1% Al and Al-group (III) 0.5% Al. Rats were sacrificed by the near-freezing method and the dissected brain parts were prepared for the determination of cAMP and biogenic amine levels and adenylate cyclase (AC) and cAMP-phosphodiesterase (cAMP-PDE) activities. In addition, plasma Al concentration was determined. In different brain regions of the Al-treated rats (e.g., cerebral cortex, striatum and midbrain), significant increases compared to control were observed in their dopaminergic and serotonergic systems. Increases in cAMP levels were also observed in group III rats (cerebellum 125% of control, midbrain 75%, pons-medulla 66% and hippocampus 30%) and in group II rats (cerebellum 24% of control, pons-medulla 29% and midbrain 23%). The changes in cAMP levels are studied further by determining the activities of both AC and cAMP-PDE in the different brain regions. In vitro studies on the effect of Al on brain AC showed that in the presence of 5 mM NaF, 5 μM Al caused a significant increase in AC activity. In the absence of NaF, Al did not have any effect on enzyme activity. Plasma Al concentration was found to be elevated in the Al-treated rats when compared with the control group. (Supported by grants from Indiana Dept. of Mental Health and A-Mideast).

159.9 REVERSIBLE CHANGE IN THE BLOOD-BRAIN BARRIER PERMEABILITY INDUCED BY INTRAVENOUS ALUMINUM IN THE RAT. Yong Sun Kim, Moon H. Lee and Henry M. Wisniewski*. New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314

We have previously reported that an intraperitoneal injection of aluminum increased the permeability of the blood-brain barrier (BBB). The change, which lasted for a few hours after aluminum administration, appeared to be closely related to the blood aluminum concentration. In this experiment, we attempted to determine the time course of the BBB change in response to aluminum injected intravenously.

Adult Sprague-Dawley rats, under pentobarbital anesthesia, were given an injection of 10 mg/kg of aluminum lactate in a volume of 0.5 ml into the left femoral vein, followed by 0.5 ml of saline containing 5 μCi of ^{14}C-sucrose injected in the right femoral vein at either 5 min, 20 min, or 60 min post aluminum injection. The control animals received the same volume of ^{14}C-sucrose 5 min after 0.5 ml of an intravenous injection of isotonic saline. Plasma radioactivity was measured until the animals were decapitated 5 min after the tracer injection, at which time brain radioactivity was measured from five different brain regions. The cerebrovascular permeability and surface area (PA) were calculated using the Rapoport model (Brain Res.,150:653-657, 1978). As indicated in the table below, the BBB permeability became increased immediately (within 5 min) following the intravenous aluminum injection. The PAs remained elevated at 20 min post-injection. By 60 min, the BBB in the forebrain had returned to normal, while the brain stem still had an increased BBB permeability. The response of the BBB to aluminum at shorter time intervals is currently under investigation.

	Mean PA X $10^6 \cdot s^{-1}$			
Brain Regions	Control (n=7)	Al 5 min (n=5)	Al 20 min (n=4)	Al 60 min (n=5)
Cortex	54.4	77.1**	89.5**	66.6
Diencephalon	36.9	52.3**	49.2*	45.1
Mesencephalon	46.1	61.6**	68.9**	35.6
Cerebellum	30.8	50.5**	63.1**	40.6*
Pons-medulla	44.8	87.5**	90.4*	65.6*

*p<.05 **p<.01

159.10 DENTATE GYRUS GRANULE CELL RESPONSES TO LOCAL APPLICATION OF TRIMETHYLTIN. D.L. Armstrong, H.L. Read*, and M.J. Wayner. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78285

The mechanism of action of the neurotoxin trimethyltin (TMT) is still unknown despite extensive investigation. The high sensitivity of specific brain regions, particularly within the hippocampus, suggests that TMT is interacting with systems within these regions or affecting input to these regions and thus producing local necrosis. Our laboratory has utilized iontophoretic application of TMT to investigate the immediate response of mouse hippocampal neurons to toxin exposure. The present study employed the pressure ejection application method since this procedure has yielded much more reliable dose-response curves for this compound.

Two-, three-, and four-barrel microelectrodes were used to apply trimethyltin chloride in concentrations ranging from 10 to 100 mM. 1.0 M glutamate and 0.15 M NaCl applications were used as control ejections. Thirty-eight spontaneously active granule cells in mouse hippocampal slices were tested. Seventeen of these cells responded to TMT application with increases in spontaneous activity. Spikes per second records revealed a 100 to 200% increase in activity with a latency of 0.5 to 1.0 seconds. AT lower doses the duration of effect corresponded to the 5 sec period of toxin ejection, however, higher doses produced a more sustained increase in activity. Ten cells displayed a variable response to the toxin that included spontaneous bursting activity or biphasic increases then decreases in activity. Eleven cells displayed no response to the doses employed in this study. Direct postsynaptic interactions or alterations in interneuron inhibition of a subpopulation of granule appears to occur with a short latency following TMT application. The mechanism by which this immediate effect could lead to cell death is being investigated.

159.11 IRON-INITIATED LIPID PEROXIDATION IN MURINE SPINAL CORD CULTURES. D. K. Anderson & E. D. Means, VA Medical Center, Cincinnati, OH 45220.

We have previously demonstrated (Trans Am Soc Neurochem 15 (1): 130, 1984) that incubation of 50μM $FeCl_2$ with dissociated murine spinal cord cultures (11-13 day embryos) for 1 hr resulted in a significant decline in Na^+, K^+-ATPase activity; an effect that was partially reversed by methylprednisolone sodium succinate (MPSS), alpha tocopherol and mannitol. This suggested that iron inhibited Na^+, K^+-ATPase, at least in part, by initiating peroxidation in membrane lipids. The purpose of the present study was to confirm the occurrence of lipid peroxidation in cultures exposed to $FeCl_2$ by assaying for thiobarbaturic reactive (TBAR) materials and to correlate the TBAR material changes with those of Na^+, K^+-ATPase in untreated and MPSS, alpha tocopherol, or mannitol-treated cultures. TBAR materials were elevated 5.3 and 6.2-fold in cultures incubated with 50μM $FeCl_2$ for 1 and 2 hr, respectively. 100μM MPSS or 50μM alpha tocopherol, or 50μM mannitol partially blocked the rise in TBAR materials at both times. There was a strong negative correlation (r = -0.87) between levels of TBAR materials and Na^+, K^+-ATPase activity at both 1 and 2 hr. Additionally, incubation of cultures with either MPSS for 1 hr or alpha tocopherol for 24 hrs resulted in a 1.5 and 1.3-fold increase in Na^+, K^+-ATPase activity, respectively. After 120 min incubation with MPSS, there was an additional 1.3-fold increase in Na^+, K^+-ATPase activity but no further increase in Na^+, K^+-ATPase activity in cultures incubated with alpha tocopherol. This secondary MPSS stimulated increase in Na^+, K^+-ATPase activity was blocked by the protein synthesis inhibitor, cyclohexamide and the RNA synthesis inhibitor, actinomyosin D. These results suggest that iron initiates peroxidative reactions in neuronal membranes. Since both alpha tocopherol and MPSS are known antioxidants, these agents appear to protect by limiting the iron-initiated lipid peroxidation. The partial protection by mannitol suggests that $OH^\cdot$ may have been involved in the initiation of peroxidation. MPSS may also protect against the iron-initiated partial inactivation of Na^+, K^+-ATPase by inducing the formation of additional Na^+, K^+-ATPase molecules.

(This work was supported by the Veterans Administration.)

159.12 NEURONAL NECROSIS AND PHAGOCYTOSIS BY NEUTROPHILS AND PROSTANOID CHANGES FOLLOWING PEROXIDATION. E. D. Means, R. Saunders*, D. K. Anderson, and L. Horrocks. VAMC, Cincinnati, OH 45220 and Ohio State Univ. Col. Med., Columbus, OH 43210.

Murine spinal cord cultures were exposed to ADP/Fe^{2+} (0.5mM/0.1 mM) for 20 min after removal of the growth media. The media containing the ADP/Fe^{2+} was replaced and neutrophils were added ($0.5\text{-}1.0 \times 10^4$). Controls consisted of normal cultures, peroxidized cultures and cultures with neutrophils added. At 2, 4, 6 and 24 hr the media was removed and frozen at -70°C. The cultures were fixed and stained with cresyl violet. Other cultures were pretreated with alpha tocopherol (50μM), methylprednisolone sodium succinate (MPSS) (0.1mM) or mannitol (1%). Controls consisted of pretreated, pretreated-peroxidized, and pretreated peroxidized with neutrophils added. Peroxidized cultures showed minor fragmentation of processes and irregularity of the neuronal membrane. Peroxidized cultures with neutrophils showed significant neuronal necrosis and phagocytosis by neutrophils. Pretreated cultures showed cytological protection (alpha tocopherol > mannitol > MPSS). Radioimmunoassay (RIA) was performed on the individual media for prostaglandin E_2 (PGE_2), $F_{2\alpha}$, ($PGF_{2\alpha}$) & slow reactive substances (SRS; primarily leukotrienes C_4, D_4 and E_4). SRS's were undetectable in the media from all cultures. The lowest values for PGE_2 and $PGF_{2\alpha}$ (100-200pg) occurred in media from normal cultures and from cultures with neutrophils and with alpha tocopherol, mannitol and MPSS. Highest values (700-1000pg) were noted in media that had been pretreated with alpha tocopherol and peroxidized or pretreated with either alpha tocopherol, mannitol or MPSS, peroxidized and neutrophils added. These data suggest that (a) lipoxygenase may be inhibited making more arachidonate available for conversion to prostaglandins, or (b) high levels of lipid hydroperoxides inactivate cyclooxygenase while alpha tocopherol, mannitol and MPSS decrease the levels of lipid hydroperoxides resulting in higher concentration of PGE_2 and $PGF_{2\alpha}$.

(This work was supported by the Veterans Administration.)

159.13 TREMORGENIC MYCOTOXINS ENHANCE DIHYDROPYRIDINE BINDING IN RAT CORTEX. J.J. Valdes, R.J. Cole* and D.H. Ross. Biotechnology Div., Aberdeen Proving Ground, MD 21010; Dept. Agriculture, Dawson, GA 31742; Dept. Pharmacology, Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

The flux of Ca++ through voltage-dependent channels is important for contractile processes in cardiac and smooth muscle and for transmitter release from nerve terminals. Although these channels may differ pharmacologically in their degree of coupling to dihydropyridine receptors and sensitivity to organic Ca++ channel blockers such as nifedipine, dihydropyridine receptor binding nevertheless has utility for the identification of compounds with potential consequences for Ca++ channel activity, and subsequent relevance to convulsive behaviors. Recently, economically important mycotoxins from the fungal genera Penicillium, Aspergillus and Claviceps have been found to induce tremors and seizures in humans and cattle. These tremorgens belong to several chemically distinct groups, including the fumitremorgens, the paspalitrems, and the tetramic acids. The best known compounds within these groups are verruculogen (P. verruculosum), aflatrem (A. flavus), and cyclopiazonic acid (P. cyclopium), respectively. The fumitremorgens and paspalitrems have, in common, an indole moiety derived from tryptophan, while the tetramic acids, though chemically related, are not considered true tremorgens. All three share, however, the ability to induce tremors and seizures which are similar to those induced by the naturally occurring marine toxin and Ca++ channel agonist, maitotoxin. Therefore, dihydropyridine receptor binding , defined as nifedipine displaceable 3H-nitrendipine binding, was assessed in cortical synaptic membranes from rats injected with a single intraperitoneal tremorgenic dose of verruculogen (1mg/kg), aflatrem (3mg/kg), cyclopiazonic acid (3 mg/kg), or DMSO vehicle, and decapitated 1 hr post-injection. There was an 88% increase in the number of binding sites following aflatrem treatment (Bmax = 100 fMol/mg protein), and a 43% increase in rats given cyclopiazonic acid (Bmax = 76 fMol/mg protein) relative to controls (Bmax = 53 fMol/mg protein). Verruculogen had only marginal effects (Bmax = 61 fMol/mg protein). These results suggest that aflatrem and cyclopiazonic acid act at dihydropyridine receptors and may, like maitotoxin, fix the Ca++ channel in an open configuration.

159.14 DIFFERENTIAL VULNERABILITY TO INSULT DURING THE BRAIN GROWTH SPURT IN RAT PUPS. J. Diaz,J. Hwang* and L. Jones*. Dept. of Psych., Univ. of Washington, Seattle, WA 98195.

The brain growth spurt (BGS) which occurs relatively late in mammallian brain development is thought to be a period of heightened vulnerability to a variety of insults. The BGS encompasses a long period of time, from mid-gestation to the fourth postnatal year in humans and approximately from postnatal day 4 through day 21 in rats. Few studies have examined insults which occur at different times within the BGS. The purpose of the present experiment is to describe the effects of RNA synthesis inhibition during the early accelerating phase, the peak velocity phase or the later decelerating phase of the BGS.

Long-Evans rats were assigned by weight on postnatal Day 4 to one of six groups: 1) pups injected with methlyazoxymethanol acetate (MAMA) daily from day 4 through day 7 (n=14); 2) pups injected daily with saline from day 4 through day 7 (n=16); 3) pups injected with MAMA daily from day 8 through day 11 (n=12); 4) pups injected daily with saline from day 8 through day 11 (n=15); 5) pups injected with MAMA daily from day 12 through day 15 (n=8); 6) pups injected daily with saline from day 12 through day 15 (n=15). MAMA was injected at a dose of 10 mg/kg (sub.cu.) and controls received an equal volume of saline. All the animals were weighed each morning . Each pup was sacrificed on the morning after its last injection, and its brain removed, dissected and weighed; and its liver, kidney, and spleen were also removed and weighed.

RNA synthesis inhibition suppressed whole brain weight and whole body weight equally at the three time periods. However, an examination of the individual components which comprise these weights revealed that : 1) cerebellar growth was depressed more during the early and peak periods of the BGS than during the late phase; 2) brain stem growth was depressed most during the peak of the BGS; 3) rostral brain growth was equally depressed during the three periods. The pattern of drug induced growth suppression in liver weight resembled that seen in the cerebellum, whereas kidney weight showed a significant linear increase over time in the drug animals when compared to the controls. Spleen growth showed a biphasic pattern over time in the drug groups.

These data indicate that within the period of the brain growth spurt different areas of the developing brain may have different corridors of vulnerability . The suppression of cerebellar growth exemplifies this point, since its growth spurt begins sooner and is faster than the rest of the brain. The description of individual growth patterns of specific brain areas as well as the persistence and functional significance of growth disruption at different times on the BGS must be determined. Supported by NSF grant RII 8114919

159.15 OLFACTORY BULB DEVELOPMENT IN GUINEA PIGS EXPOSED TO ETHANOL DURING THE LATER HALF OF GESTATION. C. Nyquist Battie. School of Basic Life Sciences, Univ. of Missouri, Kansas City, MO 64108.

The olfactory bulb with its simple cortical organization and well-worked out anatomy and physiology is a good area in which to study teratogens. Decreased overall growth of the mouse olfactory bulb(OB) has been shown to result from perinatal exposure to ethanol (ETOH), although synaptic connections form normally (Int. J. Devel. Neurosci., in press). In that study ETOH was administered primarily after birth, which may not mimic human Fetal Alcohol Syndrome. To circumvent this problem, a guinea pig model was developed because of their longer gestation. An ETOH solution(30% v/v in ENSURE diet) was administered twice daily to 6 pregnant guinea pigs from day 35 of gestation to parturition at 69-70 days for a total of 6 gm ETOH/kg weight per day. Serum ETOH levels ranged from 170-200 mg% 1 hour after administration. The control group received the liquid diet but with isocaloric replacement of ETOH by sucrose.

No differences between the groups were found in food and water intake, nor in litter size, length of gestation and in offspring weight and length on the day of birth. No facial or somatic deformaties were seen in the ETOH newborns. Under barbiturate anesthesia, newborn guinea pigs were perfused, OBs were removed, embedded in glycol methacrylate, sectioned at 1μm and stained with cresyl violet. Histologic changes, attributable to ETOH, were seen. Abnormal clumping of granule cells, leaving larger than normal acellular areas, were common. In many sections mitral cells were in disarray, although most had the mature mitral shape. These changes could indicate abnormal migration. A few cells were swollen and had reduced nissl staining. In both control and ETOH OBs, glomeruli were not fully developed and migrating cells were seen in the external plexiform layer. The size of the mitral cell nucleus was examined in coronal sections, but no ethanol-induced change in size was noted. The percentage of mitral cells having more than 1 nucleoli was higher in ETOH OBs (53% vs. 44%), which may indicate a state of immaturity. ETOH also affected OB growth. The mean diameter of the glomeruli was lowered by ETOH (74.6± 2.0 vs. 83.8± 1.8) as was the width of the external plexiform layer (244± 8 vs. 272± 5.3 μm). It can be concluded that ETOH is detrimental to OB development in the guinea pig, a useful model of Fetal Alcohol Syndrome.

160.1 REINFORCEMENT-RELATED UNIT ACTIVITY IN BASAL FOREBRAIN AND AMYGDALA F.A.W. Wilson & E.T. Rolls, Department of Experimental Psychology, University of Oxford, Oxford, U.K.

Basal forebrain and amygdala unit activity reflecting the availability of reinforcement has been compared in behaving monkeys. In a visual discrimination task the monkeys could make a lick movement to obtain fruit juice when one stimulus (S+) was shown; responses to the other stimulus (S-) produced aversive saline. A reversal task required the monkey to respond to the former S- to obtain juice, and not to respond to the former S+ to avoid saline. In a serial recognition memory task stimuli were shown twice per day, once as novel and once as familiar. Lick responses to stimuli when novel produced saline and when familiar produced fruit juice.

A population of units (108/2004) in the substantia innominata and diagonal band of Broca responded differentially to the S+ and S-. 81 of these 108 units responded maximally to the S+ (and also to familiar stimuli) predicting fruit juice; 27 of these 108 units responded maximally to the S- (and novel stimuli) predicting saline. The differential response to novel and familiar stimuli was maintained when several trials (typically 8 tested) separated novel and familiar presentations of stimuli, reflecting memory for the stimuli. In the reversal task units responsive to the former S+ responded to the former S-; units responsive to the former S- changed response to the former S+.

Units (17/657) located mainly in the dorsal medial amygdala responded differentially to the S+ and S-. 16 of the 17 differential units responded maximally to the S+. 12/14 of these units did not respond differentially to novel and familiar stimuli, responding equally to these stimuli. Therefore when the reinforcement value of stimuli was determined by the novelty/familiarity of the stimuli, most amygdala units did not reflect the reinforcement value of the stimuli. Of the 2 units tested in the reversal task neither showed significant changes in response.

Both populations of differential units reflect the learned reinforcement value of the S+ and S-, but the responses of amygdala units are not entirely specified by the reinforcement value of the stimuli. In contrast, basal forebrain unit activity changes with changes of stimulus reinforcement value (the results from the recognition and reversal tasks); these units also reflect memory for stimulus novelty and familiarity important for specifying reinforcement within the task.

160.2 THE RESPONSES OF SINGLE NEURONS IN THE PRIMATE HIPPOCAMPUS RELATED TO THE PERFORMANCE OF MEMORY TASKS. E.T.Rolls, Y.Miyashita*, P.Cahusac* and R.P.Kesner. Dept. Exptl. Psychol., Oxford University, Oxford, England.

In order to analyze the functions of the hippocampus of the primate, the activity of 1510 single hippocampal neurons was recorded in rhesus monkeys performing memory tasks known to be impaired by damage to the hippocampus or fornix.

In an object-place memory task in which the monkey had to remember not only which object had been seen in the previous 7-15 trials, but also the position in which it had appeared on a video monitor, neurons were found which responded differentially depending on which place on the screen objects were shown. These neurons comprised 5.7 % of the population recorded. It is notable that these neurons responded to particular positions in space (whereas 'place' cells in the rat respond when the rat is in a particular place). In addition, 1.0 % of neurons responded to a combination of place and novelty, in that they responded more to a stimulus the first time it was shown in a particular position than the second time. Most of these neurons had response latencies in the range 100-200 ms, compared to typical behavioral response latencies of 350 ms.

In tasks in which the monkeys had to acquire associations between stimuli and motor responses, 10.6 % of neurons responded to particular combinations of stimuli and responses. For example, in a task in which the monkey had to perform a touch response 3 times when one visual stimulus was shown, but had to perform a witholding response for 3 sec to obtain reward when a different stimulus was shown (FR-DRO, Gaffan and Harrison, 1984, Quart. J. Exp. Psychol. 36B: 223-234), 9.2 % of neurons responded to one of the stimuli if it was linked to one of the responses in this task. The same neurons typically did not respond if the same stimuli or the same responses were used in different tasks.

In recognition memory tasks, a small proportion of neurons (0.7 %) responded differently to novel as compared to familiar stimuli. In addition, some further neurons responded to novel stimuli in other memory tasks such as a delayed match to sample.

These results show that hippocampal neurons in the primate have responses related to certain types of memory. Their responses appear to reflect holding information in memory for tasks which involve memory for where in the environment stimuli have been seen, and which motor responses should be made to particular stimuli.

160.3 VISUAL PROPERTIES OF PREFRONTAL CORTICAL NEURONS. S. Funahashi*, C.J. Bruce, and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven CT 06510.

According to previous reports, many prefrontal (PF) neurons respond to visual stimuli and have visual receptive fields (RFs). We further examined visual activity in PF cortex by studying single units there while monkeys performed a visual fixation task. Their heads were fixed and a magnetic search coil was used to monitor gaze. Both the fixation spot (0.2 degree diameter) and test stimuli (0.7 degree square) were presented on a monochrome CRT. Test stimuli were presented for 1 sec during the 3 sec fixation period and stimulus location was systematically varied within a polar coordinate framework. For directional tests, stimulus eccentricity was fixed and different angular directions, often spanning 360 degrees, were interspersed. For eccentricity tests, direction was fixed and different eccentricities out to 20 degrees were interspersed.

Of 135 PF neurons studied, 76 responded to peripheral test stimuli and visual RFs of 37 were mapped. Nearly all RFs (32/37) were bilateral; only 3 were ipsilateral and 2 contralateral. Most RFs subtended large portions of visual space, although some appeared to be discontinuous or patchy. Many RFs encompassed the center of gaze as judged both by responses to the fixation light and to test stimuli superimposed on it. Overall, these RFs differed somewhat from previous studies, which have found many visual RFs of PF neurons to be unilateral or discrete. We also examined the influence of direction of gaze on visual responses of PF neurons because PF cortex is reciprocally connected with posterior parietal cortex where visual activity can be influenced by direction of gaze. We simply moved the CRT 20 degrees to the left or right, and retested using the same fixation point and stimulus arrays, thereby stimulating the same retinal coordinates while the gaze was eccentric. For 7 PF neurons visual responses depended only on the retinal coordinates, but for 6 PF neurons responses depended upon direction of gaze as well in that visual responses from the same retinal locations were greater during eccentric fixation.

These visual properties of PF neurons indicate considerable convergence from the much smaller, exclusively contralateral visual RFs of neurons in striate and prestriate cortex, and suggest that visual processing in PF cortex might more resemble that described in higher-order visual association cortices such as posterior parietal and inferotemporal cortex. Supported by MH38546, EY04740, and a grant from the H.F. Guggenheim Foundation.

160.4 BEHAVIORAL RELEVANCE, A DECISIVE FACTOR IN THE RESPONSE OF PREFRONTAL NEURONS TO VISUAL STIMULI. J. Yajeya*, J. Quintana* and J.M. Fuster. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

The activity of 150 single units was recorded from the monkey's lateral prefrontal cortex (sulcus principalis area) during performance of two visual discrimination tasks with delayed choice; in one task, the choice was between colors, in the other, between positions. In both, a trial was initiated by a diffuse low-intensity flash which served as a signal for ocular fixation (verified by EOG) on a centrally located 2.5-cm translucid disk (8°), on which 2 sec later a color light, the cue for the trial, was projected for 0.5 sec. After an 18-sec delay, the choice stimuli were presented on two lower disks. In one task, red or green cue called for the choice of that color when the two appeared after the delay; in the other task, yellow or blue cue called for choice of response side (right or left, respectively) between two white-light disks. A fifth color-cue, violet, was not followed by choice-stimuli. All cues were of equal brightness. Some of the units showed color- or task-differential reactions, but these were generally overshadowed by differences of reaction as a function of the behavioral significance of the cue. The behaviorally irrelevant stimulus (violet) tended to elicit unit responses of different magnitude than the other four. In a relatively inferior location of prefrontal cortex, the relevant stimuli induced protracted cell reactions lasting for much or all of the delay period. No such reactions were induced by the irrelevant stimulus. In conclusion, prefrontal units, at least within the area so far explored, seem to differentiate visual stimuli more by their behavioral significance than by their color or the particular task they cue. This is most evident during the delay period of delay tasks. It is well known that the functional integrity of the lateral prefrontal cortex is essential for normal performance of such tasks. The unit findings reported here provide further evidence of participation of the neurons of that cortex in the performance of these tasks and, thus, in the mediation of cross-temporal contingencies of behavior.

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160.5 ADAPTIVE PLASTICITY IN THE PRIMATE SPINAL STRETCH REFLEX: PERSISTENCE. J. A. O'Keefe* and J. R. Wolpaw (SPON: A. J. Popp). Wadsworth Ctr. for Labs and Research, New York State Dept. of Health, Albany, NY 12201; and Depts. of Neurology and Anatomy, Albany Medical College, Albany, NY 12208.

Previous work (J. Neurophysiol. 50:1296-1319, 1983) showed that monkeys can gradually change the amplitude (amp) of the wholly segmental, largely monosynaptic, spinal stretch reflex (SSR) when confronted by a task requiring change. We studied the persistence of SSR change after non-performance periods of 2-38 days.

Eleven animals (Macaca nemestrina) with chronic EMG electrodes in biceps and related muscles learned to maintain elbow angle and a given level of biceps EMG against constant extension torque. At random times, a brief additional extension torque pulse elicited the biceps SSR. In the control mode, reward always followed. Under the SSR↑ or SSR↓ mode, reward occurred only if the absolute value of biceps EMG in the SSR interval (15-24 msec after the pulse) was above or below a set level. Animals completed 3,000-6,000 trials daily. Animals worked first under the control mode for up to 60 days and then under the SSR↑ or SSR↓ mode for up to 100 days. Some were then exposed to sequences including mode reversal (SSR↑ to SSR↓ or vice versa) and re-exposure to the previous mode. They responded to each SSR↑ or SSR↓ mode exposure with gradual mode-appropriate change in SSR amp. Mode exposures were interrupted by gaps in performance of 2-38 days.

Short gaps of 2-4 days had no discernible effects on SSR amp under any mode. Long gaps of 10-38 days produced transient 10-15% decrease in SSR amp under the control mode. This nonspecific decrease disappeared over the first post-gap week. Long gaps under the control mode had no other effects.

Under the SSR↑ mode, long gaps caused a significant loss of adaptive SSR increase. Pre-gap amp averaged 168% of control, while post-gap amp averaged 128%. Thus, in the absence of performance, SSR increase appeared to decay with a half-life of about 17 days. Unlike SSR↑ gaps, gaps during the SSR↓ mode had no consistent effect on SSR adaptive change. The average pre-gap amplitude of 63.6% of control was not significantly different from the average post-gap value of 66.4%. Thus, in the absence of performance, SSR decrease usually persisted without significant change for at least one month. The data did not show rapid adaptive change in the early post-gap period under either mode. SSR behavior in this period was a combination of recovery from the modest nonspecific gap-induced decrease and resumption of slow mode-appropriate adaptive change.

These results further support the hypothesis that adaptive SSR change involves persistent segmental alterations and thus may provide a technically accessible substrate of memory.
(Supported by NIH NS22189 and by United Cerebral Palsy.)

160.6 VISUAL RESPONSES IN MONKEY LATERAL HYPOTHALAMIC AREA DURING FEEDING BEHAVIOR. T. Ono, M. Fukuda* and H. Nishino. Dept. of Physiol., Fac. of Med., Toyama Med. & Pharmaceu. Univ., Sugitani, Toyama 930-01, JAPAN.

Single neuron activity was recorded from monkey lateral hypothalamus to investigate correlation of neuronal events with food discrimination and initiation of procurement motion in operant bar press feeding behavior. The operant feeding task consisted of discrimination of food, and bar pressing behavior to obtain food. These phases were separated by a forced wait of more than 2 sec before the animal could press the bar after the visual stimulus. Of 429 neurons tested, 68 (16%) responded during the visual phase. Of these, 30 (7%) responded selectively to the sight of a food or non-food object associated with the juice reward, but not to the sight of a meaningless or non-food object, or an object associated with aversive saline. The latency of food-related visual responses was 191±58 msec (mean±S.D., n=30), which was longer than that of non-discriminative visual responses (158±61 msec, n = 38) ($p<0.05$). In extinction tests, in which the monkey could not obtain food even he did press the bar, the animal pressed progressively later in successive trials. Of 11 food-related neurons tested, neuronal responses of 9 became weaker in successive trials and finally disappeared. The latency of the visual responses tended to become later in successive trials. The disappearance of food-related visual responses was also observed in reversal tests and upon satiation by food. The strength of the visual responses was correlated with the latency of the first bar press initiation and the speed of bar pressing. Also, there was highly significant correlation between the disappearance of the visual responses and the cessation of bar pressing behavior for food. The neuronal responses tended to disappear before the cessation of bar pressing behavior for food.

The data suggest that LHA neuron activity is intimately related to discrimination of reinforcement or non-reinforcement, and drive state to get food during operant feeding behavior. These are affected by learning and internal states such as hunger and satiety.

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160.7 NEURONAL ACTIVITY OF PREFRONTAL CORTEX AND DORSOMEDIAL THALALMUS DURING A CONTINUOUS NONMATCHING-TO-SAMPLE TASK IN THE RAT. Y. SAKURAI. Dept. of Behavioral Sciences, Fac. of Integrated Arts and Sciences, Hiroshima Univ., Higashisenda-machi, Naka-ku, Hiroshima 730, Japan.

Prefrontal cortex (PFC) and dorsomedial thalamus (DMT) are thought to be involved in memory-based control of an operant response. By using a delayed go/no-go alternation task, we have suggested a functional hierarchy from DMT to PFC, that is a primary role of PFC in the response control function and that of DMT in the processing of working memory for responses (Sakurai & Sugimoto, Behav. Brain Res., in press). This study also focused attention on the memory-based control of response and elucidated the functions of PFC and DMT especially in the processing of working memory for external stimuli.

Rats were trained to perform a new delayed matching-to-sample task - the continuous nonmatching-to-sample task with Go/No-Go responses. A variable number of trials with low tone (1 kHz) stimulus alternated with trials with high tone (8 kHz) stimulus. A panelpress response (Go) on a trial following a stimulus change (nonmatch trial) was reinforced with food. Responses to repeated stimuli (match trials) were never reinforced. The animals were required to remember across the intertrial interval (delay period) which tone was presented on the previous trial and to emit or inhibit a response according to the memory process. Almost animals acquired the task with 3-sec of delay within 20 days of training. After the completion of training, the rats were chronically implanted with 25μ microwire electrodes into PFC and DMT and unit activity was recorded during performance of the task.

To the present four types of task-related neurons have been observed. 1) Retention-related type increased or decreased its activity during the delay period. 2) Response priming type responded to the tone presentation on a nonmatch trial for Go response. 3) Response inhibition type responded to the tone presentation on a match trial for No-Go response. 4) Tone recognition or retrieval type phasically responded to the tone both on match and nonmatch trials. We furthermore analyzed activity of those task-related neurons when the delay period was changed from 3 to 6 sec and after the animals had aquired the task with 6-sec of delay. The data from this experiment in conjunction with that from neuronal recording during a task which required working memory for responses, not external stimuli, (Sakurai & Sugimoto, Behav. Brain Res., in press) further delineates the role of PFC and DMT in working memory processing and control of an operant response.

This work was supported in part by a Grant-in-Aid for Scientific Research 59710072 from the Japanese Ministry of Education.

160.8 SEQUENTIAL DEPENDENCIES REGULATE SENSORY EVOKED RESPONSE OF SINGLE UNITS IN THE RAT HIPPOCAMPUS. R.E. Hampson*, T.C. Foster*, E.P. Christian, K.A. Campbell, and S.A. Deadwyler. Bowman Gray Sch. of Med., Winston-Salem, NC 27103

Past investigations have shown that preceding sequence of CS+/CS- trials in a 2-tone discrimination task affect the amplitude of auditory evoked potentials recorded in the outer molecular layer of the dentate gyrus. In this study, unit firing records from three major types of hippocampal cells were recorded from microdrive electrodes arranged to traverse the CA1, CA3 and dentate granule cell layers of the hippocampus. Classification of units as complex spike (ComSp), theta (T), and dentate granule cells (G) was according to criteria based on spontaneous and stimulus evoked firing characteristics. Single trial records for each cell type were collected from over 20 different sessions when behavioral performance was at criterion levels (n=15). Standard (Z) scores of pre- vs post-tone firing rate for each trial were analyzed by 2- and 3-way ANOVAs of cell type, CS+ vs CS- trials and preceding trial sequence.

Mean pre-tone firing rate for G cells was 17 spikes/sec., for T cells: 25 spikes/sec., and for ComSp cells: 1.5 spikes/sec. The mean post-stimulus histogram latency to peak discharge for CS+ trials was the same (90 msec.) for T, G and ComSp cells. Peak discharge occurred 100 msec after CS- trials for T and G cells and 80 msec for ComSp cells. All three cell types exhibited significant fluctuations in pre- vs post-tone firing rate (Z-scores) dependent on whether CS+ or CS- trials occurred. The main effect of preceding trial sequence on individual trial Z-scores was highly significant ($p<.001$). The Z-scores for runs of CS- trials were significantly decreased in relation to Z-scores for the CS+ run in all cells ($p<.01$). The average Z-scores for T and G cells decreased significantly during double alternation vs single alternation sequences ($p<.05$), and increased during runs of 3 or more CS+ or CS- trials ($p<.05$); however, T and G cells did not differ with respect to these sequences. In contrast to T and G cells, Z-scores for ComSp cells were significantly lower during single alternation and higher during double alternation sequences ($p<.01$). In addition ComSp cell Z-scores decreased to a significantly lower level than T and G cells during runs of 3 or more CS+ or CS- trials. When the current trial was not considered, ComSp cells were shown to be significantly affected by the preceding sequence of 3 to 5 trials ($p<.01$), whereas T and G cells were more strongly affected by the current trial.

Results showed a pronounced similarity between firing characteristics of G and T cells with respect to trial sequence. ComSp cells exhibited changes in firing characteristics which were opposite to T and G cells.
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160.9 HIPPOCAMPECTOMY ALTERS CONDITIONED RESPONSE ONSET IN LONG DELAY CONDITIONING. A. L. Beggs, R. L. Port, A. G. Romano, and M. M. Patterson, Dept. of Psychology and Coll. of Osteopathic Medicine, Ohio Univ., Athens, OH 45701.

It has been demonstrated that the hippocampus generates a multiple-unit "model" of the classically conditioned rabbit nictitating membrane response (Berger & Thompson, 1978). However, hippocampectomy has no deleterious effect on the acquisition of conditioned responses (Schmaltz & Theios, 1972). Therefore, any involvement of the hippocampal "model" in classical conditioning must be nonessential to the basic substrate of the association. Hoehler and Thompson (1980) reported that the hippocampal "model" adjusts to a change in the CS-US interval more quickly than the behavioral response. Thus, the "model" may modulate temporal aspects of CRs dependent upon the parameters of the stimulus configuration. In an earlier study (Port, Mikhail & Patterson, 1985), we found that hippocampectomy influenced the rate of learning in short and long intervals but had no effect in a moderate interval. Hippocampectomy also decreased response onset latency in the short interval but had no statistically significant effect in the long interval. Recent evidence (Marek, McMaster, Harvey & Gormezano, 1984) suggests that the neural pathways mediating reflexive responses to corneal air puff and periorbital shock are somewhat different. If the hippocampus modifies temporal characteristics of conditioned responses, the effects of hippocampectomy on CR timing may be influenced by the US. The present experiment examined the effect of hippocampectomy on response onset in a long delay procedure. A corneal air puff was employed as the US; the preceding study utilized periorbital shock.

Rabbits were assigned to hippocampal lesion, cortical lesion and nonoperated control groups. Bilateral aspirations lesions of the hippocampus or neocortex were produced under fluothane anesthesia. Damaged animals were permitted two weeks to recover. Subjects were classically conditioned with a tone CS and air puff US. CS duration was 850 ms, US duration was 100 ms, and the CS-US interval was 750 ms. Sixty trials were given each day for four consecutive days. Hippocampectomy had no significant effect on the rate of acquisition. However, conditioned responses given by hippocampal damaged animals began earlier in the interval than responses given by cortically lesioned or nonoperated animals.

These results are consistent with the notion that the hippocampus modulates temporal characteristics of conditioned responses. The magnitude of hippocampal lesion effects on simple acquisition appears to be influenced by the response system as well as the CS-US interval. Nonetheless, the "model" of the CR which develops within the hippocampus appears to play a "purposive" role in classical conditioning.

160.10 HIPPOCAMPAL SUBSTRATE OF SENSORY-SENSORY ASSOCIATIONS: ELECTROPHYSIOLOGICAL AND LESION ANALYSES OF SENSORY PRECONDITIONING. R. L. Port, A. G. Romano, D. O. Berger*, and M. M. Patterson, Dept. of Psychology and Coll. of Osteopathic Med., Ohio Univ., Athens, OH 45701.

Many of the learning deficits exhibited by hippocampal lesioned animals occur in situations in which no reflexive motor response is present. Hippocampectomy impairs learning in latent inhibition (Solomon & Moore, 1975), reversal learning (Berger & Orr, 1983) and extinction (Schmaltz & Theios, 1972). However, simple CS-US associations are not impeded by hippocampal lesions (Port, Mikhail & Patterson, 1985). Since the absence of reflexive behavior appears to indicate the learning tasks in which hippocampal participation is vital, we have adopted Brogden's (1939) sensory preconditioning paradigm to study hippocampal involvement in plasticity. An earlier study (Port & Patterson, 1984) had revealed that electrolytic lesions of the fimbria abolished the CS-CS association but had no significant effect during CS-US training. The present research examined hippocampal multiple-unit activity during preconditioning and the effects of hippocampal lesions on the CS-CS association.

In the first experiment, rabbits were anesthetized with fluothane and implanted with chronic recording electrodes placed in the CA1 layer of the dorsal hippocampus. Multiple unit activity was recorded during paired presentations of light and tone for the preconditioned group, or during unpaired presentations for the control group. Animals were then classically conditioned with the light CS and a periorbital shock US. After CS-US training, ten nonreinforced presentations of the tone were used to assess the effects of preconditioning. Preconditioned subjects responded more frequently to the test stimulus than the control group, which failed to exceed spontaneous response rates. The amplitude of evoked hippocampal activity during preconditioning was highly correlated (r=+.85) with subsequent performance during the test session. In a second experiment, rabbits were lesioned with kainic acid placed in the dorsal hippocampus while under fluothane anesthesia. A control group received sham operations. Both groups were pretreated with diazepam. Training parameters were identical to those used for the preconditioned group in experiment 1. KA lesions had no deleterious effect during CS-US training, however, response rates to the test stimulus were reduced to spontaneous levels. Control animals exhibited significant effects of preconditioning.

These results suggest that the hippocampus is vital for sensory-sensory but not for sensory-motor learning. While the neural substrate underlying sensory preconditioning appears to include the hippocampus, the manner in which this structure influences response systems remains unknown.

160.11 THE ROLE OF OLFACTION IN CONDITIONED FOOD AVERSION DURING AND AFTER RECOVERY FROM OLFACTORY NERVE SECTION. N.E. Kinney, J.W. Wright and J.W. Harding. Dept. of Psych., SE Missouri State Univ., & Depts. of Psych. and Vet. Comp. Anat., Pharm., & Physiol., Washington State Univ., Pullman, WA. 99164

The role of olfaction in conditioned flavor aversion was investigated. Male albino mice underwent bilateral olfactory nerve section and were allowed 7 days for recovery. Animals were then food deprived for 8 hr before given access to a novel food (almond). During this first access, mice in the nerve sectioned group ate significantly more almond than did home cage, sham surgery/rotation, or rotation only controls. This could be interpreted as a failure to recognize the novelness of the novel food in the absence of the olfactory component of flavor detection/recognition. Nerve sectioned mice were then subjected to 50 min of body-rotation (60 rpm). Forty-eight hr later, all mice were given a second access to almond. This time, nerve sectioned and other rotation groups consummed significantly less than during the first access, demonstrating conditioned aversion to almonds.

On day 15 post-surgery, nerve sectioned mice were given a third access followed by body-rotation. During a fourth access 2 days later, these mice once again showed aversion to almond. However, subsequent repetition of the procedure resulted in virtually no consumption on day 25, and none on day 26. Home cage, sham surgery/rotation, and rotation only control groups consummed no novel food after first access.

Functional recovery (90% of controls) of a food odor mediated task was demonstrated on day 15 post-surgery in nerve sectioned mice (Harding & Wright, Brain Research Bulletin, 4:17-22, 1979), with complete recovery by day 21. It is suggested that nerve sectioned mice can form a taste mediated food aversion (following consumption of a novel food paired with rotation-induced gastrointestinal malaise) without olfactory input on day 7 post-surgery, and a second flavor (taste plus olfaction) mediated aversion on day 15. The retention of the flavor aversion on day 25 by the nerve sectioned group was as expected and did not differ from olfactory normal controls. Further, it appears that recognition of a food substance as novel is greatly hampered in the absence of normal olfactory functioning.

160.12 THE EFFECT OF ANTERIOR TEMPORAL LOBECTOMY ON ENDOGENOUS EPs RECORDED DURING VERBAL RECOGNITION MEMORY TESTING. M.E. Smith, J.M. Stapleton, K.A. Moreno* and E. Halgren, V.A. Southwest Reg. Epilepsy Ctr.; Brain Research Institute, UCLA, Los Angeles, CA, 90024.

We have previously reported the sequence of medial temporal lobe evoked potentials (EPs) recorded from depth electrodes during a verbal recognition memory task (Smith et al., 1984, Soc Neurosci Abs 10:846). The present study reports the results of scalp recordings during the same memory task in epileptics who had undergone either a left (L, N=7) or right (R, N=10) unilateral anterior temporal lobectomy (ATL), and in normal control subjects (N=9). EEG was recorded simultaneously from 22 channels (bandpass .1 to 100Hz.) and digitized on-line. Behavioral measures indicated that L-ATLs had a lower hit-rate on the recognition memory test than the R-ATLs or controls. However their performance was still substantially above chance levels and improved at a normal rate as words became more familiar with repetition. There were no differences between groups in false alarm rates or reaction times. Comparison of peak measures of the averaged EP waveforms also indicated differences among the groups. A negativity peaking around 400 msec. was largest to non-repeated words, but this difference in amplitude between repeated words and foils was only significant in the control group. In both lesion groups this N400 component was relatively attenuated compared to controls. No significant topographic differences were noted. The N400 was followed by a broad positivity (P550) which was biggest to repeated words. This difference between non-repeated and repeated items was significant for controls and R-ATLs but not L-ATLs. The L-ATLs exhibited the greatest reduction in amplitude for this component. The P550 was largest posteriorly across subjects. Small topographic lateral asymmetries were also noted. The exogenous components were similar across groups. These waveforms are compared to those evoked in simple auditory P300 tasks in the same patients, which show little difference between lesion and control groups. These data suggest that the contribution temporal lobe sources make to the scalp-recorded endogenous EPs may be highly task specific.

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160.13 LONG-LATENCY COMPONENTS OF EVENT-RELATED POTENTIALS (ERPs) RECORDED FROM MONKEYS IN PASSIVE AND ACTIVE PARADIGMS DEMONSTRATE SIMILARITIES IN MORPHOLOGY AND FUNCTIONAL PROPERTIES TO THOSE RECORDED IN HUMANS. J. Pineda*, S. Foote, and H. Neville. Depts. of Neuroscience and Psychiatry, Univ. of California, San Diego, Salk Institute, and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

A number of studies have revealed similarities between ERP components obtained in some animal species and those recorded in humans. In order to investigate whether long-latency components in monkey ERPs exhibit morphological and functional properties similar to those recorded in humans, ERPs were obtained from the brain surface of seven chair-restrained squirrel monkeys (Saimiri sciureus) chronically implanted with skull-screw electrodes at midline (Fz, Cz, Pz) and lateral (F3, F4, T3, T4, P3, P4) sites. Free field auditory stimuli (2KHz and 6KHz tones, 40 msec, 60 db above threshold) were presented in two types of paradigms resembling those in which specific ERP components have been studied in humans.

In the first paradigm, the same tone (either 2 KHz or 6 KHz) was presented on average every six seconds. A light signaled "time-in" periods, during which bar-press responses to the tones, occurring within a specific latency window, were rewarded, while no rewards occurred during "time-out". ERPs to the "time-in" tones exhibited a large, frontally distributed negativity that resembled the "O" (for Orienting) wave recorded in humans during the presentation of the same stimuli with long interstimulus intervals (Rohrbaugh and Gaillard, 1983).

In the "oddball" paradigm an active and a passive condition were used. A series of tones (2 KHz and 6 KHz) was presented with one tone occurring frequently (p=0.9) and the second one less frequently (p=0.1). In the active condition subjects were trained to respond to the less frequently occurring tone by pressing a lever to get a reward. In both conditions a large, positive polarity component (P3) was elicited by the infrequent tone. This component appears to be affected by changes in global stimulus probability and moment-to-moment variations in sequential order but not by variations in stimulus pitch or intensity.

These results suggest that long-latency components of monkey ERPs demonstrate morphological and functional properties similar to human long-latency components in analogous paradigms. Since components such as the P300 have been shown to covary with attentional, mnemonic, and semantic variables, these studies provide an animal model in which to investigate the mechanisms of these specific neural events.

ENDOCRINE CONTROL OF DEVELOPMENT I

161.1 ENVIRONMENTAL, BUT NOT SEX, DIFFERENCES EXIST IN THE GROSS SIZE OF THE RAT CORPUS CALLOSUM. J.M. Juraska and M. Meyer*. Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

Sex differences have been documented in the splenium of the human corpus callosum where females have a larger cross-sectional area than males (deLacoste-Utamsing and Holloway, Sci.,216:1431, 1982). An animal model for this dimorphism would be useful to understand its fine structural basis and possible hormonal origins. Thus we examined the rat for sex differences in the corpus callosum. However, we have previously reported that sex differences in the dendritic tree of visual cortical and hippocampal dentate neurons were dependent on the rearing environment. Szeligo (Anat. Rec.,187:726, 1977) has reported that rats raised in a complex environment have thicker white matter in the visual cortex than isolated rats. Therefore, we examined the size of the corpus callosum in rats of both sexes raised for one month post-weaning in either a complex (with toys and other rats) or an isolated environment. Measurements were taken in a manner similar to deLacoste-Utamsing and Holloway (1982) from projected slides of mid-sagittal, Weil stained sections from 10 litters.

No sex differences were found. Rats of both sexes that were from the complex environment had significantly larger (15-17%) middle and posterior (splenium) thirds of the corpus callosum than isolated rats. The anterior third was marginally in the same direction. While these results indicate that the rat will not serve as an animal model of human callosal dimorphism, they do demonstrate that the environment can alter the size of a major fiber pathway.

161.2 DENDRITIC PLASTICITY IN THE ANTERIOR CINGULATE CORTEX IN RESPONSE TO DIFFERENTIAL ENVIRONMENTS: SEX AND HEMISPHERIC DIFFERENCES. G. Carty, J.M. Juraska and D.L. Washburne*(SPON: G. Frommer). Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

We have found that the sexes differ in dendritic responses in the visual cortex and hippocampal dentate gyrus to rearing in complex and isolated environments. The anterior cingulate cortex may also be sexually dimorphic since it is reciprocally connected to both the visual cortex and dentate gyrus (through the entorhinal cortex) and contains a sizable number of estrogen receptors during development. An overall size asymmetry has also recently been reported. Therefore, we quantified the dendritic field in layer II-III pyramidal neurons in the anterior cingulate cortex (area 24b) in 6 litters of hooded rats of both sexes that were raised for one month postweaning in either a complex (with objects and other rats) or isolated environment. Neurons were sampled from coronal sections of Golgi-Cox stained tissue.

A concentric ring analysis revealed that the hemisphere in which the environmental effects were most pronounced varied with the sex of the animal (i.e., significant three-way interactions between sex, environment and hemisphere). In both the basilar and apical dendritic tree females showed greater differences between the environmental conditions in the right cortex and males showed greater differences in the left. Sex differences were most pronounced in the right hemisphere of the isolated rats where males had more dendritic material than females. In the left hemisphere, the presence and direction of sex differences were more variable. Further analyses examining the dendritic branching patterns in these groups are currently being performed. Although there were no hemispheric differences in the hippocampal dentate gyrus, the pattern of sex and environment differences in the right anterior cingulate cortex are similar to those previously found in the dentate granule cells.

161.3 HORMONAL CONTROL OF NEURON NUMBER IN SEXUALLY DIMORPHIC SPINAL NUCLEI IN THE RAT. D.R. Sengelaub, E.J. Nordeen, K.W. Nordeen*, and A.P. Arnold, Dept. of Psychology, UCLA, Los Angeles, CA 90024

The sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) contains many more motoneurons in male rats than in females. Androgens produce this sex difference by regulating normally-occurring cell death, and perinatal treatment with testosterone propionate (TP) attenuates this death in females (Nordeen et al., 1984). Prenatally, before the differential decline in motoneuron number and development of the large sex difference, SNB motoneuron number increases dramatically. We have hypothesized that this increase is due to the migration of motoneurons into the SNB from the neighboring dorsolateral nucleus (DLN) (Sengelaub and Arnold, 1984), which is also sexually dimorphic. We thus examined the early development of the DLN in relation to the developing SNB in an effort to understand how androgens regulate cell survival and/or migration.

Timed pregnant females (Sprague-Dawley) received either 2 mg/day of TP in oil between embryonic days (E)16-22 or were left untreated (E23= day of birth= postnatal day (P)1). Pups born to TP-treated dams were cross-fostered to other lactating females and injected with 1 mg of TP on P1, P3, and P5. Lumbar spinal cords from males, females, and androgenized females (TP-female) were obtained at E18, E20, E22, P4, and P10, embedded in paraffin, sectioned and stained with cresylecht violet. DLN motoneurons were counted and corrections were made for split nucleoli.

At E18, when the number of motoneurons in the SNB is quite small, the DLN of all groups contains substantial numbers of motoneurons (1192-1244). However, by E22 when SNB motoneuron number for all groups has reached its maximum, the number of motoneurons in the DLN has declined, and males and androgenized females have significantly more DLN motoneurons than females (1140 vs. 884). DLN motoneuron number continues to decline to P10, especially in females, who lose 74% of their motoneurons compared to 54% in males. By P10 motoneuron numbers in males and females have reached their adult levels (552 vs. 320) and androgenized females do not differ significantly from males (596).

These results are consistent with the hypothesis that the SNB motoneurons originate in the DLN and migrate into position between E18 and E22. However, degenerating cells can also be seen in the DLN throughout this period, and thus it is possible that the decline in motoneuron number is the result of cell death. Furthermore, sex differences in DLN motoneuron number develop principally in the early postnatal period through a differential cell loss which can be attenuated by androgens, suggesting as in the SNB, an androgen regulated cell death.

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161.4 ANDROGENIC REGULATION OF DENDRITIC ARBOR IN A SEXUALLY DIMORPHIC RAT SPINAL NUCLEUS. E.M. Kurz, D.R. Sengelaub, and A.P. Arnold, Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The spinal nucleus of the bulbocavernosus (SNB) contains many more motoneurons in adult male rats than in females. The SNB innervates two sexually dimorphic muscles in the perineum, the bulbocavernosus and levator ani, and both the SNB motoneurons and their target muscles accumulate androgens. Castration of adult males results in a 13-17% decrease in SNB motoneuron soma size, but soma size can be maintained or returned to normal by treatment with testosterone. We examined the dendritic arbor of SNB motoneurons to determine whether testosterone's effect on soma size was also reflected in changes in the dendritic arbor following castration and hormone replacement.

Because it was necessary to examine the arbor of a comparable set of motoneurons in all groups over time, we used choleratoxin-horseradish peroxidase (CT-HRP) to retrogradely label only those motoneurons projecting to the bulbocavernosus. All injections consisted of .5 ul of .2% CT-HRP into the bulbocavernosus, which fills SNB motoneurons and their processes extensively, allowing a comprehensive description of the dendritic arbor of these cells. Intact male rats (Sprague-Dawley) were injected with CT-HRP at 8.5 or 18 weeks of age and males castrated or sham-castrated at 8.5 weeks were injected at 14 weeks. An additional set of animals castrated at 8.5 weeks, and implanted at 14 weeks with Silastic capsules either containing testosterone or left blank, were injected at 18 weeks. All rats were sacrificed 48 hours after injection and the spinal cords were removed, sectioned at 40 um, and processed with TMB. To estimate the length of SNB cell processes, every fourth section through the SNB region was examined under darkfield illumination and all HRP-filled fibers were drawn with a camera lucida. The lengths of all filled fibers were then measured and summed. The number of labeled cells and density of their labeling was also determined.

While it is possible that HRP transport efficiency was affected by the androgen depletion in castrates, we found no differences in the density of HRP labeling, or the maximal distance from the SNB at which labeled fibers could be observed. The length of the dendritic arbor per filled cell in intact males and sham castrates was similar, ranging from 4400-5500 um. At 14 weeks (5.5 weeks post-castration) dendritic length in the castrates was decreased to less than 50% of normal or sham levels. Castrated animals treated with testosterone for 4 weeks showed a substantial increase (50%) in dendritic length over that of castrates. These results suggest that androgens are critical for the maintenance of dendritic arbor in SNB motoneurons. (Supported by USPHS HD15021)

161.5 BRAIN GROWTH AND BEHAVIOR IN DEVELOPING FULLER BWS MICE: EFFECTS OF THYROID HORMONE. R. Benno, D. Desroches*, M. Hahn* and J. Salinas*. Dept. of Biol. Wm. Paterson College of N.J. 07470

Previous studies performed on Fuller Brain Weight Selected (BWS) mice have shown that the H-line (heavy brains) and L-line (light) differ on a variety of developmental tasks including surface righting and rotor rod riding ability, with L mice developing more rapidly (Chen and Fuller, Dev. Psychobiol., 8(4):355,1975). It was also shown that thyroid hormone (Th) administration decreased brain size and sped up development of behavior in both H and L mice. Our study investigated whether Th administration to H mice would make them similar to the L-line mice in terms of brain structure and behavior. We specifically focused on cerebellar related behaviors, e.g. righting reflex and rotor rod riding because of differences we have recently found in cerebellar foliation pattern between the two lines.

Three groups of H mice received daily subcutaneous injections of either 0.5 or 1.0µg L-thyroxine (Th-.5 and Th-1 groups) or saline (control), from day 1 to day 20. All were tested for surface righting and rotor rod riding ability, and the day of eye opening was recorded. On day 21, all mice were killed, total body, thyroid and brain weighed. Brains were immersion-fixed in formalin, cut in the mid-sagittal plane, stained with cresyl violet and analyzed for differences in cerebellar foliation patterns. The 1 µg treatment was effective in changing body and brain size. Th-1 mice had lower body weights ($p<.01$) and brain weights ($p<.01$) than the controls. The 0.5µg Th treatment had similar but smaller effects. Thyroid weights were unaffected by Th treatments. Brain partitions and anatomical features were also affected by Th treatments, particularly by 1.0µg Th. Th-1 mice had decreased total brain length ($p<.05$), cortical length ($p<.001$) and cerebellar width ($p<.002$). The number of cerebellar folia were also significantly reduced ($p<.0001$). Behavior was also found to be sensitive to Th treatments. All Th injected mice took less time to meet criterion for righting reflex ($p<.0003$) and rotor rod ($p<.02$). In addition, Th injections sped up the time of eye opening ($p<.0001$).

In conclusion, Th injections in the Fuller BWS H-line mice were shown to be effective in decreasing total body and brain size as well as in altering the cerebellar foliation pattern. In addition, the hormone was able to speed up the development of behavioral tasks dependent on a functional cerebellum possibly by increasing the rate of cellular maturation. These changes in brain structure and behavior make the H mice appear similar to control L mice both physically and behaviorally. It is therefore possible the Th sensitivity is involved in selective breeding for brain size. This hormonal difference may account for some of the differences in brain structure and development of behavior.

161.6 MORPHOMETRIC ANALYSES OF ALTERATIONS IN THE HYPOGASTRIC GANGLION SUBSEQUENT TO NEONATAL CASTRATION: ANDROGEN RECEPTOR REGULATION? J.E. Melvin, T.H. McNeill and R.W. Hamill. Depts of Anatomy and Neurology, Monroe Community Hospital, Univ. of Rochester Medical Center, Rochester, NY 14642.

Neonatal castration of male rats inhibits the development of postsynaptic tyrosine hydroxylase (T-OH) activity and diminishes the development of both presynaptic choline acetyltransferase (CAT) activity and total ganglion protein in the sympathetic hypogastric (HG) ganglion (Society for Neuroscience Abstract 10:457, 1984). To determine if the loss of neurotransmitter synthesizing enzyme activities and ganglion protein reflects decreased cell size and/or cell loss, morphometric analyses were performed. Additionally, cytosolic androgen receptor binding in the HG was studied according to the methods of McGinnis et al. (Brain Res. 275:75, 1983).

Neonatal male rats were castrated at 10-11 days of age and sham-operated littermates served as controls. Castration resulted in a significant decrease in both nuclear and cell body cross-sectional areas (nucleus, control $68 \pm 4\ \mu^2$, castrated $31 \pm 3\ \mu^2$, $P<.001$; cell body, control $293 \pm 17\ \mu^2$, castrated $124 \pm 12\ \mu^2$, $P<.001$). In contrast, there was no significant loss in the estimated total nerve cell population subsequent to neonatal castration (control 3700 ± 173, castrated 3262 ± 290).

To examine possible direct interactions between androgens and neurons of the HG, cytosolic receptor characteristics were studied. Castrated and adrenalectomized male rats, 65-75 days old, were maintained on 0.9% saline for 48 hours prior to assay. The HG and pituitary (Pit), which was previously shown to contain androgen receptors (McGinnis et al., 1983), were removed and immediately frozen in liquid nitrogen. HG and Pit from 18-21 rats were pooled for each assay and 4 nm ^{3}H-R1881 used as ligand. Competition studies suggested specific binding (10^{-9} - 10^{-5} M). DHT and testosterone inhibited receptor binding in the HG in a similar manner as the Pit. Corticosterone showed no tendency to compete with receptor binding, while progesterone and estradiol only competed at the highest concentrations.

These results suggest that the diminished development of presynaptic CAT activity and total ganglion protein in the HG is related to decreases in the size of neurons and not to the loss of neurons. Additionally, the lack of cell loss indicates that the inhibition of postsynaptic T-OH activity would reflect a loss of enzyme activity per cell. Furthermore, the presence of specific cytosolic androgen receptors in the HG possibly implies that altered morphological and biochemical development following neonatal castration is at least partially due to the loss of direct hormonal regulation.

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161.7 SEX DIFFERENCES IN NEURONAL NUMBER OF TRIGEMINAL MOTOR NUCLEUS IN GUINEA PIGS BUT NOT RATS. Kate Townsend-Merino & S.M.Breedlove. Psychology Dept., U. of California, Berkeley, CA 94720.

In guinea pigs the temporal muscle is much larger in males than in females and castration of males drastically reduces masticatory muscle size. In fact, of the 46 muscles tested only the temporal, digastric and masseter respond as strongly to androgens as do perineal muscles. Masticatory muscle changes in castrated rats are merely proportional to changes in body weight (C.D.Kochakian, I. Pharm. Ther. B., 1 149). In rats the sexual dimorphism in perineal musculature is accompanied by a sex difference in the number and size of innervating motoneurons. We now report a sex difference in the number of neurons in the trigeminal motor nucleus (MoV) innervating the sexually dimorphic masticatory muscles of guinea pigs.

The brains of 5 male and 5 female age-matched (57-60 days) Simonsen-Hartley guinea pigs were frozen sectioned and alternate 40 µm sections were thionin stained. MoV was examined bilaterally for the number of motoneurons with visible nuclei in the dorsal and ventral subpopulations, as well as overall MoV. Cell counts were corrected for split-nuclei error. The temporal muscle is the most highly androgen sensitive of the masticatory muscles, so the magnitude of any sex difference might be greater in the dorsal half of MoV, which contains temporal motoneurons.

There were sex differences in the number of motoneurons in the dorsal half ($p<.05$, t-test), ventral half and the entire MoV nucleus ($p<.01$):

Mean # ±S.E.M.:	Dorsal half	Ventral half	Overall MoV neuron #
MALES:	1006.7 ±49.6	1254.0 ±37.3	2260.9 ±76.6
FEMALES:	793.0 ±20.5	913.0 ±32.6	1706.4 ±43.4

Male Hartley guinea pigs begin to outweigh females at 45 days. Accordingly, males were heavier than females in the present study, but there was no significant correlation within either sex between body weight and the number of MoV motoneurons (Males: r= -.09; Females: r= -.33). Rats also display a sex difference in body weight, but we found no sex differences in the number of MoV neurons in rats (M: 1292.5 ±50.5; F: 1125.6 ±63.8, N=16, $p>.05$). Guinea pig motoneurons in the dorsal half of MoV are larger than those in the ventral half, therefore the cross-sectional areas of 15 somata from each anatomical location were measured from each animal. No sex differences in mean cell size were found in either the dorsal or ventral portion of MoV:

MALES: Dorsal, 929.0 ±26.8; Ventral, 636.5 ±21.8 µm2.
FEMALES: Dorsal, 925.4 ±80.8; Ventral, 675.1 ±17.7 µm2.

There was no difference in neuronal density (# MoV neurons/total MoV volume) between males and females. However, there was a significant sex difference in the total volume of the MoV nucleus (M: 1.399 ±.05; F: 1.229 ±.01 mm3, $p<.05$), apparently because of a greater rostral-caudal extent in males. The sex difference in the number of MoV motoneurons and the androgen sensitivity of their target muscles suggest a sex-related behavioral function of these muscles.

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161.8 NEONATAL ANDROGEN MAINTAINS SEXUALLY DIMORPHIC PERINEAL MUSCLES IN THE ABSENCE OF INNERVATION. Renata B. Fishman and S. Marc Breedlove, Department of Psychology, University of California, Berkeley, CA 94720.

The spinal nucleus of the bulbocavernosus (SNB) and its target muscles, the bulbocavernosus (BC) and levator ani (LA) are present in adult male, but not female, rats. SNB motoneurons and the BC/LA muscle complex are present in females at birth but disappear by the third week of life. Neonatal testosterone propionate (TP) treatment maintains SNB motoneurons and prevents involution of the BC/LA muscles. It is not yet determined whether androgen's primary site of action is the SNB motoneurons or BC/LA muscles. The purpose of the present study was to choose between these two alternatives by determining whether SNB motoneurons are necessary for the testosterone induced sparing of BC/LA muscles. We now report that removing SNB innervation to the BC/LA by neonatal lumbosacral spinalectomy does not prevent the androgenic maintenance of these muscles.

Sprague-Dawley female rats aged 0-24 hours received either a lumbosacral spinalectomy or sham operation. Spinalectomies were performed under a dissecting microscope by removing the dorsal surface of 2-3 mid-thoracic vertebrae, inserting a hypodermic needle down the vertebral column, and aspirating the entire lumbosacral spinal cord. Either TP (1mg) or sesame oil vechicle (.05cc) was injected s.c. immediately following surgery and again on the third day of life. At 26-30 days, rats were sacrificed and their spinal cords and perineal muscles were examined. Inspection of spinal cords indicated all lumbosacral spinalectomies were complete. BC/LA muscles were present in all TP (N=6), but none of the oil treated (N=6), spinalectomized females.

Additional experiments were conducted to determine whether or not BC/LA muscles were innervated by neurons replacing SNB cells. Spinalectomized (N=5) and sham operated (N=4) females were given additional TP on days 26 and 28 of life to increase muscle mass. Subsequently, perineal muscles were removed at day 30 and examined for motor endplates with either flourescently labeled alpha bungarotoxin (Sigma, N=3 spinalectomized and 2 sham) or an acetylcholinesterase stain (N=2 spinalectomized and 2 sham). Male BC/LA muscles were stained concurrently for comparison. The BC/LA muscles of both males and sham operated, TP treated females showed motor endplate band formations characteristic of innervated muscle. However, BC/LA muscles of spinalectomized animals showed only diffuse labeling with no evidence of a motor endplate banding pattern.

These results demonstrate that BC/LA muscles survive when neonatal females are treated with androgen immediately following complete denervation. Thus androgen can spare BC/LA muscles in the absence of SNB motoneurons, suggesting that androgen's primary site of action in maintaining SNB system structures may be the BC/LA muscles themselves.

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161.9 ONTOGENY OF FUNCTIONAL INNERVATION OF BULBOCAVERNOSUS MUSCLES IN MALE AND FEMALE RATS. Mark N. Rand & S. Marc Breedlove. Dept. Psychology, U. of California, Berkeley, CA 94720.

In male rats, the bulbocavernosus and levator ani muscles (BC/LA) are innervated by the spinal nucleus of the bulbocavernosus (SNB). Adult female rats lack both the SNB and its target muscles. However, both the SNB and its BC/LA targets are present in females at birth. Furthermore, the injection of retrograde markers into the BC labels SNB cells in newborn females, indicating that the motoneuron axons have reached their target. Nonetheless, both SNB cells and BC/LA muscles involute in females unless they are perinatally treated with androgen. It has not yet been determined how androgen maintains the SNB system, but a reasonable hypothesis is that androgen triggers or accelerates the maturation of the neuromuscular junction, and thereby prevents the dissolution of motoneurons and target. One measure of the status of the neuromuscular junction is the ability of SNB motoneurons to elicit contractions in BC/LA muscles. We have found that electrical stimulation of the appropriate ventral root elicits contractions of BC muscles in female rats perinatally.

Cesarean sections were performed on timed-pregnant Sprague-Dawley rats (Simonsen) under urethane anesthesia (1.6g/kg, im). Fetuses were rapidly decapitated and their viscera removed. The bodies were placed in a chamber with oxygenated Ringer's solution and carefully dissected under a microscope to reveal BC/LA and related structures. Ability of the muscles to contract could be confirmed by direct electrical stimulation. The ventral midline of the vertebral column was split to reveal the spinal cord, allowing access to the ventral roots. Proximal connections of individual ventral roots were cut and the roots were drawn into a suction electrode for electrical stimulation. Contraction of the BC muscles could be directly observed through the dissecting microscope, and was sometimes accompanied by isolated, ipsilateral deflection of the phallus to which the muscles attach. In some cases d-tubocurarine (Sigma) was added to the bath (.16mg/ml) to reversibly block BC/LA contractions.

BC/LA contraction could be reliably elicited in all newborn male rats by electrical stimulation of the sixth lumbar ventral root (L6). Stimulation of L5 and S1 also elicited weak contractions. Contractions of BC were observed as early as day 20 of gestation (d20g) in males. By d21g, medium to large contractions could be elicited in all males, and small but reliable contractions could be driven in the BC of females. On d22g all males showed strong responses and in 5 out of 5 females BC also clearly contracted following electrical stimulation of L6. By d23g (day of birth) BC contractions were weaker and less reliably elicited in female rats.

Our studies indicate that the BC/LA muscles of perinatal females can be functionally driven by SNB cells, yet both motoneurons and their target muscles will die without androgen. Thus it seems unlikely that androgen spares the SNB system by establishing functional contact between them, since that occurs to some extent in normal females. Apparently androgen averts dissolution of the SNB system by some other mechanism.

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161.10 SEXUAL DIMORPHISM IN ONUF'S NUCLEUS OF DOGS: ANDROGEN REGULATION OF MOTONEURON NUMBER. F.A.Beach*, Nancy G. Forger & S.M.Breedlove (Spon:I.Zucker). Psych. Dept., U.C.Berkeley, CA 94720.

Onuf's nucleus in dogs, cats and primates is a slender column of small to medium-sized motoneurons in the ventrolateral aspect of the ventral horn in segments 1-2 of the sacral spinal cord. Retrograde labelling studies in cats and dogs demonstrate that the neurons in Onuf's nucleus innervate striated perineal muscles, including the external anal sphincter, ischiocavernosus and bulbocavernosus muscles. Onuf's nucleus may, then, be homologous to the spinal nucleus of the bulbocavernosus (SNB) and the dorsolateral nucleus (DLN) in rats which innervate the bulbocavernosus and ischiocavernosus muscles, respectively. In rats, the number of SNB and DLN motoneurons is greater in males than in females, which lack the target muscles. Perinatal androgen treatment of female rats spares SNB and DLN motoneurons as well as their target muscles. Here we describe a similar sexual dimorphism in Onuf's nucleus of dogs, and report that early androgen treatment of females reduces the dimorphism.

Spinal cords from purebred beagles were examined. Normal females (N=4) and males (N=4) were compared with five androgenized females born to dams that received 20 daily injections of testosterone propionate (1.1 mg/kg dam's body weight) during the middle third of pregnancy (day 24-43 post coitum). Four of these five treated females also received a 37.5 mg subcutaneous implant of crystalline testosterone on the day of birth. The dogs were sacrificed as adults (age 3-6yrs), and perfused with saline and formalin. The spinal cords were paraffin-embedded, serially sectioned at a thickness of 15 µm and treated with a Klüver-Barrera stain. As reported by others, Onuf's nucleus stands out sharply in this stain as a pale area demarcated from the surrounding network of myelinated fibers. Bilateral counts in every third section were made of all motoneurons within this pale, ovoid area. The resulting sum for each dog was tripled to estimate the total number of Onuf's nucleus motoneurons.

Male dogs had significantly more neurons in Onuf's nucleus than did females ($p<.02$, t-test). Androgen treated females were phenotypically masculinized; external genitalia consisted of a male-like phallus and no external vagina. Similarly, the number of motoneurons in Onuf's nucleus of androgenized females was not different from that of males ($p>.05$), and was greater than the number of motoneurons in normal females ($p<.05$).

	Males	Females	Androgenized Females
Mean # neurons ±SEM:	1407 ±133	823 ±122	1134 ±56

The sex difference in the number of perineal motoneurons is less pronounced in dogs than in rats, perhaps because female dogs normally retain perineal muscles in modified form. These results suggest that, as in rats, the number of motoneurons innervating dog perineal muscles is under early androgen control. Androgen rescues motoneurons of the rat SNB during the period of normal cell death; it may be anticipated that a similar mechanism operates on the neurons of Onuf's nucleus in dogs.

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161.11 SEXUAL DIMORPHISM IN ONUF'S NUCLEUS OF HUMANS. Nancy G. Forger and S. M. Breedlove. Dept. Psychology, U.C. Berkeley, CA 94720.

Onuf described a spinal nucleus which, based on human neurological data, he believed to innervate striated perineal muscles. Onuf's nucleus is morphologically very similar in humans, cats, dogs and monkeys, consisting of small to medium-sized motoneurons in the ventrolateral aspect of the ventral horn of the sacral spinal cord. Retrograde labelling studies in cats and dogs confirm Onuf's suggestion that the nucleus innervates the external anal sphincter, m. bulbocavernosus and m. ischiocavernosus. We have recently demonstrated an androgen-regulated sexual dimorphism in Onuf's nucleus of dogs: males and androgenized females have a significantly greater number of motoneurons than do normal females (adjoining abstract). A sex difference in the number of HRP labelled pudendal motoneurons in Onuf's nucleus has also been described in macaque monkeys (Ueyama et al. JCN 232 '85). In the current study, we sought to establish whether a similar dimorphism exists in Onuf's nucleus of humans.

Spinal cords of 8 human females and 9 males from the Yakovlev collection of the Armed Forces Institute of Pathology were examined. Ages at autopsy ranged from 2-87 years in females (mean ± SEM, 22 ±10 yr), and 1.5 mos-60 yr in males (22 ±8 yr). Each cord was embedded in celloidin, cut coronally at 35 µm, and every 5th (N=1), 10th (N=10) or 20th (N=6) section was mounted and Nissl-stained. Adjacent Weigert-stained sections were also available to aid nucleus identification. The total number of Nissl-stained neurons in Onuf's nucleus for each subject was adjusted according to the sampling ratio in order to estimate the total motoneuronal number. In accord with previous reports, distinct dorsomedial (DM) and ventrolateral (VL) cell groups could be seen in Onuf's nucleus of humans. VL cells were small, dark and uniformly present, while medium-sized intermittently present cells characterized the DM division.

A sex difference was observed in the number of motoneurons of the VL group; males had significantly more motoneurons than did females ($p<.025$, t-test). The number of DM neurons was small and no sex difference was seen in this division or in the total number of motoneurons ($p>.05$).

Mean # neurons ±SEM	VL	DM	Total
Males:	2032 ±121	473 ±138	2505 ±212
Females:	1569 ±142	533 ±110	2102 ±165

A single additional spinal cord was examined in a case of amyotropic lateral sclerosis (female). As previously reported, the cells of Onuf's nucleus were preserved, despite widespread loss of other motoneurons.

The VL portion of Onuf's nucleus in cats innervates the ischiocavernosus. Onuf's nucleus is not present in rats, however in the homologous nuclei innervating the ischiocavernosus and bulbocavernosus muscles a sex difference in the number of motoneurons, favoring males, has been established. Perinatal androgen treatment of female rats reduces the dimorphism by sparing motoneurons from death during development. The present observation of a sex difference in motoneuronal number in VL Onuf's nucleus is the first description of sexual dimorphism at the cellular level in the human CNS, and one for which well established animal models are available. Supported by NIH grant # NS19790.

161.12 NEURAL CONTROL OF LORDOSIS AND EAR WIGGLING IN 6-DAY-OLD RATS. C.L. Williams and D. Lorange. Dept. of Psychol., Barnard College, Columbia Univ., NY, NY. 10025.

Six day old rats display lordosis and ear wiggling in response to tactile stimulation of the flanks and rump. These precocious behaviors are displayed by both male and female infants when they are deprived of their dam for 3-4 hrs and tested at (33 ± 2°C). The involvement of various brain regions in precocious lordosis and ear wiggling was examined by making transections along the neuraxis from the anterior hypothalamus to the pons in 6-day-old pups.

The transections were made by inserting a 30-ga., blunted, hypodermic needle through a hole in the skull on an angle so that it could be drawn laterally across the brain. A control operation was carried out in male and female pups from each litter; a hole was placed in the skull near lambda but no transection was made. Following the transection procedure, pups were placed in individual plastic tubs in an incubator for 4 hrs. Behavioral testing was conducted by stroking pups on the flanks and lower back for 30 sec every 30 min for 2 hr, and tests were videotaped. Following testing pups were sacrificed and the brains were sliced and stained. Sagittal sections were examined microscopically to determine the locus and extent of the brain cuts.

Using these techniques, we found: 1.) Cuts through the pons completely eliminated lordosis and severely reduced ear wiggling in both male and female rats. 2.) Cuts in the mesencephalon slightly reduced the frequency, duration and intensity of lordosis and the frequency of ear wiggling, however, this may have been due to an overall reduction in activity in these animals, rather than a specific effect on the neural systems underlying precocious sexual behaviors. 3.) Lordosis was severely reduced in pups with cuts in the diencephalon, while ear wiggling and overall activity were relatively normal.

These data suggest: 1.) In the infant as in the adult, supraspinal facilitation is required for the display of sexual responses. 2.) Hindbrain postural control centers support lordosis in the absence of input from the midbrain and forebrain. 3.) Hypothalamic facilitation in the infant occurs without the addition of exogenous estrogen.

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161.13 EFFECT OF PRENATAL ESTROGEN DEPRIVATION ON THE DEVELOPMENT OF FEMININE SEXUAL BEHAVIOR IN FERRETS OF BOTH SEXES. M.J. Baum, S.A. Tobet and L.A. Lundell*, Dept. of Biology, Boston University, Boston, MA 02215

We tested the unorthodox hypothesis that estrogenic stimulation of the perinatal brain is required for the development of feminine proceptive and receptive coital capacity. On gestational day 30 pregnant ferrets were either ovariectomized and implanted s.c. with a Silastic capsule containing the aromatase inhibitor, 1,4,6-androstatriene-3,17-dione (ATD) or with ATD plus estradiol (E_2). A third group of pregnant ferrets was sham-ovariectomized. All groups received a Silastic capsule containing progesterone. Litters were delivered by cesarian section on gestational day 42. All offspring were cross-fostered to lactating mothers prior to being gonadectomized on postnatal day 5. In adulthood animals were injected s.c. daily with different dosages of estradiol benzoate (EB; 0,5,10 and 15 ug/kg) prior to tests of proceptive (approach latencies in a runway) and receptive (acceptance of neck grip) responsiveness to a sexually-active male ferret.

Ovariectomy and administration of ATD to pregnant ferrets caused significant reductions in plasma E_2 and hypothalmic aromatase activity in fetal offspring killed on gestational days 37 or 41.

Female ferrets displayed similar dose-dependent reductions in latency to approach a male after adult EB treatment (proceptivity), regardless of whether they had been deprived prenatally of estrogen. Normally male ferrets are proceptively defeminized, i.e., they show no EB-induced reduction in latency to approach a stimulus male. Prenatal estrogen deprivation caused males to run faster to a stimulus male in response to adult EB. This suggests that prenatal estrogenic stimulation normally contributes to proceptive defeminization in male ferrets.

Females deprived prenatally of estrogen were significantly less receptive than control females when tested with the 2 lower dosages of EB. This effect was reversed in females whose mothers received ATD + E_2. However, females in all three groups were equally receptive in response to the highest dosage of EB. Previous work showed that male ferrets are as receptive as females after gonadectomy and EB treatment in adulthood. Males deprived prenatally of estrogen were less receptive than control males in response to the lowest EB dosage whereas males exposed prenatally to ATD + E_2 were more receptive than controls in response to all three adult dosages of EB. The results suggest that estrogenic stimulation of the fetal brain enhances later receptive responsiveness to E_2; however, prenatal estrogen is not an absolute requirement for the development of receptive or proceptive coital capacity in ferrets of either sex.

(Supported by HD-13634, MH-00392, and MH-08937)

161.14 SEXUALLY DIMORPHIC NUCLEUS OF THE MALE FERRET PREOPTIC/ANTERIOR HYPOTHALAMIC AREA IS DEPENDENT UPON PRENATAL EXPOSURE TO ESTROGEN S.A. Tobet, D.J. Zahniser* and M.J. Baum, Dept. of Biology, Boston University, Boston, MA 02215 and Image Analysis Laboratory, Tufts-NEMC Boston, MA 02111

Sex differences in brain morphology have been described in many species. In birds and rodents several sex differences in neural morphology depend on the developmental action of androgen or estrogen in the male. Similar developmental experiments have not been done in any higher mammalian species. In a carnivore, the ferret, a nucleus is present in the dorsal portion of the preoptic/anterior hypothalmic area (POA/AH) only in males. In the present experiments pregnant ferrets were administered different treatments to alter the fetal steroidal milieu. In an attempt to keep postnatal hormone exposure comparable in all groups, offspring were gonadectomized on day 5, the earliest age compatible with survival. After 18-20 days of testosterone-priming at 7-8 months of age, all animals were anesthetized with sodium pentobarbital before perfusion via the heart with saline followed by 10% neural buffered formalin. Coronal, 40-micron cryostat sections were cut through the POA/AH of all animals and stained with thionin. A bilateral, dorsal nucleus containing large cells, similar to that seen in males, was apparent in the POA/AH of female offspring of pregnant ferrets which received s.c. implants of testosterone over days 30-41 of gestation. A dorsal nucleus of the POA/AH was also present in males from litters treated with the anti-androgen, flutamide, over days 30-41 of gestation even though these males showed clear genital signs of androgen antagonism. By contrast, no dorsal nucleus was present in the POA/AH of males whose prenatal exposure to estrogen was reduced over days 30-42 of gestation by maternal ovariectomy and s.c. implantation of progesterone plus the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD). Male offspring of ovariectomized, ATD-treated mothers which received a low replacement dosage of estradiol also had no dorsal nucleus in adulthood. A higher dosage of estradiol was generally toxic; however, a dorsal nucleus was apparent in a single, surviving male whose mother received this treatment plus ATD. Thus, the development of a dorsal nucleus in the POA/AH of male ferrets depends upon prenatal exposure to estrogen derived form the neural aromatization of circulating androgen. Data from studies on the ferret as well as rodent species suggest that the neural aromatization of androgen to estrogen may be required in diverse orders of mammals for the development of sexually dimorphic features of the male forebrain.

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161.15 DIRECT EVIDENCE THAT MALE AND FEMALE CELL DEATH RATES DIFFER IN THE DEVELOPING ZEBRA FINCH SONG SYSTEM. J. R. Kirn and T. J. DeVoogd, Psych. Dept., Cornell Univ., Ithaca, NY 14853

The zebra finch song system is sexually dimorphic in cell number, density, and dendritic elaboration, resulting from hormonal influences early in development (reviewed by (DeVoogd, 1984). To elucidate possible mechanisms by which hormones act, we have begun to investigate the normal development of the song system at a cellular level. A ubiquitous feature of nervous system development, including the telencephalon, is cell death (Finlay & Slattery, 1983). This report describes cell death in 3 components of the song system: Hyperstraitum ventrale pars caudale (HVc), and its principle targets, Robustus Archistriatalis (RA) and Area X. In 10-20μ cresylecht violet stained coronal brain sections from 15, 19 and 25 day old (post-hatch) finches, all cells in three representative sections through HVc and RA were counted. Degenerating cells, recognized by their pyknotic, darkly staining appearance, were counted in these and 3 additional sections. The total number of degenerating cells was expressed as a fraction of normal cells. Since Area X was visible in males but not females at all ages examined, it was analyzed differently. In males, the position of X in Lobus Parolfactorius (LPO) was determined and this was used to define putative X in same-age females. In 3 sections, the entire LPO, including X, was scanned for degenerating cells.

In 19 day old male HVc, a cell death rate of 1.4 degenerating cells/1000 normal was observed. The rate in females was substantially higher (7.6/1000). At 25 days old, HVc cell death rates increased in both sexes, but again were higher in females (♂ = 5.2/1000 normal; ♀ = 12.2/1000). In contrast, RA cell death rates were lower and not dimorphic at either 19 or 25 days (19, 25 day ♂ = 0.91, 2.2/1000 respectively; ♀ = 0.7, 3.3/1000). These results demonstrate that a) as in the rat spinal cord (Nordeen, et al., 1984), HVc undergoes subtantially more cell death in female finches compared to males; b). High, dimorphic cell death rates in HVc are coincident with low nondimorphic cell death rates in RA suggesting a possible temporal asynchrony in the development of sex differences in these structures that parallels asynchronious developmental changes in the volumes of these nuclei (Bottjer, et al., 1984). At day 15 and 19 more degenerating cells (dc) were found in LPO of females than in males (day 15-19 males = 16-22 dc; females = 40-36 dc). Although some dying cells were within male area X or its putative location in females, the majority were located outside of X and highest densities were adjacent to the ventricular zone in medial and dorsomedial LPO. This region may partially overlap with known hormone-concentrating areas in adult finches (Arnold, et al., 1976). Thus, the present data might indicate the death of hormone-sensitive cells in medial LPO. Alternatively, the survival of migrating cells may be dependent on hormones. The absence of a male-like endocrine milieu in the developing female might then increase cell death so as to create a spatially distant sexual dimorphism in area X.

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161.16 MASCULINIZATION OF SONG-CONTROL NUCLEI MAY INVOLVE SELECTIVE RETENTION OF ANDROGEN TARGET CELLS. E.J. NORDEEN, K.W. NORDEEN* AND A.P. ARNOLD. DEPT. OF PSYCH, UCLA, LOS ANGELES, CA 90024.

Male zebra finches produce an androgen dependent courtship song that females lack. Consistent with this sex difference two neural song regions, magnocellular nucleus of the anterior neostriatum (MAN) and hyperstriatum ventrale pars caudale (HVc) have more androgen-accumulating cells in adult males than in females. Estradiol (E2) treatment of females shortly after hatching increases androgen target cell number in MAN and HVc to male-typical levels, and these masculinized females respond to androgens as adults with neural growth in song nuclei, and male-typical song. The critical period for this influence of E2 on androgen accumulation extends at least until 20 days after hatching, but not into adulthood. To help distinguish whether E2 masculinizes androgen accumulation in females by promoting the retention of androgen target cells or by promoting their development, we compared the numbers of androgen target cells present in MAN in 20-day-old females, and in adult females masculinized by E2 treatment on day 20.

Six females (ages 19-21 days) were implanted subcutaneously with silastic ropes containing 200 ug of E2. Three to six months later these E2 females and 4 normal females (ages 19-20 days) were gonadectomized. The following day, each bird was injected intramuscularly with 20 ng/gm body weight of 3H-dihydrotestosterone (DHT). The animals were decapitated 1 1/2 hours later and coronal brain sections (6 um) were processed for autoradiography. Reference sections (18 um) were taken at regular intervals and stained with thionin. Thin sections were exposed 4-8 weeks and stained with thionin. The reference sections indicated that in 3 of 6 E2-females, song nuclei were morphologically masculinized. The incidence of cells labelled by 3H-DHT or its metabolites was determined within MAN in these 3 masculinized females and in the juvenile females using the Poisson criterion. Neuronal density and total nuclear volume of MAN was also determined.

In E2-females 53% of MAN cells were labeled by 3H-DHT or its metabolites. This is significantly more than in normal adult females (17%, Soc. Neurosci. Abst., 10;454, '84). In juvenile females only 20% of MAN cells were labeled. However measurements of total nuclear volume and neuronal density indicated that MAN contains 3-4 times as many cells in juvenile females as compared to E2-females. Thus, young females possess more than enough androgen-accumulating cells in MAN than are necessary to account for the masculine pattern obtained in adult females treated with E2 early in life. These data are consistent with the hypothesis that during sexual differentiation, E2 regulates androgen target cell number in song related nuclei by promoting the retention of androgen-accumulating cells that would otherwise be lost.

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161.17 LECTIN BINDING TO SEXUALLY DIMORPHIC BRAIN REGIONS INVOLVED IN SONG LEARNING THE THE ZEBRA FINCH. A.W. Coquelin, E.J. Nordeen, K.W. Nordeen*, and A.P. Arnold. Depts. Anatomy and Psychology, Lab. Neuroendocrinology, Brain Research Institute, UCLA, Los Angeles CA 90024.

To find markers for specific cells or tissue components which are sexually dimorphic and may be involved in song learning in zebra finches, we have incubated zebra finch brain sections with peanut agglutinin (PNA), a lectin which binds specifically to d-galactose moieties of glycoproteins. We find that many song control brain regions are bound by this lectin, as are some other brain regions. Binding in the song system is most distinct in MAN (magnocellular nucleus of the neostriatum), a nucleus which seems crucial for juvenile song learning but not for maintenance of song in adult zebra finches (Bottjer et al., Science, 1984).

Adult zebra finches were perfused with buffered 4% paraformaldehyde or buffered 10% formalin. Brains were removed and stored in fixative at 4°C until sectioning with a vibratome or frozen-sectioning with a sliding microtome at 30-40 um. Sections were incubated with bovine serum albumin and then with biotinylated PNA (20 ug/ml) overnight. Using the ABC technique (Vector), the lectin was localized with biotinylated HRP and diaminobenzidine and was viewed under the bright field microscope. We compared staining in adult males, adult females, and adult females which had been masculinized at hatching by an implant of Silastic containing 40 ug estradiol (E2).

In general, PNA lectin binding was associated with neuropil and not with cell bodies. Within the telencephaic song system, very distinct binding was seen in MAN, Area X, hyperstriatum ventrale pars caudale (HVc), and robust nucleus (RA), with heaviest labeling in MAN and RA. Other song or auditory nuclei showed distinct but light labeling (field L, nucleus interface, nucleus ovoidalis, lateral mesencephalic nucleus dorsomedial nucleus of intercollicularis). Outside the song system, heavy binding was seen in various nuclei including the ectostriatum, medial habenula, septum, nucleus of the stria terminalis, preoptic area, hypothalamic nuclei, and pretectal nucleus. In several brain regions, PNA binding formed very clear rings which closely apposed the cell surface of specific neurons. For example in Area X, such dense rings surrounded large cells (12-15um diam) in males and E2 females, but not in untreated females. Thus, PNA may bind to a specific synaptic structure which is sexually dimorphic. Dense rings were also seen in other brain regions (e.g., gigantocellular reticular formation) in both sexes. This technique promises to be useful for recognizing sexually dimorphic tissue components to study their differentiation and functional significance.

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161.18 NEONATAL FOREBRAIN NOREPINEPHRINE(NE) AND REARING ENVIRONMENT INFLUENCE LASHLEY III MAZE PERFORMANCE IN RATS. DOPAMINE(DA) DEPLETION DOES NOT PROTECT AGAINST ISOLATION INDUCED MAZE DEFICITS. Matti Saari, Susan Murtha*, David Murray *, Ken Stange* and Bruce A. Pappas. Dept. of Psychol., Nipissing Univ. Coll., North Bay, Ontario, P1B 8L7, and Carleton Univ., Ottawa, Ontario, K1S 5B6.

We have previously suggested that neural and behavioural alterations induced by rearing environment may be mediated by central NE since NE depletion leads to lack of sensitivity to rearing conditions (O'Shea et al., Europ. J. Pharmacol., 1983).

We report on two experiments showing that: 1) neonatal NE depletion reduces the debilitating effects on maze performance of several kinds of rearing environments and 2) the debilitating effect of the isolation rearing condition is unaffected by neonatal DA depletion.

In experiment 1 newborn male Wistar rats were injected subcutaneously (s.c), either with 6-hydroxydopamine (6-OHDA, 50mg/kg) or vehicle (Veh, 1.0 mg/ml ascorbic acid in saline) twice within the first 24 hours after birth and cross fostered. At 24 days of age the rats were reared for 35 days in one of four environments: 1) large colony cages with rotated toys and daily 30 minute exposures to an open field 2) large colony cages with handling 3) large colony cages with no handling 4) isolated and undisturbed in single cages. At 60 days the rats were tested on four Lashley III mazes. Five trials per day were run with computer controlled randomization and data collection. Analysis of variance of the log median daily latencies revealed a significant drug treatment by environment by day interaction. This arose from the superior performance of the NE depleted rats in comparison to the vehicle injected rats from all but the enriched rearing conditions.

In experiment 2 one group with bilateral intraventricular injections of 6-OHDA (50 ug. in .5 ul. ascorbic acid/saline vehicle) and s.c. desmethylimipramine (DMI), 25mg./kg. was added to the above with their DMI-sham operated controls. These rats were all housed in isolation. The behavioural testing (as above) revealed a significant drug by control by day interaction with Ne depleted rats significantly faster and no differences among the other groups.

162.1 CHANGES ON MUSCARINIC RECEPTOR POPULATION IN SOME BRAIN AREAS OF RATS DURING AN AUTOSHAPED INSTRUMENTAL TASK. A. Ortega*, A. Meneses*, A. Oscós* and V. Alemán, Departamento de Neurociencias Centro de Investigación y Estudios Avanzados del IPN, México, D.F. 07000.

It is currently thought that the encode of learning is probably found in a modification of the organization or function a the synaptic level. Under certain demanding conditions, receptors localized at the postsynapsis could be a limiting factor in the synaptic function. Receptors in this region seems to be a plausible protein candidate regulating the synaptic function. Because of this, we have thought the possibility that during learning of an appetitive autoshaping lever pressing response paradigm, we could detect changes in the number of muscarinic receptors in different brain areas as compared with control animals. We used 30 female rats of 90 days of age. During a week, animals were fed ad libitum with tap water and Purina chow pellet diet. At this time 85% of the animals weight was programmed to be reach in the five subsequent days. Rats were divided ramdomly in 3 different groups: a passive control, a group trained during three sessions (one session of 50 associative trials per day) and a group overtrained during 10 sessions. Binding determinations were carried according to a previous report. (^{3}H)-scopolamine methyl chloride (Amersham) was used as the ligand. The amount of muscarinic receptors in hippocampus of control rats was 0.56 pmol/mg prot but this value statistically decreased to 0.40 pmol/mg prot in trained rats. There was not such difference in the overtrained group. Instead in the amygdala we found a statistic increase in the receptor number of trained animals 0.81 pmol/mg prot as compare to the value of the control group 0.39 pmol/mg prot. A similar change was not singificant in overtrained rats (0.64 and 0.60 pmol/mg prot respectively). In another brain area the septum, the population of receptors in trained animals decreased to 0.90 from 1.0 pmol/mg prot in the control group. This change was greater in overtrained rats, that is 0.34 pmol/mg prot from 0.86 pmol/mg prot in the control group. In the temporo-parietal cortex, as in the amygdala, the receptor population increased statistically from 0.48 pmol/mg prot in the control rats to 0.70 pmol/mg prot in trained animals. A similar but smaller change was found in overtrained animals that is 0.50 and 0.59 pmol/mg prot respectivelly. Finally in caudate nucleus we found a statistically significant decrease in the amount of receptors from 0.62 pmol/mg prot in control group to 0,53 pmol/mg prot in trained rats. A similar and significant change from 0.63 pmol/mg prot in the control rats to 0.50 pmol/mg prot in overtrained animals was also found. Affinity constants changes were only observed in caudate and septum of trained animals as well as in septum and temporo-parietal cortex of overtrained animals but these changes were rather small.

162.2 DSP4 ALTERS REARING ODOR EFFECTS ON DEVELOPING RATS. C.A. Cornwell-Jones, T. Giannulli* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

Odor preference modifiability declines in rats with depleted central and peripheral norepinephrine (NE), but investigation of novel object is not affected (Cornwell-Jones, C.A. et al., Behav. Neural Biol., 35:1982). In the present experiment, odor preference modifiability and general investigatory behavior were examined after depletion of central but not peripheral NE by the neurotoxin DSP4. Odor preferences of two-week and five-week old male rats were examined 10 days after systemic injection of 50 mg/kg of DSP4 or water. Seven days after injection, half the animals in each age group were placed in cedar shavings and the other animals remained in pine. Three days later, odor preferences were measured for both age groups, and on the day after preference testing investigatory behavior of the older rats was examined.

DSP4 treatment appeared to increase odor preference modifiability. Pine-reared rats in both treatment groups preferred pine to cedar odor at both developmental stages. Exposing water-treated rats to cedar induced indifference to the odors in the younger, but not the older group. In contrast, cedar-housed DSP4 rats were indifferent to the odors at both testing ages. Pine-reared infants in both treatment groups preferred the odor of soiled pine-nest shavings to the odor of clean pine shavings. Exposing rats to cedar eliminated this preference in pups injected with DSP4, but not water.

DSP4 treatment decreased rearing effects on general investigatory behavior. Exposure to cedar increased investigation of a novel cup for rats treated with water but not with DSP4. In addition, for water- but not DSP4-treated cedar-housed rats, the mean duration of investigatory bouts decreased during the testing session (i.e. habituation occurred).

NE depletion restricted to the central nervous system increased rearing-induced changes in odor preferences but reduced changes in investigatory behavior. The contrast with previously observed effects of combined central and peripheral NE depletion suggests that both NE sources normally help mediate experiential influences on these behaviors.

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162.3 APPARENT MEMORY DEFICITS IN RATS TESTED FOR DELAYED ALTERNATION AFTER SELF-ADMINISTRATION OF DIAZEPAM. K.E. Gaston and M.A. Perrah*. Pitzer College, Claremont, CA 91711.

Acute treatment with diazepam produces transient anterograde amnesia in human subjects (Hinrichs et al, 1982, Pharm. Biochem. Beh., 17; Romney & Angus, 1984, Psychopharm. Bull., 20). Animals also show performance decrements on various tasks while under the influence of diazepam (Dantzer, 1977, Biobehavioral Reviews, 1). Most of these findings, however, do not unambiguously reflect memory dysfunction and can be interpreted in terms of other known properties (e.g., anxiolytic or disinhibitory) of the drug. The present study attempts to more directly examine the effects of diazepam on memory by testing rats on a delayed alternation task in which correct performance depends on the rat's remembering, over various periods of time, where it has just been.

The training and testing procedure was similar to that described by Roberts (1972, JCPP, 78). Each rat was pretrained to turn to opposite sides of a T-maze on successive trials in order to obtain food reward. When consistent alternation was established, nominal delays were introduced such that, after each trial, the rat was confined in the start box for 0, 5, 15, 30 or 60 sec. Each rat was given 5 test trials per day at each delay for several days. As expected, the percent of correct choices made was an inverse function of the delay, ranging from a high of about 95% at 0 sec to a low of about 65% at 60 sec. Next, on several separate occasions, each rat was allowed to drink a .625 mg/ml solution of diazepam in water and was tested for delayed alternation while under the influence of the drug. Self-administered doses of diazepam ranged from about 14 to about 39 mg/kg. The results showed that diazepam did not noticeably disrupt performance at the 0 sec delay, but that at each of the longer delays there was a dose-dependent decrement in percent of correct choices, as compared with pretest scores.

These findings suggest that the delayed alternation task is a useful paradigm for assessing the effects of diazepam on memory. The fact that diazepam did not interfere with alternation at the 0 sec delay indicates that the progressive decline in accuracy seen at the longer delays was due to diazepam-induced memory impairment and not to other, non-amnestic, properties of the drug. (Supported by Pitzer College Research & Awards Committee Grants.).

162.4 POST-LEARNING ETHANOL EFFECTS ON A WATER-FINDING TASK IN RATS. K.F.Melia*, C.L.Ehlers*, C.J.Lebrun*, and G.F.Koob. (SPON: L.Henderson). Div. of Neurosci., Alcohol Res. Ctr., Res. Inst. of Scripps Clinic, La Jolla, CA. 92037

In alcohol and cognition research, post-hoc administration of ethanol has been shown to enhance human memory for newly-learned material. Current animal research has replicated this phenomenon but with aversively-motivated tasks. Unfortunately, in such paradigms, ethanol's putative retroactive effects are potentially mediated by its lingering autonomic effects. An alternative paradigm, the water-finding task, offers the advantage of being an appetitively-motivated latent learning paradigm. Additionally, it has been used previously to study post-learning drug effects. To this end, the present study employed this paradigm to address the issue of post-hoc alcohol effects on memory enhancement.

Sixty male rats (138-264g) were assigned to one of three groups: control saline, a low dose ethanol (.75 mg/kg) and a higher dose ethanol (1.5 mg/kg). For latent learning, a rat was individually placed into the corner of an open field (37 x 64 x 46 cm) containing a drinking tube recessed into an alcove (11 x 13 x 46 cm). The subject was then allowed 5 min to explore the environment. During this time, the rat's initial latency to enter the alcove and lick the water tube was obtained (learning latency), and subsequent trips into the alcove accompanied by at least one lick were recorded (latent learning bouts). Immediately afterward, a subject was injected with one of the three doses and returned to its home cage. Approximately 30 minutes later, ad lib water was withdrawn, and 48 hours later, each animal was re-introduced to the open field and a second latency (test latency) to make contact with the drinking tube was measured.

A repeated measures analysis of variance revealed a statistically significant reduction in test latencies compared to learning latencies for all subjects ($p \leq .001$). The mean test latencies of the ethanol animals were the shortest, but not statistically significantly so (mean latencies = 42.44, 27.59 and 40.62 sec for control, low ethanol and higher dose ethanol, respectively). Linear regression analyses were performed on both test latency and the difference between learning and test latencies for each group. While the ethanol rats' behavior at time of test was significantly correlated with, and could be predicted from, behavior during latent learning, this was not true of the control animals. The control rats' test latencies were essentially random with respect to all measures of their previous behavior. Bouts and learning latency together were correlated significantly with test latency for the higher dose ethanol rats ($r=.81$, $p \leq .001$), and bouts were significantly correlated with the change in latency scores for the low dose ethanol rats ($r=.53$, $p \leq .05$). That is, for low dose ethanol, intraindividual latency reduction was predicted from the number of times the subject engaged in a drinking bout during latent learning.

These results indicate that ethanol administration can modify the effects of previous experience such that past behavior can come to more fully predict future behavior. It is suggested that the group-related orderly changes in behavior found may have implications for current research on the facilitating effects of post-learning ethanol on memory. (Supported by AA06059 and, 06420).

162.5 **EFFECTS OF CENTRAL DEPLETION OF NOREPINEPHRINE OR SEROTONIN UPON MEMORY TASKS USING THE RADIAL MAZE.** K. Pang[1], A. Chavez[2*] and G. Rose[1,2]. [1]Dept. of Pharmacology, University of Colorado Health Sciences Center and [2]Medical Research, VAMC, Denver, CO 80262

The hippocampal formation is known to play a critical role in the processes of learning and memory. One recent theory of hippocampal function suggests that this structure is particularly important in spatial memory; another theory suggests that the hippocampus participates in the recall of recent events (i.e., working memory). Both these types of memory may be demonstrated using the radial arm maze. Acquisition and performance of tasks using this maze are profoundly disrupted by damage to the hippocampus or its major afferents, but are unaffected by lesions of nonlimbic areas. Many studies have also implicated CNS monoaminergic systems in memory. However, the central site(s) of influence of monoamine neurotransmitters in learning or memory remain undefined, primarily because the brain loci governing performance in the tasks used have not been determined. In the present study, the effect of depletion of central norepinephrine (NE) or serotonin (5-HT) upon learning and memory was examined using the radial maze, since for such tasks CNS substrates are known.

Central NE or 5-HT were depleted by injection of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4; 2 injections of 50 mg/kg, i.p., at a 7 day interval) or 5,7-dihydroxytryptamine (5,7-DHT; 150 ug, i.c.v.), respectively. Rats treated with 5,7-DHT received prior treatment with desmethylimipramine (20 mg/kg, i.p.) to protect noradrenergic terminals. Control rats received equivalent injections of 0.9% NaCl. All animals were allowed 2-3 weeks to recover before maze training was begun. Rats were trained on the radial maze using the 8-arm spatial memory and the 4-arm working/reference memory paradigms; reversal learning in the 4-arm task was also examined. At the conclusion of behavioral testing, all rats were sacrificed and the hippocampus removed for analysis of tissue levels of monoamines using HPLC analysis.

Rats treated with 5,7-DHT (>70% depletion of 5-HT) had profound deficits in acquisition of the spatial and working/reference memory tasks. By contrast, treatment with DSP4 did not influence acquisition of these tasks; nor was performance of the spatial memory task or the work/reference reversal affected. NE depletions were, in all cases, greater than 90%. Thus, 5-HT, but not NE, is required for normal limbic system functioning in memory tasks using the radial maze. We are currently examining the effect of DSP4 and 5,7-DHT upon hippocampal long-term potentiation, a putative electrophysiological correlate of memory. (Supported by the Veterans Administration Medical Research Service.)

162.6 VINPOCETINE: NOOTROPIC EFFECTS ON DISRUPTED MEMORY RETRIEVAL IN RATS. V. J. DeNoble, L. W. Gelpke*, S. J. Repetti*, L. M. Wood*, and K. L. Keim. Dept. Pharmacology, Ayerst Laboratories Research Inc., Princeton, NJ 08540.

Vinpocetine (3α, 16α eburnamenine-14-carboxylic acid ethyl ester), vincamine, aniracetam and hydergine, compounds with purported cognitive activating profiles, were evaluated for their ability to prevent scopolamine-induced and hypoxia-induced impairment of retention of a step-through passive avoidance response. During the acquisition of the avoidance response, male Sprague-Dawley rats were placed in a lighted compartment and given access to a dark compartment. Two sec after entering the dark compartment, the rats were given a 2 sec inescapable 750 μA shock; retention was tested 24 hr later. For memory disruption, scopolamine (2 mg/kg sc) was administered 30 min prior to acquisition; all test drugs were given 90 min prior to acquisition.

Rats receiving scopolamine entered the dark compartment during the retention test with an average latency of 58 ± 7 sec, whereas vehicle control rats failed to enter (180 sec maximum latency). Vinpocetine (Peak Effect Dose [PED] = 200 mg/kg po) and aniracetam (PED = 100 mg/kg po), and hydergine (PED = 1 mg/kg po) prevented disruption by scopolamine of the passive avoidance retrieval. Hypoxia (7% O_2, 93% N_2) 20 min before and 20 min after avoidance training also impaired retention: > 75% of the rats did not retain the passive avoidance task. Vinpocetine (PED = 3 mg/kg po) and aniracetam (PED = 30 mg/kg po) were effective in preventing disruption of retrieval. Furthermore, in both tests the drug protection was dose-related in a typical inverted U-shaped manner. In contrast, hydergine (0.05 to 3 mg/kg po) and vincamine (0.3 to 100 mg/kg) were not effective against hypoxia-induced impairment. Hydergine at doses > 3 mg/kg po, markedly impaired motor function and could not be tested. The PED for vinpocetine and aniracetam in hypoxia impairment was significantly lower when compared to the doses effective in the scopolamine-impaired test. (-)-nicotine (0.1 to 0.4 mg/kg sc) and mecamylamine (1, 3, 10 mg/kg sc) given at various pretreatment times did not protect against memory impairment induced by either procedure.

These data support the view that vinpocetine has cognitive activating abilities as defined in models of both scopolamine-induced and hypoxia-induced memory impairment in rats. Disruption of retrieval by scopolamine is considered a specific antagonism of cholinergic neural substrates, whereas hypoxia-induced impairment is a more global brain insult. Since vinpocetine was active in both tests, this drug may be effective in a broad range of conditions which compromise memorial function.

162.7 THE ATTENUATION OF PAVLOVIAN EYEBLINK CONDITIONING BY PIZOTIFEN (BC-105) MAY BE DUE TO ITS EFFECTS ON AUTONOMIC SYSTEMS.* Sheryl R. Ginn and D. A. Powell. Neuroscience Lab., Dorn Veterans' Hospital, Columbia, SC 29201.

Experimentally naive New Zealand albino rabbits received Pavlovian eyeblink conditioning in which 75 db, 500 msec duration tones served as the conditioned stimulus (CS) and 200 msec paraorbital electric shock trains served as the unconditioned stimulus (UCS). Thirty-two animals, divided into four groups, received 0.0, 2.5, 5.0 or 10.0 mg/kg of the central serotonin (5-HT) antagonist pizotifen (BC-105) subcutaneously (s.c.) 15 minutes prior to session onset. Two pseudoconditioning control groups (N=16) received a random sequence of explicitly unpaired tones and shocks. These animals received 0.0 (n=8) or 5.0 (n=8) mg/kg BC-105 s.c., 15 min prior to session onset. Both heart rate (HR) and eyeblink (EB) conditioned responses (CRs) were assessed.

All three doses of pizotifen attenuated acquisition of the EB CR compared to animals which received saline injections, but these differences were not dose related. Both the 2.5 and 5.0 mg/kg groups showed significantly slower eyeblink acquisition than the saline group. The 10.0 mg/kg group also revealed fewer EB CRs than the saline group, but these differences were not significant. The cardiac CR, which accompanied EB conditioning, consisted of bradycardia and was attenuated in a dose-related fashion by BC-105. The groups which received explicitly unpaired tones and shocks showed virtually no EB or HR conditioning. The finding that the effects of BC-105 on the HR CR were dose related but its effects on EB conditioning were not, suggests that BC-105 may affect nonspecific processes related to autonomic conditioning, which in turn interferes with the acquisition of the somatomotor eyelid conditioned response. Interference with visceral systems by BC-105 may, for example, indirectly lead to cognitive impairments by causing confusion or motivational deficits in drugged animals.

*Supported by VA Institutional Research Funds

162.8 EVIDENCE FOR MULTIPLE STAGES OF MEMORY FORMATION IN THE CHICK. T.A. PATTERSON, M.R. ROSENZWEIG, E.L. BENNETT, and S.E. LUCEY*. DEPARTMENT OF PSYCHOLOGY, UNIVERSITY OF CALIFORNIA, BERKELEY, CA. 94720.

Gibbs & Ng (1977) proposed a three stage model of memory formation based on work with the chick: short-term memory, due to hyperpolarization dependent upon K+ conductance changes; intermediate-term memory due to hyperpolarization dependent upon sodium pump activity and long-term memory, dependent upon protein synthesis. In preparation for further study in this field, we have attempted to replicate some key findings of Gibbs & Ng.

Two-day-old male Leghorn chicks were trained in a one-trial taste avoidance task. Dose response functions were determined for glutamate, a depolarizing agent; ouabain, a Na+/K+ ATPase inhibitor; and anisomycin (ANI), a protein synthesis inhibitor. Intracerebral injections (10 ul/hemisphere) of saline or drug were given 5 min pre-training, and chicks were tested 24 hr post-training. Compared to saline solution, 37.5mM- 75.0mM glutamate, .014mM-.041mM ouabain, and 11.3mM- 18.8mM ANI produced significant amnesia. Lower doses of each drug were not effective, while higher doses produced behavioral side-effects.

The duration of protein synthesis inhibition after intracerebral injection of ANI (10 ul/ hemisphere) was examined. A non-amnestic dose (7.5mM) produced 70% whole brain (minus cerebellum) inhibition 30 min after injection; this dropped to 50% by 2 hr. An amnestic dose (15.0mM) produced more than 80% inhibition from 30 min to 1 hr; inhibition dropped to 60% by 3 hr.

The development of amnesia following pre-training injection (10 ul/hemisphere) of glutamate, ouabain or ANI was determined. Glutamate (50mM) produced amnesia which developed by 10 min post-training. Ouabain (.027mM) produced amnesia which developed between 15 and 30 min post-training, while ANI (15.0mM) produced amnesia which developed by 90 minutes post-training. Amnesia produced by these agents appears permanent; chicks tested at later times are also amnesic.

These results are similar to those obtained by Gibbs and Ng and support a sequentially dependent three-stage model of memory formation. Doses required for amnesic effect, and the time which amnesia appears after injection of ouabain and ANI differ from their report; we are attempting to determine if these differences are due to training parameters or housing conditions.

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162.9 DECREASE BY THC OF MEDIAL SEPTAL MEDIATED AUGMENTATION OF RESPONSE AT HIPPOCAMPAL CA1. N.J. Pontzer,* and D.M. Wilkison* (SPON: L.F. Tseng). Dept. of Pharm. and Tox., Medical College of Wisconsin, Milwaukee, WI 53226.

Decrements in learning and memory are among the most prominent behavioral effects of THC, the active constituent of marijuana. Both hippocampal lesions and anticholinergic drugs produce memory deficits similar to those observed after THC. THC has been shown to potently and specifically reduce spontaneous acetycholine turnover in the septo-hippocampal cholinergic system. This action is manifested by a decrease in cholinergic-mediated hippocampal theta rhythym. Pre-stimulation of the septum augments evoked monosynaptic discharge at the hippocampus in an atropine sensitive manner. We tested the hypothesis that THC would decrease evoked as well as spontaneous septo-hippocampal function.

Male Sprague-Dawley rats anesthetized with urethane (1.5 g/kg, ip) were implanted with 16 mil concentric bipolar stimulating electrodes in the medial septum (MS) and ventral hippocampal commissure (VHC). Semi-micro recording electrodes were positioned in the contralateral CA1 pyramidal cell layer. Eight successive CA1 responses (0.2 Hz) were averaged and digitally filtered to separate the cell discharge (spike) and synaptic (slow positive wave) components of the VHC-evoked field potential. Averaged responses were obtained for VHC stimulation levels which produced near-threshold, half-maximal and supramaximal spike amplitudes. The percentage increase in spike amplitude produced by septal pre-stimulation (400 Hz, 15 mS) was measured before and 15 min after THC given iv in cumulative doses of 0.3, 1.0 and 3.0 mg/kg.

Septal augmentation of CA1 spike amplitudes was greatest at near-threshold VHC stimulation (200 - 500%), much less for half-maximal (20 - 80%) and not detectable at supramaximal stimulation levels. There was little or no direct septal response or augmentation of synaptic components. THC reduced half-maximal VHC-mediated responses to levels at which greatly increased septal augmentation would be expected. THC also reduced the predicted septal augmentation of the spike response based on spike amplitude. This effect was sometimes seen at the lowest dose of THC when there was little change in spike amplitude. Physostigmine (0.3 mg/kg iv) produced a partial recovery of septal augmentation, but not VHC responses when given after THC. These actions of THC on septal augmentation at CA1 are similar to those we previously demonstrated for perforant path mediated responses in the dentate.

162.10 THE UTILITY OF A LATENT INHIBITION PARADIGM IN COMBINATION WITH A DELAYED REINFORCEMENT AUTOSHAPED BEHAVIOR. C.A.Cohen*and S.B. Sparber. Depts. of Psychology and Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Latent inhibition is a term coined by Lublow and Moore which can be used to describe the inhibiting effect upon learning by exposing subjects to environmental cues, which will be used for conditioning stimuli, prior to the conditioning procedure (Lublow and Moore, J. Comp. Phys. Psych.,1959,52:415). It may be thought of as habituation or suppression of the saliency associated with novelty and/or the arousing effect of exposure to the novel cues. Acquisition of a forward autoshaped task is retarded by interposing a delay between the required lever-touch response and delivery of the food pellet reward (Messing,Kleven, and Sparber, submitted). Facilitatory effects of agents upon acquisition of autoshaped behavior may be due to nonspecific motoric or arousing properties, thus there is a need to establish a reliable task which requires response inhibition as a measure of learning, so that we can control for such variables.

During the delayed autoshape procedure, the retractable lever was extended into the chamber on a 45 second random time schedule and withdrawn either when the animal made a lever touch or 15 seconds had elapsed. When the lever was retracted under either of the above mentioned conditions, a food pellet was delivered 6 seconds later. Adult male Sprague-Dawley rats were randomly assigned and placed in modified Skinner boxes with retractable levers for 2 or 4 sessions of 24 trials without reinforcement. A third group was never placed in the operant chambers prior to the autoshape sessions. The animals placed in the chambers for the latent inhibition sessions were exposed to the identical procedure as in the subsequent autoshape sessions, except the food dispenser was disconnected. Three days after the last nonreinforced session, all subjects were presented with the delayed autoshape task for four consecutive sessions. A repeated measures ANOVA of the number of correct lever touch responses indicated that animals exposed to the chamber for 2 or 4 sessions prior to acquisition of the autoshape task did not acquire the response, as did the control subjects.

Exposure to Chamber	Acquisition Sessions (M±SE) 1	2	3	4
0	9.00± 1.07	8.50± 1.69	12.44±2.60	16.80± 2.92
2	6.73± 1.14	6.18± 2.23	5.70±2.63*	8.36± 2.78*
4	6.18± 1.15	4.09± 1.65	5.45±2.48*	7.64± 2.83*

*Different from controls by Dunnett's test.

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162.11 AF64A IMPAIRS WORKING MEMORY IN RADIAL-ARM AND T-MAZE TASKS. J.J. Chrobak*, I. Hanin & T.J. Walsh* (SPON: R.A. King). Univ. of North Carolina, Chapel Hill, NC 27514 and Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Ethylcholine mustard aziridinium ion (AF64A) is a select cholinotoxin which produces a persistent reduction in presynaptic cholinergic markers (Mantione et al., Science, 213, 1981). This compound has been shown to impair retention of a passive avoidance response and disrupt acquisition of a radial-arm maze (RAM) task (Walsh et al., Brain Res., 321, 1984). The studies presented here attempted to further characterize the nature of the cognitive deficits produced by AF64A.

Male Fischer rats were trained in a modified RAM task. In this task rats had access to only 4 out of 8 maze arms during a predelay session. After entering these arms the rats were returned to their home cages for a 2 hr. delay period. Following the delay they were returned to the maze and allowed to freely choose among all 8 arms. Arms not previously entered were now baited and entry into previously visited arms constituted an error. Following 20 days of training rats were injected with AF64A (3 nmols/side/icv.) or an equivalent volume of saline and allowed 2 weeks to recover. Rats treated with Af64A performed at chance level, making 2 or more errors in their first 4 post-delay choices and making significantly more total choices in the post-delay session, throughout 20 post-operative trials (one per day, five days a week). The behavioral deficits were associated with a selective decrease (38%) of choline acetyltransferase activity in the hippocampus.

In a second experiment rats were trained on a modified T-maze task. Animals were placed into one of two start/goal boxes located on either arm of the maze. They were trained to run down the stem of the maze and return to the alternate start/goal box for food reward (i.e., a non-match to sample task). The location of the start box was randomly alternated on each trial. The different aspects of this task may be characterized as a) a reference memory component which involves an invariant response of running down the stem of the maze and b) a working memory component in which the animal must maintain some representation of the previous stimulus event (i.e., the start location) to perform accurately. Following training rats were injected with AF64A or saline according to the above protocol. Rats treated with AF64A were impaired on the working memory task, while their performance of the reference memory task, within the same trials, was unimpaired.

These data suggest that hippocampal cholinergic mechanisms are critical for allowing an animal to differentially respond on the basis of recently experienced stimulus events.

162.12 INTERACTIONS BETWEEN UNCONDITIONED STIMULI THAT PRODUCE TASTE AVERSION LEARNING. B. M. Rabin, W. A. Hunt* and J. Lee*. Behav. Sci. Dept., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20814-5145, and Dept. Psychol., Univ. Maryland Baltimore County, Catonsville, MD 21228.

A conditioned taste aversion (CTA) is produced when a novel tasting solution is paired with a wide variety of unconditioned stimuli, including injection of lithium chloride or amphetamine, or exposure to ionizing radiation. The diversity of unconditioned stimuli that can lead to CTA learning raises the question of whether or not there may be a common factor underlying the capacity of these stimuli to produce a CTA. This question was studied by investigating whether or not it would be possible to produce a CTA in rats by combining subthreshold doses of radiation, lithium chloride or amphetamine.

A CTA was established in rats using a two-bottle procedure with a 10% sucrose solution as the conditioned stimulus. The first experiment determined the doses that were just below threshold for producing a CTA: lithium chloride, 0.3 mEq/kg; amphetamine, 0.5 mg/kg; radiation, 15 rad (at 20 rad/min). These in turn became the standard doses for the second experiment. On the conditioning day, naive rats were treated with one unconditioned stimulus immediately following ingestion of the sucrose solution. 30 min later, without further access to the conditioned stimulus, they were given a second treatment with either the same or with a different unconditioned stimulus. Controls were treated with only a single unconditioned stimulus following sucrose ingestion followed by either saline injection or sham exposure 30 min later. In contrast to the controls, combining an initial subthreshold exposure to ionizing radiation with a second exposure 30 min later or with injection of a subthreshold dose of amphetamine or lithium chloride produced a significant decrease in test day sucrose preference. Similar results were obtained with combined subthreshold doses of amphetamine, but not with combined doses of lithium chloride.

The observation that two subthreshold doses of different unconditioned stimuli, following a single exposure to the conditioned stimulus, can be combined to produce a CTA suggests that there is a common factor underlying the capacity of lithium chloride, amphetamine and radiation to produce a CTA. The results also suggest that treating an organism with ionizing radiation or amphetamine produces a physiological change in the organism that remains active for at least 30 min following treatment.

162.13 EFFECTS OF TRIMETHYLTIN (TMT) ON THE LICL DOSE-RESPONSE FUNCTION FOR TASTE AVERSION LEARNING IN THE RAT. J. P. Mastropaolo, R. J. Dacanay & A. L. Riley. Psychopharmacology Lab, The American University, Washington, D.C. 20016

Recently, TMT has been shown to disrupt the acquisition of a conditioned taste aversion (CTA) when a delay is imposed between the presentation of the taste and the administration of a toxin (Riley, A., Dacanay, R., & Mastropaolo, J., Neurotox., 5:291-296, 1984). Specifically, TMT- and non-TMT-treated rats presented with a novel saccharin solution and injected with LiCl immediately, 3-h or 6-h post-saccharin ingestion displayed differential aversions. In both groups, the strength of the aversion decreased as the interval between ingestion and LiCl administration increased. Relative to the non-TMT-treated rats, however, aversions were weaker at the longer delays in the TMT-treated rats, a disruption consistent with memory deficits reported in other procedures (e.g. Walsh, T., Miller, D., & Dyer, R., Neurobehav. Toxicol. Teratol., 4:177-183, 1982). Concluding that this disruption is a memory deficit requires consideration of recent evidence that TMT also affects sensory systems (Dyer, R., Howell, W., & Wonderlin, F., Neurobehav. Toxicol. Teratol., 4:191-196, 1982; Howell, W., Walsh, T., & Dyer, R., Neurobehav. Toxicol. Teratol., 4:197-202, 1982). Since the acquisition of a CTA increases with the dose of the toxin, it might be that the effect reported by Riley et. al is due to changes in the intensity of the toxic effects of LiCl, which could interact with the delay gradient, rather than to memory impairment. The present experiment, therefore, assessed the effects of TMT on the ability of various doses of LiCl to produce CTAs. Specifically, 48 water-deprived rats were randomly assigned to two groups, one intragastrically administered TMT (6 mg/kg) and the other receiving an equivolume intubation of distilled water. Twenty one days following this treatment, all subjects were given 20-min access to a novel saccharin solution (0.1% w/v), followed immediately by an injection of either 0, 0.3, 0.6 or 1.8 mEq of LiCl. In total, there were four saccharin/LiCl pairings and a final saccharin aversion test. Three water recovery days separated successive exposures to saccharin. TMT- and non-TMT-treated rats showed equally large dose-related aversions, i.e., the higher the dose the greater the aversion. More importantly, no differences were found in the degree of CTA produced in TMT- and non-TMT- treated rats. Therefore, the earlier reported deficit in the acquisition of CTAs over long delays is not the result of changes in stimulus intensity. As such, the interpretation of this effect as a memory deficit is strengthened.

BIOLOGICAL RHYTHMS II

163.1 OLFACTORY BULBECTOMY PREVENTS THE ACYCLICITY ASSOCIATED WITH SHORT PHOTOPERIOD IN FEMALE GOLDEN HAMSTERS. D.R. Pieper and R.N. Heverly*, Department of Biology and Health Sciences, University of Detroit, Detroit, MI 48221

Exposure of golden hamsters to a short photoperiod (SP) for 10 weeks results in testicular regression in the male and acyclicity in the female. Exposure of rats to SP alone has no effect, but if the rats are pre-pubertally olfactory bulbectomized (BX) and then placed on SP or blinded and BX, the testes become smaller and the females have irregular estrous cycles. All of these effects in rats or hamsters are prevented if the animals are pinealectomized in addition to the other manipulations. We have recently shown that pre-pubertal BX of male golden hamsters will prevent the testicular regression associated with SP (Brain Res 321:183, 1984), an effect opposite that predicted by the results of BX in rats. The experiments described here test whether pre-pubertal or adult BX will prevent the acyclicity associated with SP in female hamsters. In the first experiment, 23 day old hamsters were divided into 4 groups: LD 14:10 sham BX; LD 14:10 BX; LD 6:18 sham BX; and LD 6:18 BX. Cells from vaginal washings were observed under the microscope for 11 weeks. At least 90% of both groups on LD 14:10 had regular estrous cycles for the last 7 weeks of the experiment. In the LD 6:18 sham BX group, 7 of the 19 animals (37%) were cycling regularly by 11 weeks after being placed in LD 6:18. On the other hand 21 of 23 (91%) of BX animals on LD 6:18 had regular estrous cycles. These results were very interesting and indicated that BX may prevent the acyclicity associated with SP. However it was somewhat perplexing that only 57% of the control group on LD 6:18 became acyclic. It was felt that this may have been due to the animals being exposed to the SP pre-pubertally. The experiment was therefore repeated except that this time the hamsters arrived in the laboratory at 63 days of age and they were sham BX or BX and placed in LD 6:18 at this age. This time, only 2 of 10 (20%) of the sham operated animals had regular estrous cycles 11 weeks after being placed in LD 6:18. On the other hand, 9 of 10 (90%) of the BX animals had regular estrous cycles. These results indicate that either pre-pubertal or adult BX will prevent the acyclicity associated with SP in female golden hamsters. The neural or humoral relationship between the olfactory bulb and the reproductive response to photoperiod is not clear at this time and is now being investigated.

163.2 HYPOTHALAMIC KNIFE CUTS THAT PREVENT SEASONAL REPRODUCTIVE CYCLES: DO THEY INTERRUPT HYPOTHALAMO-SPINAL CONNECTIONS IN HAMSTERS? M. H. Brown and A. A. Nunez. Dept. of Psychology and Neurosci. Prog. Michigan State University, E. Lansing, MI, 48824

Previous results from our laboratory (Nunez et al., submitted for publication) have shown that bilateral horizontal knife cuts placed ventral to, through, or dorsal to the paraventricular nucleus of the hypothalamus (PVN) prevent testicular regression in golden hamsters housed in a short photoperiod (6 hr light:18 hr dark) without disrupting entrainment of circadian activity patterns to the light-dark cycle. To further investigate the hypothalamic neural pathways involved in the gonadal response to photoperiod, an additional group of hamsters was given bilateral horizontal cuts aimed dorsal to the PVN and housed in short photoperiod (6L:18D). One group of control hamsters was also housed in short photoperiod while a second group was kept in long photoperiod (16L:8D). Begining 4 weeks after introduction of the hamsters to short photoperiod, biweekly measurements of the width and length of the right testis of each hamster were made. As in the previous experiment, most of the knife cuts were effective in blocking testicular regression. Possible explanations for the efficacy of the cuts dorsal to the PVN include:(i), interruption of projections from the SCN to the habenula (Neurosci. 6:2625,1981) which in turn projects to the pineal gland (Exptl. Neurol., 79:858,1983); (ii), severing of fibers that project from the PVN to the habenula (JCN., 169:221,1976) and/or pineal (Cell Tiss. Res., 205:11,1980); or (iii), interruption of efferent fibers that project dorsally out of the PVN and then caudally to preganglionic sympathetic cell bodies in the thoracic spinal cord. Support for the third possibility is provided by Luiten et al. (Br. Res., 329:374,1985) who described one bundle of paraventriculo-spinal fibers that leaves the nucleus laterally and then turns caudally near the medial forebrain bundle, and a second bundle of paraventriculo-spinal fibers that travels dorsally out of the PVN and passes dorsocaudally through the thalamus and the periaqueductal gray. To investigate the possibility that knife cuts dorsal to the PVN interrupt paraventriculo-spinal projections, we have employed unilateral horizontal knife cuts and subsequent bilateral injections of horseradish peroxidase (HRP) into the upper thoracic spinal cord. In one case, a knife cut just dorsal to the PVN and very near the dorsal boarder of the PVN substantially reduced the number of labelled cells in the PVN ipsilateral to the cut as compared to the contralateral PVN. Preliminary results obtained using bilateral injections of HRP into the spinal cord of hamsters in which bilateral cuts blocked gonadal regression indicate that the effective cuts interrupt some but not all of the paraventriculo-spinal projections. (Sponsored by NIMH grant MH 37877 to A. A. N.)

163.3 EFFECTS OF VENTROMEDIAL HYPOTHALAMIC LESIONS ON CIRCADIAN DRINKING AND TEMPERATURE RHYTHMS IN RATS. Stephen Kent* and Evelyn Satinoff. Dept. of Psych., Univ. of Illinois, Champaign, Il. 61820

Ventromedial hypothalamic (VMH) lesions eliminate the circadian rhythms of feeding (Kakolewski et al., 1971) and sleeping (Danguir and Nicolaidis, 1978). It has been suggested that they also eliminate the circadian temperature rhythm (CTR) (Krieger, 1980).

In this study we examined the effects of VMH lesions on body weight and circadian temperature and drinking rhythms in unrestrained female rats. Body temperature (Tb) was measured every 10 minutes through implanted telemetry devices and drinking via a drinkometer circuit. The rats were weighed 3-4 times a week for the first 2 months post-lesion and once a week after that. Tb retained its rhythmicity in all animals after VMH lesions in a 12:12 light-dark cycle, and the rhythm free-ran with a period of 24.12 ± .03 hours in constant dark (N=9). Some of the CTR parameters were altered postoperatively. During the dynamic stage of hyperphagia (days 6-20) (mean weight gain 4.77 ± .74 g/day) the acrophase advanced an average of 67 ± 19° compared to prelesion values ($p<.01$). The individual amount of phase advance was positively correlated with the maximum increase in body weight ($r=.844$, $p<.01$). This advance decreased slightly over time. Mean daily Tb slowly decreased after surgery so that by days 84-99 postsurgery, when the rats were in the static stage (mean weight gain 269 ± 30 g) it averaged 0.52 ± .16°C lower than prelesion values ($p<.02$). The amplitude increased slightly, but not significantly, over the post-lesion period.

Drinking became aperiodic in the six rats in which it was measured. In one animal drinking became rhythmic after 10 days, and in two others after 6-8 weeks. In the remaining three animals drinking remained arrhythmic for the duration of the study (4 months). Water intake increased 236 ± 25% over control levels ($p<.01$) during the dynamic stage and 133 ± 19% during the static stage.

When the rats were in the static stage, with body weights 238 ± 20% over controls, we tested their ability to maintain normal Tb in the heat (3 hrs. @ 40°C). They were removed from the heat earlier if their Tb increased above 41.75°C. Six of the seven lesioned rats had to be removed from the stress before 3 hours were up (avg. time = 117 ± 13 min.). In contrast, only 1 of the 7 controls had to be removed (avg. time = 177 ± 3 min.) ($p<.001$). Preliminary data with 4 VMH-lesioned animals reduced to pre-lesion weight levels suggest that this deficit may be due to the lesion and not to the increased weight.

These results indicate that in rats the CTR remains after VMH lesions with a decreased daily mean and a phase advance that is correlated with the weight gain. Neither the presence of the CTR nor any changes in it depend on the drinking rhythm.

Supported by NSF BNS 8311466

163.4 Melatonin and Photoreceptor Metabolism: Interactions of Melatonin, Dopamine and GABA in the Regulation of Cone Movement. M.E. Pierce* and J.C. Besharse*. (SPON: J. Sutin). Dept. of Anatomy, Emory School of Medicine, Atlanta, GA 30322.

Using an in vitro eye cup preparation from Xenopus laevis, we have previously demonstrated that cone retinomotor movements are influenced by melatonin and by dopamine and have suggested that melatonin may regulate cone retinomotor movement via modulation of dopamine release. In eye cups from four day constant light-treated animals, melatonin mimics darkness and induces cone elongation. Dopamine not only mimics light and induces cone contraction, but also blocks melatonin-induced cone elongation. Dopamine also induces cone contraction in eye cups from cyclic light animals (12 hour light:12 hour dark), but melatonin does not reproducibly elongate cone in eye cups prepared at light offset from cyclic light-treated animals. The difference in melatonin sensitivity between cyclic and constant light-treated eye cups may be related to decreased melatonin biosynthesis and down-regulation of dopamine sensitivity expected in constant light (DeMello et al., PNAS 79: 5708, 1982; Lucas et al., Soc. Neurosci. Abstract 10(2):328.11, 1984). Another possibility is that an additional mechanism for the suppression of dopamine release is needed in cyclic-light animals.

There is considerable evidence that GABA-containing cells exert an inhibitory influence on dopaminergic amacrine cells in rat retina. Muscimol, the GABA agonist, inhibits the activation of tyrosine hydroxylase, a key enzyme in the synthesis of dopamine, and the GABA antagonists picrotoxin and bicuculline activate tyrosine hydroxylase and increase dopamine turnover in dark adapted retinas (Marshburn and Iuvone, Brain Res. 214:335, 1981). Therefore, we designed experiments to test the effects of GABA on cone movements. In eye cups prepared from constant light-treated animals, muscimol (50 µm) stimulates cone elongation which is blocked by the addition of dopamine. Muscimol does not stimulate cone elongation in eye cups prepared from cyclic light-treated animals. However, when both muscimol and melatonin are added to eye cups prepared at light offset from cyclic light animals cones elongate although this treatment is not as effective as darkness. In our cyclic light preparation, the GABA antagonist picrotoxin (100 µm) blocks dark induced cone elongation and induces cone contraction. These results are consistent with a model in which the suppression of dopamine is a necessary part of the dark signal for elongation. Melatonin and GABA may exert their influence through modulation of the dopaminergic system.

163.5 FLUCTUATIONS IN VISUAL SENSITIVITY OF GOLDFISH. C.J. Bassi* and M.K. Powers. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240

Although a number of species exhibit circadian rhythms in visual physiology, only a few have been shown to manifest such changes behaviorally (e.g. Limulus, rat and human). In this experiment, we measured fluctuations in the ability of goldfish, an animal that has long served as a model for retinal function, to detect a visual stimulus over prolonged periods of constant darkness.

Adult goldfish (9-15 cm standard body length) were maintained on a 12L:12D schedule for several weeks before the experiment began. They were classically conditioned to inhibit respiration when presented with a large, diffuse, 532 nm., 5 second stimulus.

Once trained a fish was placed in constant darkness and threshold was measured approximately every four hours for the duration of the experiment. Absolute threshold was measured by recording the probability of response to successively dimmer stimuli, in a staircase procedure. Threshold was determined by standard psychophysical techniques. The four fish tested so far remained in the dark for 40, 56, 92 or 168 continuous hours. During this time, the only light available was the dim, near-threshold stimulus.

All fish showed fluctuations in visual threshold over the course of the experiment. These fluctuations were periodic, with peak sensitivity occuring at approximately 24 hour intervals; five continuous cycles were observed in the fish tested for 168 hours. Maximum sensitivity generally occurred during the late subjective evening. The average change in sensitivity over the day was .50 log unit.

The rhythm in sensitivity observed in this psychophysical experiment does not correlate with our measurements of rod outer segment shedding under constant conditions. Nor does it match the patterns of locomotor activity that occur under constant conditions in this species. Nonetheless, the results are consistent with the hypothesis that the detectability of dim lights fluctuates on a circadian cycle; i.e., the rhythm in visual sensitivity persists for multiple cycles under constant conditions, with a period of approximately 24 hours.

(Research supported by EY07007, EY03352 and a Vanderbilt dissertation grant. MKP supported by EY00246.)

163.6 SEROTONINERGIC INNERVATION OF MOLLUSCAN EYES CONTAINING CIRCADIAN PACEMAKERS. J.S. Takahashi AND A. Eskin. Dept. Neurobiol. and Physiol., Northwestern Univ., Evanston, Il. 60201; Biol. Dept., Univ. Houston-Univ. Park, Houston, TX., 77004.

Our previous studies indicated that 5-HT (Serotonin) is used as a transmitter of circadian information in the Aplysia eye. An important extension of these studies is to determine the anatomy of the 5-HT system. Goldstein et al (1984) examined 5-HT-immunoreactivity in whole mounts of juvenile Aplysia eyes. They observed a dense plexus of stained fibers surrounding the eye and in preliminary studies on adult sectioned eyes, found that most of the stained fibers were in the outermost region of the retina. The source of "5-HT" fibers in the eye appears to be somewhat controversial. Olson and Jacklet (1984) described accessory optic nerves (Acc. ON) carrying efferents up to the eye. Fluorescence studies indicated that the Acc. ONs contained some "5-HT" fibers and, based on other studies, Olson and Jacklet stated that the optic nerve probably did not contain "5-HT" fibers. On the other hand, Goldstein et al mentioned that the optic nerve contained two stained fibers.

Aplysia californica eyes were fixed in paraformaldehyde, sectioned on a cryostat, and then hydrated with porcine serum. Eyes were exposed to 5-HT antibody from either Immunonuclear or Accurate Scientific and then treated with a secondary antibody conjugated with FITC. A number of controls were performed to establish the specificity of the staining for 5-HT. No staining was observed when eyes were exposed only to the secondary antibody, only to normal rabbit serum, or to 5-HT antibodies pretreated with 5-HT. Also, the two different 5-HT antibodies gave similar patterns of staining. Antibody stained fibers were observed in the optic nerve, in the Acc. ONs, in the connective tissue capsule surrounding the eye, and within the eye itself. No stained cell bodies appeared in either the connective tissue sheath surrounding the eye or in the eye. We observed up to six large "5-HT" fibers in optic nerves and these "5-HT" ON fibers were continuous with "5-HT" fibers in the eye neuropil. "5-HT" fibers from Acc. ONs formed a dense network in connective tissue surrounding eyes. Some "5-HT" fibers in connective tissue were continuous with "5-HT" fibers within the retina. In the retina, "5-HT" fibers ran mainly around the periphery of the eye and penetrated not much beyond the cell body layer of photoreceptors. The retinal "5-HT" fibers did not appear to be restricted to certain "poles" or regions of the eye as described for certain cell types by Herman and Strumwasser, 1984. Our observations indicate that cells in the Aplysia retina receive 5-HT innervation from two sources, the optic nerves and the Acc. ONs, and that perhaps this anatomical separation correlates with different functions of 5-HT in the eye. Similar studies on Bulla eyes were performed. The connective tissue sheath surrounding Bulla eyes contain a network of "5-HT" fibers but no "5-HT" fibers were observed either within the optic nerves or within the retina.

163.7 SEROTONIN PHASE SHIFTS THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN THE COCKROACH. Terry L. Page, Department of General Biology, Vanderbilt University, Nashville, TN 37235.

The pacemaker that regulates the circadian rhythm of activity in the cockroach Leucophaea maderae is composed of two oscillators, one located in each optic lobe of the brain. Two sources of input to each oscillator function in entrainment. The first is from the retina of the ipsilateral compound eye, and the second is from the oscillator in the contralateral optic lobe. On the hypothesis that at least one of the two entrainment pathways utilizes chemical synaptic transmission an effort was made to identify putative transmitters capable of phase shifting the pacemaking system.

Animals freerunning in darkness were removed from their activity monitors and restrained in a light-tight box. A small canula, placed in the head capsule near one optic lobe, was used to infuse putative transmitters in a series of 4 µl pulses (one pulse per 20 minutes) for a period of either 3 or 6 h. At the end of the infusion animals were returned to activity monitors and the phase of the freerunning activity rhythm was compared to the phase of the rhythm prior to treatment. Transmitters were dissolved (.05M) in physiological saline plus 1 mg/ml ascorbic acid. Three hour infusions were begun at circadian time (CT) 16-18; 6h infusions were initiated at CT 12-14.

For 3h infusions, serotonin (5HT) was the only substance tested that was found to generate phase shifts significantly different from saline controls ($p<.05$, t-test). The average phase shift for 5HT was -4.0 ± 1.29 h (mean+SD, N=6) while the average shift in controls was -1.4 ± 1.3 h ($\bar{N}=9$). Dopamine (N=6), norepinephrine (N=6), octopamine (N=7), carbachol (N=4), gamma-amino-n-butyric acid (N=4) and glutamate (N=4) had no phase shifting effect. Average shifts for these substances ranged from -0.2 h to -1.3 h.

Results were similar for 6 h pulses with the exception that dopamine as well as 5HT caused significant ($p<.05$) phase shifts compared to saline controls. Average phase shifts were: 5HT, -3.7 ± 1.11 h (n=7); dopamine, -3.8 ± 2.87 h (N=6); control, -0.9 ± 1.6 h ($\bar{N}=8$). Neither octopamine ($\bar{N}=6$), carbachol (N=4), norepinephrine (N=1), GABA (N=4) glutamate (N=5) or histamine (N=2) caused significant shifts.

Preliminary results indicate the effect of 5HT is dependent on concentration. Six hour treatments with .01M 5HT caused significantly smaller shifts (-1.33 ± 0.29 h, N=3) than the .05M 5HT infusions. Specificity of the 5HT effect was examined by treating animals with structurally related compounds. Neither tryptophan (N=3), tryptamine (N=3) or 5-hydroxyindole-3-acetic acid (N=3) caused average phase shifts greater than 1 h. (Supported by PHS-NIH Grant GM30039).

163.8 EFFECTS OF CONSTANT ILLUMINATION ON THE CIRCADIAN RHYTHM OF THE HORSESHOE CRAB Limulus polyphemus. A. Blaber, and P. Mack*
Biophysics Interdepartmental Group, University of Guelph, Guelph Ontario, Canada. N1G 2W1

Horseshoe crabs, Limulus polyphemus, are nocturnal animals and have endogenous circadian rhythms with free running periods of approximately 24 hours in constant darkness.

Monitoring of electroretinogram responses in the lateral eyes of four horseshoe crabs, under conditions of constant illumination, revealed a lengthening of the period in accordance with Aschoff's Rule. In all cases the length of the subjective day decreased by 1.2 to 2.7 hours and the length of the subjective night increased by 2.1 to 4.3 hours, giving total period changes of 0.9 to 1.6 hours, with respect to constant darkness.

These results contradict the velocity response curve hypothesis (Daan,S., and C.S.Pittendrigh, J.Comp.Physiol.,106:267-290,1976), developed to explain period changes in animal circadian rhythms under constant illumination, since it does not allow for changes in the length of subjective day. A new hypothesis is presented, using the phase response curve, which allows for changes of the lengths of both subjective day and subjective night.

163.9 A PROTOCEREBRAL CIRCADIAN PACEMAKER FOR HYPERGLYCEMIC HORMONE RELEASE IN THE CRAYFISH: Is there a common clock circadian system driving both light-adapting and crustacean hypergycemic hormones (CHH) from the sinus gland? B. Barrera-Mera, S. March-Mifsut*
Depto. de Neurociencias C.I.F.C., UNAM & Deptos. de Fisiología y Bioquímica, Fac. Medicina, UNAM A-Postal 70250 México, D. F.

A circadian clock is bilaterally located in the protocerebral eyestalk supraesophageal ganglion in crayfish (J. Interdiscipl. Cy. Res. 10, 79, 1979). By the surgical bilateral deafferentation of this structure from the rest of the nervous system we could isolate in vivo the main neuroendocrine complex of these animals. Its anatomical integrity and cyclical circadian ability for release the light-adapting hormone from the sinus gland of both eyestalks (Brain Res. Bull. 5, 667, 1980), offers us as an atractive model for the study of neuroendocrine circadian rhythms regulation. Aside from many other hormonal substances of the sinus gland-neurohemal complex in these crustaceans, the CHH also seem to be under the potent effects of a cephalic pacemaking system of glucose level regulation in the crayfish Astacus (J. Comp. Physiol. 89, 197, 1974). CHH is synthetized in specific neuronal elements of the eyestalk and the exact anatomical site controlling their cyclical release is presently unknown. Since the original publications, it is known that glycemic levels in crayfish readily increase not only by the action of stress, by toxic or pharmachologic substances, but also by the simple handling of the animals during experimental manipulation, by feeding, moulting, or by the action eyestalk extracts.

Nine in vivo protocerebral preparations were used. These preparations were daily feed with fresh or cooked chicken meat;4 mg/g of body weight. The retinal sensitivity measured as electroretinogram (ERG) was correlated with the haemolymphatic glucose concentration. For this purpose 25 µl haemolymph samples were obtained every 6 hours while ERG rhythm was continously recorded. Glucose was measured using a glucoxidase method. In all the animals tested an increase from 4.1-7.3 mg/% to 6.5 to 9.2 mg/% in haemolymphatic glucose concentration was obtained during the activity phase of ERG. Both the ERG rhythm and the cyclical variations of glucose concentration occurred with a periodicity of 26-27 hours. Glucose circadian variations dissappeared and the ERG rhythm became damped after the complete removal of protocerebrum.

Based in the present results we belive that protocerebral circadian pacemaker in crayfish would be driving among many others, the light adapting and CHH hormones from sinus gland. In accordance with the fact that both ERG and glycemic rhythmic variation show the same periodicity, we postulate that a single one pacemaking system command visual and glycemic circadian rhythms.

163.10 ONTOGENY OF LIGHT-DARK ENTRAINMENT IN THE RAT. M.J. Duncan*, M.J. Banister*, and S.M. Reppert (SPON: R.D. Hall). Children's Service, Massachusetts General Hospital, Boston, MA 02114.

Previous studies in rats indicate that the circadian system is functional and entrainable by the mother during the prenatal and early postnatal periods. The retinohypothalamic tract, which transmits light information for entrainment to the SCN in adults, is anatomically in place between postnatal days 3-6. We determined the onset of light-dark entrainment by using the rhythm of pineal N-acetyltransferase (NAT) activity to monitor the developing circadian system. This rhythm is the first readily measurable circadian rhythm overtly expressed in rats and is characterized by greatly elevated levels of enzyme activity during the dark period.

Timed pregnant Sprague-Dawley rats were exposed to a light-dark cycle (LD, lights on 0700-1900) throughout pregnancy. Following parturition, all dams were blinded. Groups of 6 dams and their litters were exposed to various lighting cycles postnatally. On postnatal day 10, pups (N=4-6/time point) were killed and their pineal glands removed for determination of NAT activity (method of Deguchi and Axelrod).

In Experiment 1, rats were exposed at birth to either constant darkness (DD) or to a reversed lighting cycle (DL, lights on 1900-0700) for 4, 6, or 8 days followed by DD. Pineal glands were collected at 2-h intervals throughout day 10 and the 24-h profiles of NAT activity were examined. For the DD group and the group exposed to DL from 0-4 days of age, the profiles were rhythmic and entrained to the prenatal LD cycle, with elevated levels from 2100-0500. In contrast, the NAT rhythm of the group exposed to DL from days 0-8 was re-entrained to the postnatal DL cycle (elevated NAT values from 0900-1700). The profile from the group exposed to DL from days 0-6 of age was arrhythmic, suggesting partial entrainment had occurred. Experiment 2 was conducted to determine more precisely the age at which LD entrainment begins. Animals were exposed from birth to a 6-h phase-delayed LD cycle for 4, 6, or 8 days followed by DD. The 6- and 8-day groups exhibited phase-shifted NAT rhythms, while the 4-day group did not. Furthermore, in another study, exposure to the phase shift from days 4-8 was sufficient to shift the NAT rhythm.

Thus, the data show that the potential for LD entrainment of the circadian system begins between postnatal days 4-6, the approximate age when the retinohypothalamic tract is innervating the SCN. (Supported by PHS Grant HD 14427)

163.11 ENTRAINMENT OF CIRCADIAN OSCILLATIONS IN THE HAMSTER FETUS BEFORE DAY 12 OF GESTATION. F.C. Davis, Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

In rodents, circadian oscillations appear to already be present and entrainable in the fetus. As in adults, these oscillations are most likely generated by the suprachiasmatic nucleus (SCN) of the hypothalamus. This is based on day/night differences in metabolic activity of the fetal rat SCN on gestational day 19, three days before birth (Reppert and Schwartz, Science 220: 269, 1983), and on the observation that prenatal phase affects the postnatal phase of rhythms controlled by the SCN (e.g. hamster locomotor activity; Davis and Gorski, Neurosci. Abs. 8, 35, 1982). The activity rhythms of a hamster mother and her pups are approximately synchronous at weaning even when raised in dim constant light (LL). Davis and Gorski (Neurosci. Abs. 9: 625, 1983) found that lesions of the mother's SCN on day 7 of gestation disrupted the synchrony among pups, but that lesions on day 14, two days before birth, did not. This suggests that entrainment of fetal oscillations occurs before day 14. This age is just at the end of SCN neurogenesis (Davis and Gorski, unpublished; Crossland and Uchwat, Dev. Brain. Res. 5: 99, 1982). In the present study, lesions were performed on day 12 (when neurogenesis of the SCN is still occuring) to determine if the completion of SCN neurogenesis is required for entrainment of fetal oscillations. Locomotor activity records were used to select four lesioned mothers with severe disruption of their circadian rhythms. Seven pups from each of these litters were placed in individual cages to determine the phases of their freerunning activity rhythms. The within litter phase distributions were analyzed by the Raleigh test for orientation data. Three of the four litters were clearly synchronized ($P < 0.05$) while the fourth was not ($P=0.1$). Although the completeness of the maternal SCN lesions has not yet been histologically confirmed, it is unlikely that three of the four were incomplete, especially given the disruption of their activity rhythms. Therefore, it appears that by day 12 of gestation, before neurogenesis of the SCN is complete, entrainment of a fetal pacemaker has already occured. Either complete formation of the SCN is not required for it to generate oscillations and to become entrained, or entrainment of some other tissue occurs initially with phase being transferred to the SCN at some later age. Supported by NIH grant 5 R23 HD18686 to FCD and by the Joseph P. Riley Zoology Fund of the Univ. of Virginia.

163.12 CHARACTERIZATION OF THE CIRCADIAN TEMPERATURE RHYTHM (CTR) OF FEMALE AND MALE RATS. I. Rykaszewski* and E. Satinoff. (SPON: F. R. Brush). Dept. of Psychology, University of Illinois, Champaign, IL 61820.

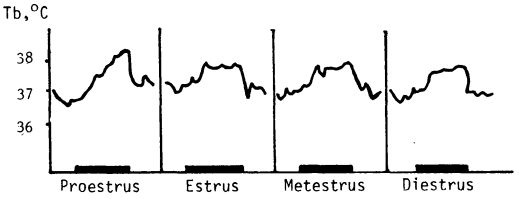
Adult rats have prominent 24-hour rhythms in body temperature (Tb), with the trough occurring during the lights-on portion of a 12:12 LD cycle, and the peak occurring during lights-off. Here we describe the CTR of cycling females and compare it to that of intact males.

Tb's were recorded at 10-min intervals from implanted telemetry devices in intact male and female hooded rats of the Blue Spruce strain. Data were collected for 5 estrous cycles in the females, and for 15-20 days in the males.

The amplitude of the CTR varied from about 2.7°C on proestrus to 2.2°C during metestrus, diestrus, and estrus. In 3 out of 4 females, the increased amplitude was produced by both an elevation in the peak Tb and a lowered trough. There was a less marked tendency for the trough of the CTR to be elevated on the day of vaginal estrus. These changes in amplitude resulted in a decrease and increase in mean daily Tb on proestrus and estrus, respectively.

There were changes in the waveform and phase of the CTR across the cycle (Fig). The peak of the CTR was phase-delayed on the night of proestrus, and the rhythm was sawtooth in shape when compared to the squarewave pattern observed during the other days of the cycle.

The characteristics of the the CTR of the males (mean daily Tb, amplitude, and phase) were most similar to that of diestrous females.

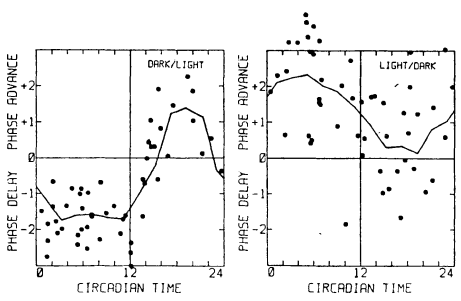


Averaged wave forms of hourly means of Tb across 5 estrous cycles of a female rat. Black bars = 12 hr lights off.

Supported by NSF grant BNS8311466

163.13 RESPONSE OF THE HAMSTER CIRCADIAN SYSTEM TO TRANSITIONS BETWEEN LIGHT AND DARK. H.E. Albers. Dept. of Physiol., Univ. of Mass. Med. Sch., Worcester, MA 01605.

Much of our current knowledge of how light-dark (LD) cycles entrain circadian clocks has come from studies documenting how brief pulses of light can phase shift circadian rhythms. However, the specific components of the light pulse, i.e. the transition between dark and light (D/L transition), light and the transition between light and dark (L/D transition) to which the circadian system responds have not been thoroughly defined. To determine how D/L and L/D transitions phase shift circadian rhythms, adult male hamsters were switched between constant light and constant dark at 10 day intervals at various times within the circadian cycle. When exposed to D/L transitions during the 12 hr prior to activity onset (subjective day) the activity rhythm was consistently phase delayed by approximately 1.5 hr. In contrast, L/D transitions provided during the subjective day produced phase advances ranging from approximately 1.5 to 2.0 hrs. During the early subjective night (until approx. 3 hrs. after activity onset) D/L transitions produced phase delays, however during the remainder of the night D/L transitions produced advances of up to 2 hrs. L/D transitions tended to produce small advance shifts during the subjective night. In summary, both D/L and L/D transitions produce distinct shifts in circadian phase throughout the circadian cycle. When combined additively, the phase shifts produced by D/L and L/D transitions mimic those produced by brief pulses of light. (Supported by NIH GM 34798).



163.14 CIRCADIAN PHASE-REPONSE CURVE BASED ON OSCILLATING VISUAL SENSITIVITY. Michael Terman and Jiuan Terman*. Dept. of Psychiatry, College of Physicians & Surgeons, Columbia Univ., and New York State Psychiatric Inst., New York, NY 10032.

Mammalian and avian circadian phase-response curves (PRCs) have typically been time-referenced to motor-activity rhythms measured in continuous darkness (DD). A discrete bright-light pulse presented early in the subjective night induces a lasting phase delay; late in subjective night, an advance; and during the subjective day, little or no response. This profile reveals the underlying mechanism of entrainment of non-24-hour free-running rhythms to daily light-dark cycles: animals with free-running periods greater than 24 hours, for example, adjust their daily pattern to accommodate phase-advances correcting for the deviation.

We have previously reported that visual sensitivity, measured in continuous signal-detection performance, shows circadian rhythmicity exclusive of motor-activity rhythms controlled by the suprachiasmatic nucleus (SCN) pacemaker (Terman, M. & Terman, J. Ann. N.Y. Acad. Sci., 1985, in press). Rats with and without SCN lesions show similar oscillations of dark-adapted detectability given dim light signals, under both DD and skeleton-photoperiod entrainment. The signals themselves are of insufficient intensity to induce phase responses.

In the present experiment, bright Vita-Lite fluorescent pulses (approx. 350 lux, 1-h duration) were occasionally presented against the DD background in unlesioned animals, at varying phases of free-running visual sensitivity, without reference to concurrent behavioral activity. Phase adjustments of the sensitivity rhythm were monitored for at least two weeks before another pulse was given. The resulting PRC is similar to that obtained by activity measurements.

Since ocular input induces phase responses in both motor activity and visual sensitivity, it is possible that there are redundant PRC processes governed by separable SCN and visual pacemakers. Alternatively, the two classes of PRC may be hierarchically organized, with SCN phase responses deriving from visual phase responses.

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163.15 SUPPORT FOR THE NON-PARAMETRIC MODEL OF ENTRAINMENT IN THE DIURNAL PRIMATE Saimiri sciureus. J.S. Ferraro and F.M. Sulzman*. Dept. of Biol. Sci., SUNY-Binghamton, Binghamton, NY 13901.

Feedback lighting (LD_{FB}), which provides illumination only during the subjective night (i.e. the photosensitive portion of the circadian cycle) in response to a given behavior, has previously been used to test the non-parametric model of entrainment in nocturnal rodents (Ferraro, PhD Thesis, 1984). In three species (Rattus norvegicus, Mesocricetus auratus, and Mus Musculus), the free-running period (tau) of the locomotor activity rhythm was similar whether the animals were exposed to continuous light (LL) or discrete light pulses occuring only during the subjective night (i.e. LD_{FB}). The results of these experiments support the non-parametric model proposed by Pittendrigh and Daan (J. comp. Physiol. 106:291, 1976). In the current experiment, LD_{FB} was presented to 4 squirrel monkeys so that light fell predominately during the subjective night. LD_{FB} was linked to the drinking behavior in this diurnal primate so that when the animal drank the lights went out. Despite this seemingly adversive predicament the monkeys maintained regular circadian drinking rhythms. Furthermore, just as the free-running activity rhythms of nocturnal rodents exposed to LL or LD_{FB} were similar, the drinking rhythms of the squirrel monkeys in LL and LD_{FB} were comparable. Mean taus for squirrel monkeys exposed to LL and LD_{FB} were similar (25.6 and 25.8 respectively), despite a substantial decrease in the total amount of light exposure associated with LD_{FB}. Therefore, discrete light pulses given predominately during the subjective night are capable of simulating the effects of LL on the free-running period of the drinking rhythm of a diurnal primate as would be predicted by the non-parametric model and as was previously shown for three nocturnal rodents. These results thus lend support for the model and further suggest that the major entrainment mechanism are similar in nocturnal rodents and diurnal primates. (Supported by BRSG Grant SO7RR07149-11.)

163.16 ELECTROCONVULSIVE THERAPY: EFFECTS ON SPLIT CIRCADIAN ACTIVITY RHYTHMS OF HAMSTERS. J.D. Hallonquist and J.S. Brandes. Dept. of Psychiatry, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada.

Abnormal circadian organization has been reported in patients with severe mood disorders. An abnormally fast circadian oscillator and/or altered coupling between at least 2 oscillators may be present in some uni- and bipolar patients. If a causal relationship exists between mood disorders and circadian dysfunction, then clinically effective therapies should correct such abnormalities. Although lithium and antidepressant drugs are reported to slow or delay circadian rhythms in animals, no such reports exist for electroconvulsive therapy (ECT). This study examined effects of ECT on abnormal coupling between 2 oscillators that is sometimes observed in hamsters housed in constant light (LL).

Twenty-eight male hamsters had been individually housed in cages with activity wheels, in LL of approx. 75 lux, and had demonstrated a stable 180 degree split of the normal free-running activity rhythm for at least 1 month. Seventeen hamsters were randomly selected to receive a single administration of ECT (75 mA, 1 msec pulses @ 100 pps for 0.8 sec) via saline-soaked, gauze-covered ear clips. The remaining 11 hamsters received a single administration of sham-ECT during which no current was passed. Hamsters were anesthetized with a mixture of N_2O (2L/min) + O_2 (1L/min) passed over ethrane (2% V/V) during administration of ECT or sham-ECT, both of which were distributed over circadian time. Rhythms were then monitored for at least one additional month.

Overall, ECT had obvious chronotypic effects on the split activity rhythms of 10 (59%) of 17 hamsters, while sham-ECT had but a small effect (1 lengthening, 1 phase-delay) in only 2 (18%) of 11 hamsters -- a difference that is significant at the $p.05$ level (Chi-square). ECT lengthened (3), phase-delayed (2), phase-advanced (2) or actually fused (3) the split activity components. Most interesting was the fusing, which presumably reflected a differential effect on the 2 oscillators, never followed sham-ECT, and only very rarely occurs spontaneously in hamsters with split circadian rhythms. Further experiments are examining to what extent the type of response to ECT reflects the timing of its administration relative to the split components.

These results with ECT and our previous results with lithium (Soc Neurosci Abstr 1984, 10, 503) suggest that the split circadian rhythm preparation of hamsters may be particularly useful for assessment of chronotypic properties. The slowing of rhythms and normalization of coupling following ECT support the circadian dysfunction hypothesis of mood disorders. This work may contribute to a more effective clinical use of ECT, and the eventual development of less invasive therapies that correct circadian abnormalities (Supported by the Ontario Mental Health Foundation).

163.17 CIRCADIAN FACTORS IN MONKEY PERFORMANCE: EFFECTS OF TASK, PHASE SHIFTS AND CONSTANT LIGHT. W. N. Tapp*, B. H. Natelson, D. Creighton*. (SPON: S.D. Cook). Primate Neurobehavioral Unit, VA Medical Center & New Jersey Medical School, East Orange, NJ 07019.

We have developed a task where rhesus monkey performance exhibits important features of circadian influences on human performance. Monkey performance on this chained vigilance-discrimination task exhibits task dependent differences in the time of peak performance (discrimination peaks several hours before vigilance, $p<.01$) and in the time required to recover from performance deficits following a 6 hr phase shift (discrimination recovers significantly faster than vigilance, $p<0.01$). These task differences correspond to differences in humans where performance on "more cognitive" tasks peaks earlier during the day and recovers more rapidly after a phase shift than performance on simple repetitive tasks. Similarly, phase shifts produced the same sort of transient internal desynchronization in monkeys that they do in people, with activity resynchronizing significantly faster than temperature.

After characterizing performance in LD 12:12, we released the monkeys into constant light (LL). The task was available in LL from 0900 to 1700. Monkeys showed significant performance deficits in LL. Average vigilance performance was 15.1% worse in LL than in LD 12:12 ($p<.01$). Average discrimination performance was 14.6% worse in LL than in LD 12:12 ($p<.01$). While these deficits were not as severe as those associated with phase shifts, they were much longer lasting, persisting for over 100 days without sign of recovery. Performance deficits in LL were not due to internal desynchronization. During the period studied, the monkeys' circadian temperature and activity rhythms maintained stable periods and phase relationships. Indeed, both rhythms were synchronized to the time of the task with 24 hr periods and positive phase angles with respect to the task. Thus, LL represents a light schedule that produced significant performance deficits despite the fact that the monkeys were internally synchronized.

The pattern of behavioral change and physiological rephasal shown by rhesus monkeys after phase shift closely resembles that seen in humans. Thus, this model provides a unique opportunity to study performance and physiological function after chronobiological manipulations that would be too expensive or impossible to study in humans.

Supported by USARMDC

164.1 NORADRENERGIC INHIBITORS MODULATE THE CONCENTRATION OF FUNCTIONAL ESTROGEN RECEPTORS IN FEMALE RAT HYPOTHALAMUS. J.D. Blaustein, T.J. Brown* and E.S. Swearengen*. Division of Neuroscience and Behavior, University of Massachusetts, Amherst, MA 01003.

We have reported previously that after injection of the dopamine-β-hydroxylase (DBH) inhibitor, U-14,624, in ovariectomized female rats, the concentration of cytosol ERs in the mediobasal hypothalamus (MBH) and anterior pituitary gland (AP) decreased. This decrease, however, was mirrored by an increase in the concentration of cell nuclear ERs. U-14,624 appears to be anomalous in this action, because we now report that a variety of noradrenergic inhibitors decrease the concentration of cytosol ERs in the MBH (and in some cases the AP) without increasing the concentration of nuclear ERs. The DBH inhibitors, FLA 63 and diethyldithiocarbamate (DDC) both caused a biphasic effect on the concentration of cytosol ERs in MBH; the concentration first increased (by 13 - 27%) and then decreased (by approximately 21%), compared with the level in vehicle-injected rats. This biphasic effect occurred in the absence of any detectable change in the concentration of nuclear ERs. Inhibition of DBH decreased the concentration of cytosol ERs in the MBH and preoptic area-septum, but not in the amygdala, suggesting that the decrease is not due to a nonspecific effect on all cytosol ERs. Finally, an injection of estradiol-17β in rats that had been pretreated with either DDC or FLA 63 resulted in less accumulation of cell nuclear ERs than in vehicle-injected controls only in those tissues in which the drug first caused a decrease in the concentration of cytosol ERs. Therefore, under some conditions, pretreatment with a DBH inhibitor decreased the concentration of functional cytosol ERs resulting in subsequent decreased nuclear ER accumulation in response to an estradiol injection.

Treatment with the α_1-adrenergic antagonist, prazosin, also caused a decrease in the concentration of cytosol ERs in MBH and AP by 8 hours after injection, suggesting that the decrease seen after injection of DBH inhibitors is dependent on decreased α_1-adrenergic receptor stimulation. Similarly to the case using DBH inhibitors, injection of estradiol-17β caused a decreased cell nuclear accumulation of ERs in rats that had been pretreated with prazosin. The results of these experiments support the hypothesis that the catecholamines have a role in modulating the sensitivity to estradiol in some hypothalamic neurons and, in some circumstances, in the AP. (Supported by NS 19327 from the N.I.H.)

164.2 HORMONAL MODULATION OF NOREPINEPHRINE-INDUCED CYCLIC AMP ACCUMULATION IN SLICES FROM FEMALE RAT HYPOTHALAMUS AND PREOPTIC AREA. Anne M. Etgen and Nicola Petitti*. Department of Biological Sciences, Rutgers University, New Brunswick, NJ 08903.

To examine the interactions between ovarian hormones and brain noradrenergic systems, cyclic AMP (cAMP) accumulation in slices from the preoptic area (POA), anterior hypothalamus (AH), middle hypothalamus (MH), and posterior hypothalamus (PH) were incubated in vitro for 20 min in the presence or absence of 10 uM norepinephrine (NE). Prior to slice preparation, the endocrine state of the animals was varied. In the first experiment, slices were obtained from animals in mid to late diestrus or in proestrus (after the preovulatory LH surge). NE treatment of tissue from diestrous females produced robust increases in cellular cAMP content in slices from all regions except the PH. Of the remaining regions, the POA and AH showed significantly greater cAMP elevations than the MH. In contrast, slices taken from proestrous females showed small, statistically insignificant changes in cAMP content in all four brain regions. A second experiment evaluated NE-induced cAMP accumulation in ovariectomized (OVX) females. The basal level of cAMP in most slices from OVX rats was significantly higher than that observed in either diestrous or proestrous animals. NE treatment produced significant increases in cAMP in POA, AH and PH, but not in MH slices from OVX animals, but the levels were not as great as those in NE-treated slices from diestrous females. A third experiment evaluated the effects of estrogen and estrogen plus progestin treatment in OVX rats. Females which had been OVX for at least 4 days received 2 ug of estradiol benzoate 24 and 48 hr before sacrifice (EB) or this same estrogen treatment followed by 500 ug of progesterone 3.5 hr prior to sacrifice (EB+P). Basal levels of cAMP were reduced in both treatment groups when compared to OVX rats. As in the diestrous rats, POA, AH and MH slices from EB rats showed large NE-induced elevations in cAMP concentration. On the contrary, the EB+P rats, like the proestrous animals, showed little change in cAMP level following exposure to NE in vitro. These data suggest that ovarian steroids can alter neural responsiveness to NE in some diencephalic regions known to participate in the hormonal regulation of behavior and anterior pituitary hormone release. Supported by Grant No. MH36041 and a grant from the Charles and Johanna Busch Memorial Fund.

164.3 IMPACT OF ADRENALECTOMY ON α1 AND α2-NORADRENERGIC RECEPTORS IN THE RAT BRAIN: RELATION TO SPONTANEOUS FEEDING. M. Bailo*, M. Jhanwar-Uniyal*, A.D. Factor*, G. Beitmirza* and S.F. Leibowitz. (SPON: S. Berl) The Rockefeller University, New York, NY 10021.

Adrenalectomy (ADX) has been shown to down-regulate α2-noradrenergic receptors specifically in the paraventricular nucleus (PVN) (Jhanwar-Uniyal et al., 1984). Furthermore, α2-noradrenergic receptors of this nucleus are known to be involved in the stimulation of feeding behavior, and this effect requires the presence of circulating corticosterone(CORT) (Leibowitz et al., 1984). To investigate further the modulatory role of CORT on these PVN receptors and ultimately feeding, the present study was undertaken to investigate: 1) The influence of chronic ADX on α1 and α2-noradrenergic receptor density in brain regions analyzed via Scatchard analysis; 2) The impact of short term (6h and 12h) versus chronic (7 days) ADX on α2-noradrenergic receptors in the PVN; and 3) The effect of ADX on spontaneous food intake during the light versus dark period.

Male albino rats were sacrificed by decapitation 6h, 12h and 7 days after ADX or SHAM surgery. The brains were rapidly removed, frozen on dry ice, and the brain areas, either PVN, or medial hypothalamus, lateral hypothalamus, and frontal cortex, were microdissected. Standard radioligand binding procedures were employed using the α2-noradrenergic agonist [^{3}H]p-aminoclonidine ([^{3}H]PAC, 0.5-10.0 nM; 3.0 nM for discrete PVN) and the α1-noradrenergic antagonist [^{3}H]prazosin (0.1-1.4 nM). Nonspecific binding was determined in the presence of phentolamine (50 uM). For the spontaneous food intake study, daily food intake (lab chow pellets) was measured at 12h intervals over a period of 24h in pre-operative, SHAM and ADX rats.

The results indicate that: 1) Following ADX, high and low affinity [^{3}H]PAC binding were attenuated, respectively, by 28% and 21%, specifically in the medial hypothalamus; 2) The [^{3}H]prazosin binding (Bmax), as well as the K_D for both ligands, remained essentially unchanged in all brain areas examined; 3) ADX, at 6h, 12h, and 7 days, down-regulated α2-noradrenergic receptors in the PVN by 88%, 57% and 53%, respectively; 4) Compared to SHAM scores or pre-operative baselines, the ADX showed a significant attenuation (-51%) of food intake only in the dark period.

The results suggest that ADX influences the [^{3}H]PAC high affinity binding only in the medial hypothalamus, without affecting [^{3}H]prazosin binding. The present study also concludes that ADX down-regulates α2-noradrenergic receptors in the PVN, which may in turn cause a reduction in food intake in ADX rats. These findings suggest that PVN α2-noradrenergic receptors and circulating CORT have an important role in the regulation of food intake.
(This research was supported by NIH grant MH 22879.)

164.4 PROGESTERONE MODULATES CEREBELLAR PURKINJE CELL RESPONSIVENESS TO GAMMA-AMINOBUTYRIC ACID (GABA) AND GLUTAMATE (GLUT). Sheryl S. Smith, B.D. Waterhouse and D.J. Woodward. Cell Biology, UTHSC, Dallas, TX 75235.

In a previous report we have shown that both intravenous and iontophoretic application of 17 Beta-estradiol (E2) are capable of increasing cerebellar Purkinje (P) cell responsiveness to microiontophoretically applied GLUT in the halothane-anesthetized, ovariectomized adult rat. In the present study we have examined the effects of another sex steroid, progesterone (P) and combinations of varying doses of both E2 and P on responses of individual P cells to GABA and GLUT. Extracellular activity of single Purkinje neurons was recorded using multibarrel glass micropipets. Spontaneous firing rate and responses of neurons to microiontophoretic pulses (10s pulses every 40s) of GABA (10-50 nA) and GLUT (3-40 nA) were examined before and after jugular i.v. administration of P, E2 or E2/P. In some cases animals received s.c. injections of E2 (2 μgs) at 24 and 48 h before the day of recording. This injection schedule, when followed by P administration, induces reproductive behavior. Within 5-15 min after P administration (.5 mgs) to ovariectomized rats, P cell responses to GLUT were decreased by 45% (13 of 14 cells), and inhibitory responses to GABA were increased by 50% (6/6 cells), with no associated change in spontaneous firing rate. Complete recovery was observed 20-30 min after P administration (10/14). E2 pretreatment did not alter these P-induced effects; however, the latency of E2-induced increases in P cell responsiveness to GLUT was decreased by 15 min (8/8). Combinations of E2 (2 μgs) and P (.5 or 5 mgs) injected simultaneously resulted in effects similar to those seen with P alone (10/15), while effects similar to E2 alone were observed with administration of E2 plus P at 0.05 mgs (3/3). The administration of a protein synthesis inhibitor, anisomycin (30μg/kg,i.v.), 20 min before the recording session did not prevent any of the above steroid effects (3/3 per condition). The results indicate that sex steroids can act to alter neuronal responsiveness to putative neurotransmitters in a CNS region not known to contain steroid receptors. These findings further suggest that the observed steroid-induced alterations in P cell responsiveness may be due primarily to membrane effects and do not appear to require genomic mechanisms. (Supported by MH09010 to SSS; AA3901,DA02338 & Biological Humanics Foundation to DJW.)

164.5 CHANGES IN CORTICAL SEROTONIN RECEPTORS DURING THE FEMALE RAT ESTROUS CYCLE. L. Uphouse, J. Williams*, K. Eckols*, and V. Sierra*,Dept. Biology, Texas Woman's University, Denton, Texas. 76204

Neurotransmitter changes contribute significantly to the behavioral and neuroendocrine events characteristic of the female reproductive cycle. Most such changes have been seen in the hypothalamus and other basal forebrain areas with high densities of intracellular estrogen receptors. It is also these brain regions where steroidal modulation of neurotransmitter activity has been emphasized. However, estrogen can alter serotonin receptors in cortical tissue which has only minimal quantities of intracellular steroid receptors. It is, therefore, possible that estrogen can directly down-regulate cortical 5-HT receptors. If estrogen's modification of cortical 5-HT receptors is physiologically relevant to cyclic changes in behavior, then such changes should be evidenced during the estrous cycle. Such cyclicity is readily apparent in estrogen receptor rich regions, but cortical changes have not been seen previously. We have now identified cyclic changes in cortical serotonin receptors. The major change occurs between afternoon and evening on the day of proestrus which probably accounts for earlier failures to observe the cyclic variation. In our initial studies, Fisher F-344 female rats were sacrificed during the early afternoon of diestrus, proestrus, or estrus. Cortical serotonin-1 receptors (measured as the specific binding of ^{3}H5-HT) was 30-33% higher on estrus than on proestrus or diestrus. No changes were seen in either ^{3}H-spiroperidol or ^{3}H-ketanserin binding. ^{3}H-5-HT binding was then examined in proestrous and estrous females sacrificed during the afternoon, the early evening or near midnight. These studies demonstrated that a major increase in serotonin binding occurred between 2 and 4 p.m. on the day of proestrus. By 11:00 p.m., proestrous females were not significantly different from the estrous females but were approximately 30% higher than the proestrous females killed during early afternoon. Comparison by scatchard analysis of the increased binding during late proestrus and estrus suggested that the increased binding resulted from a significant increase in B_{max} and only a small change in K_D. The results of these studies are consistent with the reported ability of estrogens to down-regulate cortical serotonin-1 receptors. Cortical 5-HT binding was lowest on the morning of proestrus when estrogen levels are highest. There followed an upregulation of serotonin receptors in the cortex as estrogen levels decrease throughout the day. Such upregulation of serotonin receptors on the evening of proestrus and day of estrus may be a useful model system for the study of hormonally related behavioral disorders.

164.6 TRANSIENT CESSATION OF THE PERSISTENT ESTROUS AFTER NUCLEUS SUPRACHIASMATICUS LESION BY LOCUS COERULEUS DAMAGE. L.P. Solano-Flores*, H.U. Aguilar-Baturoni, O.A. Donatti-Albarrán*, C. Santos-Toledo* and R. Guevara-Aguilar. Depto. Fisiología, Fac. Medicina, U.N.A.M. Apdo. Postal 70250, 04510-México, D.F.

We have shown that the electrolytic damage of the noradrenergic nucleus locus coeruleus (LC) causes a transient cessation of the estrous cyclic activity of the non-previously manipulated female rat, effect which was caracterized by a diestrous aspect of the vaginal smears. It could be seen that the number of days after lesion in which diestrous smears were observed depended upon the amount of LC destroyed. Then, it seems that LC might be participating in the modulation of the normal expression of the estrous cyclic activity. Thus, we have in one side that a cyclic activity was disturbed by the anatomical impairment of the LC function. In the other side, we have the facts that the hypothalamic nucleus suprachiasmaticus (SCN) recieves direct retinal input, that the destruction of the SCN disrupts various physiological rhythms, that the lesion of the SCN induces a persistent estrous, and, that persistent estrous can be induced by exposure to constant bright light. Our laboratory has also already reported a transient supression of the persistent estrous induced by constant bright light by damage to the LC. This fact suggests a posible participation of the LC in the activity of those brain regions which mediate the persistent estrous response to constant bright illumination. This work was done with the aim to know whether the LC electrolytic damage disturbs the persistent estrous induced by lesion of the SCN. Vaginal smears were sampled daily during 100 days from mature and virgin Wistar rats. The samples were stained and analysed at 400x magnification. After at least four control cycles, the SCN area was electrolitically damaged bilaterally with a bipolar stainless steel electrode. Then, after at least ten days of a well stablished persistent estrous, the LC was electrolitically damaged monolaterally. Inmediately after the LC damage, the vaginal smears showed a diestrous aspect, situation which was maintained during some days before the smears showed an estrous aspect again. Two rats did not reinstalled the persistent estrous. The sham operated control animals continued cycling. The extension of both lesioned areas were evaluated. The present results suggest a possible functional interaction of the LC and the SCN in the regulation of the estrous cyclicity in the female rat.

This work was supported by CONACYT Grant PCSABEU-002187.

164.7 EFFECTIVE ORAL ADMINISTRATION OF 17β-ESTRADIOL THROUGH THE DRINKING WATER OF FEMALE C57BL/6J MICE. M. N. Gordon, H. H. Osterburg*, P. C. May and C. E. Finch, Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089-0191.

17β-Estradiol (E_2) was added to the normally acidified drinking water of female C57BL/6J mice and presented ad libitum. This oral administration paradigm is effective, reliably producing a variety of E_2-sensitive responses in several different target tissues. Vaginal smear cytology changed from the predominantly leukocytic smear characteristic of ovariectomized control mice to a smear consisting entirely of cornified epithelial cells. Uterine wet weight, and the specific activities of two uterine enzymes, glucose-6-phosphate dehydrogenase and alkaline phosphatase were each elevated in a dose-dependent manner by E_2 administered through the drinking water. E_2 administration did not produce global alterations in all uterine proteins, as the specific activity of acid phosphatase was unaffected by E_2 in this dose range. Finally, the postovariectomy elevation in serum LH was suppressed in a dose-dependent manner after E_2 administration through the drinking water. A dose of 94 μg E_2/kg body weight/d administered through the drinking water for 7 d was sufficient to maximally elevate uterine wet weight 5.5-fold and uterine glucose-6-phosphate dehydrogenase specific activity 75%, and to maximally suppress serum LH to nondetectable levels.

Oral administration of E_2 through the drinking water did not produce tonic elevations of circulating E_2, but rather, produced a dynamic flux of serum E_2 qualitatively similar to that observed after subcutaneous injections of E_2. Thus, significant elevations of serum E_2 were observed when mice were sacrificed at night (2300-0300 h; lights out at 1700 h), 2-6 h after the peak in drinking rate occurred, but not when sacrificed in the late afternoon (1400-1800 h), 15-21 h after the peak drinking period.

The oral route of administration has advantages over the more conventional routes of E_2 administration, injection or surgical implantation of E_2-containing capsules, particularly for long-term experiments. Administration of E_2 through the drinking water is simpler and less labor-intensive, less stressful for experimental animals, does not require surgery, and may be more analogous to the normal, oral route of estrogen administration in women as oral contraceptives or as postmenopausal estrogen replacement therapy.

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164.8 CASTRATION INDUCES NUCLEOLAR PROLIFERATION IN NEURONS OF THE MEDIAL AMYGDALOID NUCLEUS. S.W. Newman*, B. Bramoweth, and J.M. Swann, Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI.

The output of the medial nucleus of the amygdala (M) is essential for normal mating behavior in the male Syrian hamster. It is through this nucleus that chemosensory signals from the female drive sexual behavior. The chemosensory input to M is via direct projections from the accessory (vomeronasal) olfactory bulb and indirect projections from the main olfactory bulb. Circulating testosterone is also essential for normal male sexual behavior, and the neurons in M actively accumulate androgens. We have hypothesized that castration induces morphological changes in these neurons which might alter their ability to transmit chemosensory information.

Young adult male Syrian hamsters were castrated either 1 week (n=8) or 8 weeks (n=8) prior to sacrifice. Half of the males in each group were given testosterone propionate (TP) (500 ugm in peanut oil injected SQ every other day) before sacrifice. Brains of castrated males (with or without TP) and intact control animals (n=4) were dissected into small blocks containing the corticomedial amygdala. Blocks were embedded in glycolmethacrylate (Polysciences JB4), sectioned in the coronal plane at 6 um (av. size of nuclei of neurons in M=6-8um) and stained with hematoxylin and eosin. One section each from the rostral and caudal parts of M was chosen from each brain for analysis. On each section all neurons containing nucleoli (approximately 100 neurons per section) in each of 3 nonoverlapping grid areas within M were scored as containing single or multiple nucleoli. Our preliminary results show a significant increase in the number of neurons with multiple nucleoli 1 week after castration, followed by a reduction to slightly above control levels after 8 weeks. The TP treatment either prevents this change (in 1 week castrates) or restores the neurons to their precastration condition (when given for several weeks in the regimen described, which restores mating behavior in long term castrates).

Since multiple nucleoli normally occur in cells during periods of increased protein synthesis, these data suggest that protein synthesis in the neurons of M is enhanced for a limited period after castration. Further research is needed to determine whether this is a qualitative or quantitative change in protein synthesis and in what way this change influences the structural and functional integrity of these neurons.

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164.9 QUANTITATION OF ANGIOTENSIN-1-CONVERTING ENZYME KINETICS IN INDIVIDUAL RAT PITUITARY AND ADRENAL GLANDS WITH ^{125}I-MK351A, A SPECIFIC ENZYME INHIBITOR.. L.M.Plunkett*, F.M.A.Correa and J.M.Saavedra. Section on Psychopharmacology, NIMH, Washington, D.C. 20205-1000.

We describe a quantitative autoradiographic technique which allows measurement of angiotensin-1-converting enzyme (ACE) levels as well as enzyme kinetics in discrete areas of the pituitary gland and adrenal gland in individual animals. Thin tissue sections (16μm) were incubated with ^{125}I-MK351A, a specific ACE inhibitor, and results were obtained with computerized densitometry followed by comparison of the optical densities generated with ^{125}I-standards. Compound 351A is a p-hydroxybenzamidine derivative of MK521, which is a Lys-Pro analog of MK422, the active form of the specific ACE inhibitor, Enalapril. By iodination of 351A, a specific marker for ACE is available for the compound will react in a one-to-one ratio with endogenous ACE, providing an estimate of availabe enzyme.

There were high levels of ACE present in both the anterior and posterior lobes of the rat pituitary gland. The maximum binding capacity (Bmax) was 920±62 fmol/mg protein for the anterior pituitary and 1162±67 fmol/mg protein in the posterior pituitary. The binding affinity constant (Ka) was 0.95±0.11 x 10^9M^{-1} and 1.20±0.19 x 10^9M^{-1} for the anterior and posterior lobes respectively. There was no detectable ACE binding with compound 351A in the intermediate lobe of the pituitary gland.

In the adrenal gland, there were two distinct areas of 351A binding, the adrenal medulla and the adrenal capsule-zona glomerulosa area. The Bmax for the medulla was 652±80 fmol/mg protein and 294±53 fmol/mg protein for the zona glomerulosa. The Ka for 351A was 1.04±0.19 x 10^9M^{-1} and 1.74±0.40 x 10^9M^{-1} for medulla and zona glomerulosa respectively. Other adrenal cortex areas showed no detectable ACE binding with compound 351A.

The results support the existence of a local ANG system in both the pituitary and adrenal glands. Each tissue had a distinct value for Bmax and Ka of the compound 351A/ACE interaction. With this method we can also make comparisons in ACE levels between individual animals in discrete tissue areas. Kinetic analysis of enzyme activity through an ACE-351A binding reaction provides even more information concerning ACE function.

64.10 NEURONAL CaATPase ACTIVITY AND CHANGES IN INTRACELLULAR Ca^{2+} INDUCED BY SHORT-TERM EXPOSURE TO THYROID HORMONE. L. Kragie-Ahmed*, Z. Ahmed, J. A. Connor and P. J. Davis*. Endocrin. Div. VAMC, Buffalo, NY 14215, Div. Neurobiol., SUNYAB, Buffalo, NY 14214 and Mol. Biophys., AT&T Bell Lab., Murray Hill, NJ 07974.

A thyroid hormone stimulable Ca^{2+}-dependent Mg^{2+} ATPase activity (CaATPase) has been described in RBC, reticulocyte and sarcolemma preparations. Here, we report for the first time, stimulation of a CaATPase activity by thyroxin (T_4) and triiodothyronine (T_3) in a membrane preparation obtained from a primary neuronal culture grown in serum-free, defined medium. Using Ca^{2+}-sensitive fluorescent indicators, quin 2 and fura 2, we also found a decrease in intracellular calcium upon short-term exposure to T_4. Whole cell voltage clamp experiments showed that the presence of 10^{-9} M T_3 or T_4 in the bath did not alter the primary membrane currents.

Four to six day old cultured neurons from fetal rat diencephalon and cerebral cortex were subjected to hypotonic lysis and mechanical shear. After removal of the nuclei (700xg pellet) "membrane fraction" (10Kxg pellet) was resuspended and aliquoted into isotonic saline containing 0.1 mM oubain, 1 mg/ml oligomycin, 1 mM $MgCl_2$, 0.1 mM EGTA and ± 0.1 mM $CaCl_2$. The incubation was 60 min at 37°C. The concentration of hormone was varied between 10^{-11} to 10^{-8} M. Membrane CaATPase activity in the presence of 10^{-11} M T_3 was 140% greater than the no TH control. Peak activity in presence of 10^{-10} M T_4 was 60% greater than control. Similar data were also obtained from mature rabbit synaptic plasma membrane. The CaATPase activity seems to show differential sensitivity to T_3 and T_4 according to region of origin.

Cells growing on polylysine coverslips were loaded with either quin 2 or fura 2. Fluorescence of small groups of cells was imaged using a CCD-based system (see Connor, J.A., these abstracts). Following 5 to 10 min. exposures to 10^{-9} M T_4, Ca^{2+} was significantly lower in a fraction of the cells on a given plate. In other cells on the same plate the $[Ca^{2+}]$ remained constant.

164.11 RENIN RELEASING PEPTIDE AND THE SEROTONERGIC REGULATION OF RENIN SECRETION. L.D. Van de Kar and J.H. Urban*, Department of Pharmacology, Loyola University School of Medicine, Maywood, Illinois 60153.

In previous studies (Fed. Proc. 44, 6774, 1985), we showed that administration of the serotonin releaser p-chloroamphetamine (PCA) leads to the appearance of a renin releasing factor in plasma. This factor is heat resistant and is in the molecular weight range of 500-10,000 daltons. Using an improved kidney slice method, we replicated these studies and observed that the molecular weight range is 5,000-10,000 daltons. When we fractionated plasma from PCA treated rats by ultrafiltration into two fractions, one with solutes in the 1,000-5,000 daltons and the other 5,000-10,000 dalton range, only the heavier fraction increased renin release from kidney slices. Incubation of this plasma fraction (5,000-10,000 daltons) with a non-specific protease (pronose) completely abolished its ablility to release renin from the kidney slices, suggesting that the renin releasing factor is a peptide. Co-incubation of the kidney slices with the renin releasing factor and propranolol (10^{-6} M) does not abolish the renin releasing ability of this renin releasing factor, suggesting that this effect is not mediated via a beta receptor. In conclusion, the present studies suggest that release of serotonin in the brain triggers the release of a peptide into the blood that directly stimulates renin release from the kidney. The source of this peptide is presently unknown.

164.12 EFFECT OF 1,25DIHYDROXYVITAMIN D_3 ON CHOLINERGIC ACTIVITY IN DISCRETE NUCLEI OF THE RAT BRAIN AND ON TESTOSTERONE IN PLASMA. J. Sonnenberg*†, V. N. Luine*, L. Krey* and S. Christakos†, †UMDNJ-New Jersey Medical School, Newark, NJ 07103 and The Rockefeller University, New York, NY 10021

In an effort to obtain a better understanding of the role of the calcium regulating hormone, 1,25dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) in brain function, the activities of choline acetyl transferase (CAT) and monamine oxidase (MAO) were measured in discrete brain nuclei of vitamin D deficient and replete male rats. The nuclei sampled were those in which receptors for 1,25$(OH)_2D_3$ and/or vitamin dependent calcium binding protein (CaBP) have been identified. Significant elevations in CAT ($p < 0.05$) were observed in the arcuate-median eminence of the hypothalamus (A-ME) and in the bed nucleus of the stria terminalis (St) in rats made vitamin D replete by 8 daily intraperitoneal (i.p.) injections of 100 or 200 ng 1,25$(OH)_2D_3$ as well as by constant intraventricular infusion (i.v.i.) by ALZET minipumps of 25 ng 1,25$(OH)_2D_3$ for 7 days. The percent increase ranged from 12-45% and was related to the i.p. dose administered. Constant i.v.i. of 2 mM Ca^{++} or 125 ng 25$(OH)D_3$/day for 7 days did not alter CAT activity. No significant changes in MAO or CaBP in discrete brain nuclei were observed with vitamin D repletion. Since the A-ME is an important regulatory site in the neuroendocrine axis, serum testosterone was measured by radioimmunoassay (RIA). Serum testosterone was increased 2-5 fold ($p<0.05$) in rats made vitamin D replete by constant i.v.i. of 25 ng 1,25$(OH)_2D_3$ or by i.p. injection of 100 or 200 ng 1,25$(OH)_2D_3$. Testosterone treatment of gonadectomized male rats does not similarly alter CAT activity in these nuclei. Our results suggest that 1,25$(OH)_2D_3$ effects cholinergic activity in discrete brain regions and that 1,25$(OH)_2D_3$ may play a role in the regulation of certain neurohormones.

164.13 LOCALIZATION OF SEROTONIN IN THE CAUDAL NEUROSECRETORY SYSTEM. S.L. Cohen*, K.E. Miller, and R.M. Kriebel (SPON: M. Moffroid). Dept. of Anatomy & Neurobiology, Univ. of Vermont Col. of Med., Burlington, VT 05405, and Dept. of Anatomy, Univ. of Minnesota Med. Sch., Minneapolis, MN 55455.

Several neuroendocrine systems have been shown to receive projections containing serotonin, which has been implicated as a neurotransmitter in some of these nuclei. The caudal neurosecretory complex (CNC) of poecilids is an isolated neuroendocrine nucleus that is suited for study of the effects of putative neurotransmitters on neuroendocrine cells. This study describes the serotoninergic innervation of the CNC. Fish were anesthetized with MS-222 and perfused with 4% paraformaldehyde in 0.1M phosphate buffer. Cryostat sections (8 μm) or frozen sections (20 μm) were cut from the terminal spinal cord and processed for immunofluorescence or peroxidase anti-peroxidase immunohistochemistry respectively. Serotonin was identified with an antisera raised against a serotonin/BSA conjugate (Immunonuclear). Preabsorption of the antisera with this substance eliminated all labeling in the CNC. Sections for immunofluorescence were counterstained with ethidium bromide, and sections for immunoperoxidase were osmicated, in order to visualize anatomical relationships between labeled fibers and unlabeled cells. A dense network of long parallel fibers with multiple varicosities were observed within the CNC. Close appositional relationships were noted between these fibers and CNC neurosecretory cells, but most fibers were directed toward or located within the neuroendocrine axonal tract. The apparent origin of these fibers was rostral to the CNC. Several fish were sacrificed one week after the CNC was deafferented by lesioning the spinal cord one segment rostral to the nucleus. The dense plexus of serotonin fibers was absent in these specimens. However, a population of small (5-8 μm) labeled cells was present in both control and experimental animals. Many of these cells were intermingled with the neuroendocrine cells, and a few appeared to be located in the ependymal lining of the central canal. Labeled cells were also seen individually or in clusters on the lateral edge of the CNC, and abutting the ventral portion of the neuroendocrine tract. Most of these intrinsic neurons were round to fusiform with multiple processes. The processes were short and less varicose than the long labeled fibers. The source of some serotoninergic fibers appears to be extranuclear and some arise intrinsically. The source of the extrinsic projection and the role of serotonin in regulating CNC cells are being investigated. The results of this study imply that the CNC receives a dual serotoninergic innervation.

Supported by BNS-8206452.

164.14 OXYTOCIN SECRETION FROM POSTERIOR PITUITARY LOBES OF LACTATING RATS INCUBATED IN VITRO. E. W. Haller and J. B. Gearns*. Dept. of Physiology, School of Medicine, Univ. of Minnesota, Duluth, MN 55812.

Neural lobes were excised from 2- to 10-day postpartum lactating Sprague-Dawley rats and incubated in a modified Locke's solution at 37° C. Oxytocin released into the medium during 10-minute incubation periods was measured by a radioimmunoassay using a specific antibody obtained from Dr. R. P. Elde, Dept. of Anatomy, University of Minnesota. Basal oxytocin output from the glands averaged about 60 pg/min. Cell membrane depolarization by raising K+ concentration of the incubation medium to 56 mM resulted in a 15-fold elevation of oxytocin secretion which was maintained at that level as long as incubation in high K+ was continued. When K+ concentration was restored to normal (5.6 mM), oxytocin secretion promptly returned to basal levels. Incubation of posterior pituitaries at normal K+ but in the presence of oxytocin at 200 pg/ml in the incubation medium produced a 40-fold increase of the oxytocin secretion rate during the first 10 min, which was elevated further during the subsequent five 10-min incubation periods, if oxytocin was present in the medium. On the other hand, incubation of posterior pituitaries in medium containing both 56 mM K+ and 200 pg/ml oxytocin responded in the same manner as in the presence of elevated K+ alone. The present data suggest that oxytocin may participate in the modulation of its own release not only at the level of oxytocinergic cell bodies (Freund-Mercier, M.-J. and Richard, P., J. Physiol. (London), 352: 447, 1984) but at the site of hormone release in the posterior pituitary, as well. The mechanism of such modulation remains to be elucidated. Supported by NIH NICHHD R01-13906.

164.15 Effects of hypophysectomy and arcuate and perifornical lesions on hypothalamic rat growth hormone-releasing factor. C.Y. CHIEN# and L.C. Terry (SPON: S. Berent). Neuroendocrine Lab., Univ. of Michigan and VA Medical Center, Ann Arbor, MI 48105.

The majority of growth hormone-releasing factor (GRF)-immunoreactive cell bodies in the rat brain were shown to be located in the arcuate nucleus and medial perifornical region (MPFX) of the lateral hypothalamus (Merchenthaler, I., et al., Endocrinology 114:1082, 1984). Fibers from the perifornical cell bodies form a fan-like projection to the median eminence (ME), where a dense accumulation of GRF-containing processes and terminals is found. The localization of GRF in the ME supports the hypothesis that this peptide is a regulator of growth hormone (GH) secretion. The objectives of this investigation were to determine (1) the origin of ME GRF, and (2) the effect of hypophysectomy on hypothalamic GRF. In experiment 1, adult male Sprague-Dawley rats (250g, 6-9/group) had thermal lesions placed bilaterally in the arcuate/ventromedial complex (ARC/VMN) or MPFX regions; sham controls had the electrode lowered 2 mm beneath the dura. Animals were sacrificed 7-10 days postoperatively, the ME was removed, sonicated in dist. water, placed in a boiling water bath for 10 min., centrifuged, and the supernatant and pellet were frozen (-80oC) for radioimmunoassay of rat GRF and protein, respectively. Lesion placement was verified histologically. In experiment 2, one hypophysectomized group received thyroxine/dexamethasone (HYPOX), the other received thyroxine/dexamethasone/GH (HYPOX/GH); controls received normal saline (CON) or GH (CON/GH) twice daily for 7 d, and animals were sacrificed on day 8. Hypothalami and ME were removed separately and processed as in experiment 1, except somatostatin (SS) was also assayed in the same samples. Antibodies to rat GRF were generated in rabbits, rat GRF was labelled with chloramine T and purified by HPLC. The antibody selected did not cross-react with human GRF, PHI, VIP, GIP, secretin, glucagon, or several other hypothalamic peptides, and bound 35% of labelled rat GRF at 1:60,000. ARC/VMN lesions decreased (p<.001) ME GRF by 96% (3.41 ± 0.29 vs 0.11 ± 0.08 pmole/mg protein, sham vs ARC, respectively), whereas, MPFX lesions had no effect (3.41 ± 0.29 vs 3.52 ± 0.21 pmole/mg protein, sham vs MPFX, respectively). Results of experiment 2 are tabulated below (#=p<.05).

GRF (fmole/mg prot)	CON	CON/GH	HYPOX	HYPOX/GH
ME	568 ± 61	706 ± 74#	468 ± 54	482 ± 54
Hypoth.	54.4 ± 1.9	57.1 ± 4.5	55.6 ± 6.7	45.0 ± 1.9
SS (pmole/mg prot)				
ME	3.1 ± .6	4.5 ± .4#	1.1 ± .2#	3.3 ± .7#
Hypoth.	.88 ± .11	.71 ± .08	.88 ± .07	.97 ± .08

Hypophysectomy had no effect on either ME or hypothalamic concentrations of GRF. However, the control group treated with rat GH (10 ug/kg bw twice daily ip) had increased levels of ME GRF. In contrast, hypophysectomy caused a significant decrease in ME SS levels; this effect was reversed by administration of GH, confirming previous studies. Similar to GRF, GH treatment in controls increased SS levels in the ME. Interestingly, hypothalamic levels of SS were not affected by hypophysectomy or GH administration.

These results indicate the following: 1. GRF-containing neurons in the hypothalamic ARC/VMN region project to the ME and contribute >95% of ME GRF, 2. GRF-containing cell bodies in the MPFX region do not project to the ME, and 3. hypophysectomy decreases ME somatostatin, but does not affect GRF. These findings support the hypothesis that GRF perikarya in the ARC/VMN nuclei are responsible for generation of episodic GH secretion, and that somatostatin is involved in feedback regulation of GH.

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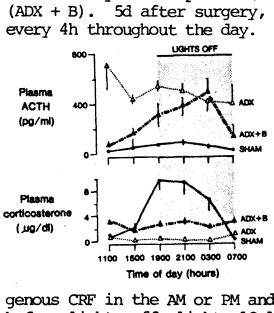
164.16 ACTH SENSITIVITY TO INHIBITION BY CORTICOSTERONE CHANGES BETWEEN MORNING AND EVENING. S.F. Akana*, C.S. Cascio*, N. Levin*, J. Shinsako*, M. Goodman*, and M.F. Dallman* (SPON: B. Libet). Dept. Physiol, UCSF, San Francisco, CA 94143.

Negative feedback regulation of ACTH by corticosterone (B) over the course of 24h is difficult to quantify because of the normally high amplitude circadian variation in plasma B in rats. Recently, we have implanted fused pellets of mixtures of B and cholesterol sc in adrenalectomized (ADX) rats and reported that constant B levels of approximately 5μg/dl provide good replacement based on restoration of AM ACTH levels, body weight and thymus weight to normal (Fed Proc 44,#3427). Furthermore, rats given either 0,20 or 40% B implants at ADX and killed 5d later either in the AM (2h after lights on) or in the PM (2h before lights off; lights 12:12h) showed a clear difference between AM and PM ACTH levels at constant plasma B levels of about 5μg/dl (20% pellet) with greater inhibition in the AM (Endocrine Soc., 1985). To further test the AM-PM shift in ACTH sensitivity to B feedback in rats with constant B levels we prepared groups of rats with either sham-adrenalectomy (SHAM), adrenalectomy + 0% B pellet (ADX) or adrenalectomy + 25% B pellet (ADX + B). 5d after surgery, 6 rats from each group were killed every 4h throughout the day. The figure clearly shows that plasma B in rats bearing the B pellet is constant and unchanging throughout the day. Plasma ACTH, while low and similar to SHAM levels in the morning, rises during the lights-out period and at peak values does not differ from ADX levels before returning to the morning low. Thus, a marked increase in PM ACTH occurs in the face of constant B levels. To test whether pituitary sensitivity to CRF varies between AM and PM, SHAM and ADX + B rats were treated with chlorpromazine-morphine-nembutal to block endogenous CRF in the AM or PM and 4h later (1h after lights on or 1h before lights off; lights 12:12h) were injected iv with either vehicle or 0.1 μg CRF. There was no AM/PM difference in the response to CRF in the SHAM (ΔACTH (pg/ml) AM:48+3, PM:58+8) or ADX + B (ΔACTH (pg/ml) AM:175+13, PM:157+15). Thus, there is greater negative feedback inhibition of ACTH by a given level of B in the AM than in the PM which is not explained by a concomitant shift in pituitary sensitivity to CRF. We conclude that operationally there is a daily "reset" of B feedback, but we postulate that functionally there is a shift in major feedback site from pituitary in the AM to brain in the PM.



(Supported in part by AM28172 and HL29714.)

164.17 RESISTANCE OF GLUCOCORTICOID RECEPTORS IN BRAIN TISSUES TO DOWN-REGULATION BY DEXAMETHASONE TREATMENT. B.B. Turner and J.E. Cole, II*. Dept. of Physiology, Quillen-Dishner Coll. of Med., East Tenn. State Univ., Johnson City, TN 37614.

Glucocorticoids are frequently used in high dosages for the treatment of a variety of pathological conditions. Despite the fact that numerous side effects of the steroid are receptor mediated, the effect of exogenous glucocorticoid on the autoregulation of receptors in different tissues has not been compared. We wished to test two hypotheses: 1) that the magnitude of cytosol receptor down-regulation is proportional to the receptor density in the tissue, 2) that receptor down-regulation is related to changes in the level of "transcortin-like" binding in the tissue. Dexamethasone (DEX; 1mg/1) was administered in the drinking water of adult male rats for 10 days. Both control and experimental animals were adrenalectomized 12 h before sacrifice. Rats were thoroughly perfused and the following tissues taken: liver, heart, kidney, pituitary, cerebral cortex, hippocampus, amygdala, and hypothalamus. The prepared cytosols were treated with charcoal to remove any remaining DEX. Aliquots of cytosol were incubated for 24 h at 4C with varying concentrations of 3H-DEX (0.5-30 nM). Bound steroid was separated from free by passage through LH-20 columns. "Transcortin-like" binding in tissues was defined as the specific binding of 3H-corticosterone occurring in the presence of excess unlabeled DEX.

The binding capacity of liver cytosol was markedly lower in the DEX-treated rats as compared with controls ($p<.01$). Decreases in the Bmax of heart and kidney cytosols were not significant. The results obtained in the brain tissues were unexpected: the four tissues were similar to each other in that the binding capacity of the DEX-treated rats was somewhat greater than that of the controls. Analysis of variance for the brain regions indicated a significant treatment effect ($p<.05$). "Transcortin-like" binding in liver, heart and kidney was significantly lower in the DEX-treated rats. No significant decrease in "transcortin-like" binding was observed in any of the brain tissues despite a decrease of 92% in plasma transcortin.

These data suggest that 1) in brain tissues, receptor binding capacity is increased, not decreased, by DEX treatment 2) the magnitude of receptor down-regulation varies markedly in peripheral tissues, and may be proportional to initial receptor density, 3) decreases in tissue transcortin are associated with glucocorticoid down-regulation. The anomolous finding in brain cytosols may be due to the inability of DEX to access or bind to these receptors in vivo. If this is the case, then glucocorticoid receptor up-regulation would result since the secretion of the endogenous corticoid, corticosterone is suppressed by DEX treatment. Supported by VA grant 1A (74) 111-430108.

164.18 PERFORMANCE OF TWO COMMERCIAL RADIOIMMUNOASSAYS (RIA) FOR PLASMA ACTH COMPARED WITH TWO REFERENCE LABORATORY ASSAYS. J.C. Ritchie, A.E. Kegelmeyer* and J.T. Walker*. Clinical Psychobiology Laboratory, Duke Univ., Durham, N.C., 27710.

Within the last year several commercial ACTH RIA's have appeared. All are performed on non-extracted human plasma and all claim to provide accurate measures of ACTH. In an attempt to verify these claims, we compared the ACTH assay kits of Radioassay Systems Laboratories, Inc. (Carson, CA.) and Immuno Nuclear Corp. (Stillwater, Minn.) with our well characterized, in-house extracted assay and the non-extracted assay of Dr. D. Orth (Div of Endo., Vanderbilt Univ.)

The determinations were performed on plasma samples (obtained from 7 normal at rest volunteers between 0800 and 1000) according to manufacturers specifications by technicians well versed in RIA methodologies. For our assay, samples and standards are extracted using a Sep-Pak cartridge (Waters, Inc.) and run in a homologous RIA using an antibody we developed against the 11 to 24 region of human ACTH 1 to 39. Details of Dr. Orth's assay have been published elsewhere (Clin. Chem. 30, #2, 259-265, 1984).

Results for the unknown plasmas (in pg/ml) are summarized below:

Sample	R.S.L.	Immuno Nuclear	Vanderbilt	C.P.B.L.
A	17	N.D.*	8	4
B	26	35	23	25
C	17	47	13	15
D	41	46	21	18
E	93	N.D.	37	49
F	51	757	38	32
G	39	N.D.	10	8

N.D.* = Not Done

As can be seen, both commercial assays gave higher values for all samples than either reference assay. This effect is probably due to the use of Bovine Serum Albumin (BSA) to "blank" out non-specific plasma interactions and is strictly dependent on the particular BSA preparation used in the assay.

We conclude that these two commercial RIA's should not be used in any area of research where measurement of absolute plasma ACTH concentrations is vital. When used in the clinical setting, (where the requirement for accuracy is not as high) samples should be run at several dilutions and checked for parallelism with the standard curve. Samples which generate binding curves that are not parallel should be referred to a reference laboratory.

164.19 BLOOD INDOLE AMINE LEVELS IN PREGNANT RATS FED TRYPTOPHAN DIETS.

M.Sakuma*, I.N.Mefford* and R.Grdy Jr.* (SPON:R.Gala). Dept. of Psychiatry, Wayne St. Univ.,Detroit MI 48207 and Dept. of Chemist. Boston Coll.,Boston,MA 02167.

We have previously shown that the blood levels of both tryptophan and 5-HT of pregnant rats were lower than prediet levels when animals fed by a 3% tryptophan diet during the third trimester, whereas in the age-matched non-pregnant rats a reduction was found only in the serotonin level (Neurosci. Ann. Meet.,1984). This suggests that pregnant and non-pregnant rats have a different response to 3% tryptophan diet. To confirm this, the effects of diets of various tryptophan contents were examined in the present project. 12 pregnant rats were assigned to 3%, 1%, 0.19% and 0.03% tryptophan containing diets. The pure tryptophan reagent (Sigma) was added to the base diet(Tryptophan Free Pregnant Rat Diet, Bio Serv.) by 3%, 1% and 0.19% by weight. Animals given each of the diets failed to show a dose dependent response in blood levels of tryptophan, 5-HT and 5-HIAA at all blood sampling times. The blood was sampled from the same animals consecutively at the time of the prediet (15th day of gestation), diet-end (delivery-end) and at weaning. Values of an analysis of variance for these sampling times revealed significant differences at 0.01% level. This was shown as sharp reductions of tryptophan and serotonin levels from prediet values throughout all diet groups, with small increases at weaning time. The levels of 5-HIAA of all diet groups showed no change at these sampling times. These results confirm those of the previous study and can be interpreted that during pregnancy of rats, both the levels of tryptophan and serotonin characteristically drop at the end of delivery. Namely, the influence of delivery is more powerful than the effects of tryptophan content in diet and able to produce some changes in blood parameters at the delivery end. The failure to show a dose dependent response to tryptophan in the diet suggests some regulatory mechanism occured at delivery end such as hemorrhage or hormone level changes. This warrants further studies. The reduction of serotonin levels from prediet values is evidence of the diet's effects which were similarly observed in the non-pregnant rat group of previous study.

165.1 BILATERAL SYRINGEAL INTERACTION IN THE VOCAL PRODUCTION OF AN OSCINE BIRD SOUND. S. Nowicki* and B. Capranica. Sect. of Neurobiol. and Behav., Cornell Univ., Ithaca, NY 14853.

The syrinx of oscine birds is two-parted and each side is presumed to operate independently of the other, enabling a bird to sing an "internal duet." Support for this theory comes both from spectrographic analyses revealing temporally-overlapping, non-harmonic acoustic elements in certain complex songs, and from the discovery that neural control of the syrinx is also highly lateralized. We here demonstrate one counter-example of a sound produced through a bilateral interaction of the two vocal sources.

The 'dee' syllable is the terminal note in the 'chick-a-dee' call of the black-capped chickadee (Parus atricapillus). It comprises 12-20 temporally-overlapping frequency components, evenly spaced at approximately 400 Hz intervals. The first and second spectral components having significant energy occur at about 1600 and 2000 Hz, with higher frequency components, spaced at the interval between these two peaks, occurring up to 7000 Hz. This orderly spectral structure resembles a harmonic series, but the lack of energy at lower frequencies and ambiguous estimates of fundamental frequencies based on the intervals between higher frequency components both suggested an alternative mechanism for production. In an effort to understand what role each side of the syrinx plays in the generation of this sound, we performed unilateral hypoglossal nerve sections on chickadees.

Sectioning either side gives rise to noise in which is embedded a true harmonic signal with a fundamental frequency in the range of 1500-2000 Hz. If the right hypoglossal nerve is sectioned, the fundamental frequency occurs at about 1600 Hz, corresponding to the first component of the intact signal; if the left nerve is sectioned, the post-operative fundamental occurs at about 2000 Hz, corresponding to the second component of the intact signal. Linear summation of post-operative signals from the left and right sides does not, however, give rise to the frequencies seen in the spectrum of a normal signal. Thus, the chickadee's two syringeal halves cannot be acting independently in the production of this sound, and must be coupled in some non-linear fashion.

The correspondence between the first two spectral components of the intact signal and the fundamental frequencies of the post-operative signals, as well as the fact that the frequency interval between all spectral components invariably equals the interval between these two frequencies, suggests that the additional spectral components of the intact call arise as sum and difference frequencies resulting from amplitude modulation between the two syringeal sources. Calculation of expected sum and difference products from the mathematical expression for such cross-modulation, using realistic values for post-operative harmonic signals, supports this hypothesis.

165.2 SIZE OF VOCAL CONTROL REGIONS CORRELATES WITH SONG COMPLEXITY ACROSS DUETTING BIRD SPECIES. E.A. Brenowitz and A.P. Arnold. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024

Song in oscine birds is a learned behavior. It has previously been shown that 2 forebrain vocal control regions (VCRs), the caudal nucleus of the ventral hyperstriatum (HVc) and the robust nucleus of the archistriatum (RA), are large in male Canaries that produce a large repertoire of song syllables. Canaries with small song repertoires generally have small HVcs and RAs (Nottebohm et al. 1981). Similar correlations between VCR size and song complexity have also been reported for female Canaries induced to sing with testosterone (Nottebohm 1980), and between populations of Marsh Wrens having different song repertoire sizes (Canady et al. 1984). In the present study we wished to determine whether this correlation is also observed between closely related species. We compared VCRs and song complexity in 2 congeneric species of Panamanian wrens, the Bay Wren (Thryothorus nigricapillus) and the Rufous-and-white Wren (T. rufalbus). In both species males and females engage in elaborate song duets with each other. T. rufalbus has a slightly larger average body size (bill to tail = 14.6 cm.) than T. nigricapillus (14.0 cm.). Song repertoire size is about the same for male T. nigricapillus (X + SD = 16.5 + 6.4; R. Levin in prep.) and male T. rufalbus (14.0 + 3.3; Farabaugh 1983). However, repertoire size is much larger for female T. nigricapillus (15.2 + 0.5; Levin in prep.) than for female T. rufalbus (6.0 + 1.4; Farabaugh 1>

Brains from 5 males and 5 females of each species were sectioned and stained with cresyl violet or thionin. We measured volumes of 4 VCRs (RA, HVc, Area X, and the motor nucleus of the hypoglossal nerve, nXII), as well as of 2 thalamic nuclei not involved in vocal control (the rotund nucleus, Rt, and the pretectal nucleus, Pt).

RA, HVc, Area X, and nXII are an average of 78%, 55%, 43%, and 26% larger, respectively, in female T. nigricapillus than in female T. rufalbus ($P < .05$, Rank Sum test). Pt is the same size in females of both species ($P > .05$), while Rt is 78% larger in female T. rufalbus ($P < .05$). Conversely, there are no differences in the volumes of any of these brain nuclei between males of these 2 species ($P > .05$). This suggests that differences observed in the size of VCRs in female wrens are not due to differences in body size, phylogenetic history, or histological preparation, but rather are due to interspecific differences in female song repertoire size. These data support and extend the hypothesis that there is a causal connection between VCR size and the complexity of song behavior that can be learned by oscine birds.

(Supported by USPHS grants NS 19645, NS 07134-01, and MH 15795-03).

165.3 INTRATELENCEPHALIC AUDITORY PATHWAYS IN THE BUDGERIGAR. C.M. McHale and S.E. Brauth. Department of Psychology, University of Maryland, College Park, Maryland, 20742.

In a previous study of auditory nuclei in the budgerigar, we have shown that the auditory relay nucleus of the thalamus, nucleus ovoidalis, is composed of two major subdivisions in this species. These are a central compact zone which projects to the primary auditory region of the telencephalon, Field "L" and a ventral and medial subdivision which projects both to Field "L" and to a dorsolateral auditory area located in the neostriatum intermedium (NIDL). As part of an ongoing study of the organization of these parallel pathways, we have investigated the efferent projections of both Field "L" and NIDL neurons. We find that neurons in both fields project laterally and ventrally to another telencephalic field located in the ventrolateral neostriatum intermedium (NIVL). Experiments utilizing 2-deoxy-D-glucose (2DG) autoradiography (Brauth et al, Neurosci. Abs., 10:401, 1984) have shown that NIVL is metabolically activated by acoustic stimuli including species-typical contact calls and warbles. Since NIVL neurons do not receive direct projections from the auditory thalamus, acoustic activation of NIVL is probably a result of projections to this region from auditory neurons in NIDL and Field "L".

All three telencephalic auditory fields (Field "L", NIDL and NIVL) project to selective portions of the archistriatum including portions of the posterior archistriatum and rostromedial archistriatum. In birds the archistriatum is a major source of descending telencephalic projections to brainstem nuclei. Of particular importance in the present context, we have found that projections derived from neurons in the posterior archistriatum terminate within both the MLd (nucleus mesencephalicus lateralis pars dorsalis - the avian inferior colliculus), and the ventral and medial subdivision of nucleus ovoidalis. Therefore pathways exist by which information processed by telencephalic auditory structures can influence brainstem auditory centers in this species. These pathways may provide a possible substrate for auditory discrimination or sensory learning processes.

Field "L" neurons also project to a small region directly abutting the medial border of nucleus archistriatalis robustus (RA). In budgerigars, RA is located within the rostromedial archistriatum (Paton et al, J. Neurosci., 1:1279-1288, 1981) and projects bilaterally to the motoneurons controlling the syrinx (nXIIts). RA therefore contains the upper motoneurons for the control of vocal behavior. Projections from Field "L" to a portion of the archistriatum immediately adjacent to RA may provide a mechanism by which information processed within the auditory nuclei of the telencephalon can provide feedback during vocal learning or vocal performance.

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165.4 ELECTRORECEPTION AND ELECTROLOCATION IN PLATYPUS H. Scheich, G. Langner, Ch. Tidemann*, R. Coles, and A. Guppy* Zoological Institute, Technical University, 6100 Darmstadt, FRG, and Australian National University, Canberra, ACT 2601

The Australian monotreme platypus subsists entirely on live food caught during nightly dives in lakes and streams. Since eyes, nostrils, and ear canals are closed under water its ability to locate and catch rather mobile prey like crayfish, shrimp and small fish appeared to depend on the tactile sense of the bill which was known to be covered with mechanoreceptor organs (Poulton, E.B., Quart. J. Micr. Sci. 36: 143, 1894). We have conducted behavioral experiments showing that platypus can detect electric field gradients as small as 50 µV/cm and that it makes use of dipole fields to locate objects. Behavioral tests with 1 male and 3 females in a 3 m Ø pool involved: 1. locating small object which emitted DC fields and discrimination of identical mechanical objects, one emitting a field and the second not; 2. probing in hollow stones with and without electric fields; 3. avoidance of unexpected obstacles with and without electric fields; and 4. eliciting a reflex-like head jerk by switching on and off a homogeneous electric field. Complementary measurements of the compound action potentials from the tail flick of local freshwater shrimps gave values at the order of 1 mV/cm at a few cm distance, thus way above threshold for platypus. Since a tail flick yields only a few cm escape distance we consider the action potential a meanigful stimulus to guide prey catching.

Cortical evoked potentials with stimulation of the bill revealed an electrosensory field ventral to the auditory field and caudal to the mechanoreceptive bill map (Bohringer, R.C., Rowe, M.J., J. Comp. Neur. 174: 1, 1977). Evoked potential thresholds with pulses of 55 ms duration were at the order of 50 µV/cm. Electroreceptor candidates in the bill may be so-called gland duct receptors, unrelated to electroreceptors in lower vertebrates (Andres, K.H., v. Düring, M., In: Sensory Receptor Mechanisms. Hamann, W., Iggo, A., Eds., World Sci. Publ. Co., Singapore, pp. 81-99, 1984).

It is worth mentioning also that platypus showed a remarkable under-water-chemosensitivity. A drop of shrimp juice but not NaCl elicited vigorous search in the pool. Since nostrils are closed chemosensitivity may relate to the large size vomeronasal organ.

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165.5 CENTRAL CONTROL OF FREQUENCY IN BIOSONAR VOCALIZATIONS OF THE MUSTACHED BAT. D.M. Gooler and W.E. O'Neill, Center for Brain Research, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

The biosonar system of the mustached bat, Pteronotus parnellii, has proven strategically advantageous for the study of audition and vocalization. In particular, this study exploits the fact that mustached bats control very precisely the frequency of their biosonar signals, based upon the frequency information present in echoes. This behavior, known as "Doppler-shift compensation", involves the production and reception of comparatively simple acoustic signals, which, however, contain acoustic elements similar to those used in human speech. These signals and their underlying motor patterns are stereotyped and repetitively produced, both in nature and via microstimulation of brain. The emphasis of this study is to examine the role of the anterior cingulate cortex (ACg) in the fine control of the frequency of emitted sounds, such as occur for Doppler-shift compensation during target-oriented flight.

Constant current cathodal pulses (20-30 μamps) were applied to a midline region of brain dorsal and anterior to the corpus callosum, presumably ACg,to elicit vocalizations. ACg appears to be divided into two areas based on the type of vocalization produced by microstimulation. The anterior portion is associated with echolocation sounds and the posterior section is associated with audible noise-bursts. The frequencies emitted by the bat during microstimulation of the anterior portion of ACg are most often 58-62 kHz, which is the normal range of frequencies dominant in the second harmonic of natural biosonar emissions in this species. This is in contrast to results of microstimulation in the midbrain periaqueductal gray where the frequencies of electrically elicited vocalizations are only near the resting frequency (61-62 kHz). Most interestingly, the frequencies of vocal pulses elicited by microstimulation of ACg vary depending on the position of the electrode. The frequency of emitted sound increases along a rostro-caudal axis. Thus, the ACg appears to be organized by place for the frequencies normally emitted during Doppler-shift compensation.

Studies are ongoing to examine the neural connections associated with elicited echolocation sounds from ACg. To date, retrograde transport of HRP from one injection site in ACg (frequency of elicited vocalization, CF_2=61 kHz) shows cell bodies of origin in the contralateral, reciprocal portion of ACg; ipsilateral, ventral ACg; ipsilateral auditory cortex; and two ipsilateral, midline thalamic nuclei. The results, thus far, indicate a potential source for an auditory influence on vocalization and thereby, potential for the control of emitted frequency of echolocation sounds in the bat.

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165.6 AUDITORY LOCALIZATION AND VISUAL FIELDS IN MAMMALS. R. S. Heffner and H. E. Heffner. Lab. of Comparative Hearing, Bureau of Child Research, University of Kansas, Parsons, KS 67357.

Mammalian sound localization acuity has been shown to vary widely from 1^0 in man and elephants to over 20^0 in some rodents and hoofed mammals. In seeking possible causes of this variation we have begun to explore the relation between auditory localization and other ecological and morphological variables.

Because it has often been suggested that an important function of sound localization is to direct the eyes to the source of a sound, we have begun to explore the relation of sound localization to some simple visual parameters. As a first step, the size of both the binocular and panoramic visual fields was determined in 19 species of mammals whose sound localization acuity is known. It was found that the size of the binocular visual field is reliably correlated with sound localization acuity ($r=0.78$ $p<.01$). That is, animals with frontally directed eyes and wide binocular visual fields are better able to localize sounds in front of them than are animals whose vision is directed laterally.

The existence of such a correlation suggests a link between sound localization and vision. Whether this correlation indicates a causal link between the amount of binocular vision and localization acuity or whether both are related through some other factor cannot yet be determined. Nevertheless, the closeness of the relationship, when taken together with the recent discoveries of congruent maps of auditory and visual space in the superior colliculus, indicates that visual space and auditory space are functionally linked and merit further exploration.

165.7 AZIMUTHAL TRACKING ACCURACY OF MOVING TARGETS BY THE BIG BROWN BAT (EPTESICUS FUSCUS). W.M. Masters* and A.J.M. Moffat* (SPON: J.A. Simmons). Institute of Neuroscience, University of Oregon, Eugene, OR 97403

The Big Brown Bat (Eptesicus fuscus) detects and captures flying insects using its sonar (echolocation) system. Photographs of capture sequences show that soon after a bat has detected a potential prey target, it aims its head at it and keeps its head pointed at the target during the subsequent approach to and interception of the target. This behavior of keeping the head aimed at a moving target is useful experimentally, because a bat can be trained to perform a similar behavior in a laboratory setting, thereby permitting us to investigate some aspects of the bat's echolocation abilities. We trained bats to sit on a small platform and keep their head aimed at a small styrofoam ball suspended in space in front of the bat and moving irregularly along an arc a constant distance from the bat. We monitored the head aim of the bat by a specially built optoelectronic device, and simultaneously monitored the angular position of the target and the time of occurance of echolocation sounds. We were interested in two questions: how accurately can a bat keep its head aimed at a moving target, and what tracking strategy does it use to keep its head aimed at the target.

When tracking a moving target, a bat's head aim lags behind the target by about 60 to 100 ms, which, at any given instant, results in an error between the direction that the bat is aiming and the direction to the target. The error waveform (head aim minus target direction) averages $\pm4.7^0$ and is strongly correlated with the target's velocity waveform. If the bat's tracking lag is removed before the subtraction, the error waveform (now corrected for lag) is reduced to $\pm2.3^0$ and no longer correlates with target velocity. The lag-corrected error waveform does, however, correlate well with target angle. The explanation for this appears to be that the bat does not track the target with unity gain, but rather with a gain of roughly 0.9, so that the farther off the midline the target is, the larger is the bat's error. This error probably is not due to the bat's uncertainty as to the actual direction to the target, and if the bat's tendency to track with non-unity gain is compensated for by dividing the head-aim waveform by the tracking gain, the resulting lag- and gain-corrected error waveform averages $\pm1.6^0$ and no longer correlates with target angle or velocity. The residual error appears noise-like, as would be expected if it is due to the bat's uncertainty as to target direction and/or imprecision in aiming its head. The significance of these observations for the bat's head-aim tracking strategy will be discussed.

165.8 TIME OF DAY IS CRITICAL IN THE ASSESSMENT OF LESION-INDUCED DEFICITS IN VISUAL ORIENTING RESPONSES. B.A. Sabel, S.L. Ayres* and G.E. Schneider, Dept. of Psychol. and the Whitaker College, M.I.T., Cambridge, MA 02139.

Normal hamsters are remarkably different in their visual responsiveness during tests carried out at different times of the day (Ayres & Schneider, unpubl.). Our animals are kept on a 14 hr:10 hr light-dark cycle, with lights going off at 4 p.m. At this time, a period of greatest locomotor activity begins each day, which probably corresponds in the wild to a period of emergence from the burrows and foraging for food. However, in tests of visually elicited turning toward sunflower seeds presented in various parts of the visual field, normal laboratory hamsters are least responsive during this active period, whereas they are much more responsive when tested earlier in the day. The major reason for this appears to be that in the most active period, hamsters show the most predator-avoidance types of responses, including escape movements and freezing, to visual stimuli. (We have found that such responses can be abolished in hamsters by lesions of the superior colliculus (SC): Merker, '80; Schneider & Ayres, unpubl.)

These facts led us to test hamsters at two different times of day in a study of recovery of visually elicited turning movements after unilateral ablation of neocortex (on the right side) plus either exposure or exposure and removal of the opposite (left) SC. (Exposure of the left SC was done by removal of the overlying cortex.) Six adult hamsters comprised each of the two groups. Systematic variation in time of testing was begun 90 days after surgery, long after both groups had fully recovered visual orienting to seeds when tested in the morning, the time when our hamsters are generally most responsive. As in the case of normals, each group of brain-damaged animals showed significantly less orienting to seeds late in the day, during their active period ($p<.05$). However, in tests conducted during this late afternoon period a lesion-group difference appeared for presentations in the upper part of the left field, the field which had been affected by the cortical lesion. The group with the addition of an opposite sided (left) midbrain lesion showed less orienting in the left (as well as in the right) field ($<.02$). At the same time of day this group showed more freezing to an overhead "looming" stimulus, but this difference was not significant.

This appearance of a group difference at one time of day which was not present at another time of day leads us to suggest that time of day may account for some discrepancies found in the literature on brain lesion effects, and should be considered a critical variable in such studies, a variable which may be quite different for different species. (Supported by NIH grants EY00126 and EY02621.)

165.9 THE NEURAL SUBSTRATE FOR VOCALIZATION IN THE DOMESTIC CAT: A 2-DEOXYGLUCOSE ANALYSIS. N.C. de Lanerolle, Sections of Neurosurgery & Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT. 06510.

The neural substrate for vocalization in mammals is as yet only poorly understood. In order to determine the brain areas metabolically active during vocalization, the pattern of 14C-2-deoxyglucose (2DG) uptake was studied in the brains of anesthetized cats made to vocalize by electrical stimulation at a site in the ventrolateral pons. In these experiments each cat was injected with 100 uCi/kg of 2DG and stimulated for a period of 45 min with current intensities slightly above threshold for call evocation. The pattern of stimulation employed was 20 sec. current ON and 10 sec. OFF. Stimulation at such sites evoked mainly meow calls with some growls and meow-growls. Animals used as controls underwent all procedures except electrical stimulation.

The brain areas that were metabolically active in experimental animals alone were the following: In Medulla/pons: nu. of cranial nerve XI; caudal lateral nu.; hypoglosssal nu.; nu. ambiguus and adjacent medial reticular formation; facial nu. and adjacent medial reticular formation; main sensory nu. and motor nu. of cranial nerve V; cochlear nu. Midbrain: the ventromedial and dorsolateral edges of the periaqueductal gray; occulomotor nu.; medial geniculate nu.; red nu.; nu. Darkschewitsch and interstitial nu. The inferior colliculi and interpeduncular area were strongly labelled compared to controls. Diencephalon: medial mammillary nu.; ventral posterolateral nu; anterior ventral thalamic nu. Telencephalon: caudate nu and some cortical areas. All of the above areas were bilaterally active. The brain areas identified through this 2DG study correspond rather well with those areas found to be connected with this ventrolateral pontine call site in silver degeneration studies. It appears that in mediating vocalization induced by electrical stimulation at this site, several motor nuclei directly controlling sound production are active. In addition areas responsible for controlling head and eye movements, areas processing sensory inputs, some limbic structures, and areas intergating sensory/motor functions are also metabolically active.

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165.10 BEHAVIOR PATTERNS EVOKED BY ELECTRICAL STIMULATION OF THE HAMSTER SUPERIOR COLLICULUS. D.P.M. Northmore* and G.E. Schneider (SPON: P. Hartline). Inst. for Neuroscience, Univ. of Delaware, Newark DE 19716 & Dept. of Psychology, M.I.T. Cambridge, MA 02139.

Evidence is accumulating that the rodent superior colliculus (SC) is involved not only in orienting movements of the body, head and eyes, but also plays a crucial role in avoidance of or escape from predators. Here we report on the variety of movements that can be evoked by electrical stimulation of SC in unrestrained hamsters.

Adult Syrian hamsters were implanted with 1-4 fixed electrodes made from teflon coated Pt-Ir wire (110μm diam) aimed at SC on the right side. The animals were observed in a circular walled arena while 1 sec trains of cathodal pulses (200 Hz, 0.1msec) were delivered to the electrodes by a constant current source.

The orienting movements evoked varied from quick turns resembling visual orienting to sunflower seeds, to slow, forced motor turns that continued for the duration of the stimulus. In either case, turning was contraversive (leftward). The most common defensive behavior evoked was freezing, ranging from a brief interruption of ongoing behavior, to prolonged immobility characteristic of one of the hamster's natural responses to visual threat. Other types of defensive behavior seen included ducking, backing and running escape.

Electrode tips were identified in sections stained for Nissl substance and degeneration. While all of the behavior patterns described could be observed from stimulation at different sites above the stratum opticum, relatively high currents (>500 μA) were required. Sites in the intermediate layers of SC yielded turns with low current thresholds (20 - 70 μA), and defensive behaviors at higher currents. Deep sites in SC tended to give brief arrests (12 - 90 μA), and other behavior patterns at higher currents.

The evocation of a range of defensive behaviors in addition to turning movements is consistent with previous observations (Merker, PhD Diss., MIT, 1980; Schneider & Ayres, unpubl.) that responses to visual threat in the hamster are diminished or abolished by SC lesions.

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165.11 BENACTYZINE POTENTIATES ALARM CALLS IN THE SQUIRREL MONKEY J.R. Glowa and J.D. Newman. National Institute of Mental Health and National Institute of Child Health and Development, Bethesda, MD 20205.

An alarm call is a naturalistic response of many mammalian species to the presence of a predator. The study of possible neural mechanisms underlying this behavior is difficult because conditions appropriate for reliably eliciting alarm calls are not easily created in the laboratory. The present experiments were initiated following the serendipitous observation that benactyzine appeared to increase the frequency of alarm calling in squirrel monkeys when laboratory personnel were nearby. Benactyzine (Sigma) is a tertiary anticholinergic little studied for its behavioral effects. Squirrel monkeys are especially useful subjects for the study of this type of vocalization because they emit a species-specific alarm call (yap or cackle) under laboratory conditions. The effects of various combinations of benactyzine dose and stimulus conditions were studied on spectrographically analyzed vocalizations of isolated squirrel monkeys. Four monkeys, weighing between 700-1000 g, were tested individually in a quiet setting. The drug was not given more often than once a week. When given saline (0.1 ml/kg, i.m.), the intermittent presentation (30 sec on - 30 sec off, for 10 consecutive min) of a monkey puppet stimulus (Hanmee Trading Co., Flushing NY) resulted in a low level of alarm calling. Benactyzine (0.1-1 mg/kg, 0.1 ml/kg, i.m.), given 5 min before the start of recording, significantly increased the frequency of alarm calling in a dose-related manner following the onset of testing. In the absence of the stimulus, benactyzine resulted in little or no vocalization. These findings indicate that benactyzine can increase rates of alarm calling in the squirrel monkey under defined laboratory conditions. Such results suggest that benactyzine may serve as a useful pharmacological probe for the study of mechanisms mediating the alarm call and that in the squirrel monkey one such mechanism may involve a cholinergic substrate.

165.12 YAWNING AS A STEREOTYPED ACTION PATTERN AND RELEASING STIMULUS. R. R. Provine, Dept. of Psychology, Univ. of Maryland Baltimore County, Catonsville, MD 21228.

Yawning was induced by instructing subjects to "think about yawning." Such yawns were reported as normal by subjects. Yawns were consistent in duration ($X = 5.9$ sec $\neq$ 1.9 sec (SD)), periodic (X interyawn interval = 68.3 $\neq$ 33.7 sec) and within subject stability of yawn duration and frequency was maintained for at least several weeks. Yawns were "all-or-none" actions that went to completion once initiated; fractional yawns were seldom if ever produced and it was difficult for subjects to stifle a yawn. Yawns were complex, relatively long lasting, independent of the amplitude of the releasing stimulus and species typical. These and other characteristics qualify yawning as a stereotyped action pattern of the type described by ethologists.

Yawning was released both by observed yawns and by yet to be defined physiological states. Although visually observed yawns were potent yawn producing stimuli, thinking about or reading about yawning also elicited yawning. The wide variety of possible releasing stimuli contributes to the "infectiousness" of yawning.

Yawns may be the best example of stereotyped action pattern and releaser in humans. The study of yawning may provide insights both into yawn function and the more general issue of how releasers trigger behavior. Yawning also offers us the opportunity to experience the sensation of having a stereotyped action pattern released and performed. The prominence of yawning in human behavior and the advantages of yawning as a subject for neurobehavioral analysis are inconsistent with the current status of yawning as a minor behavioral curiosity.

166.1 REGIONAL NEUROTRANSMITTER TURNOVER FOLLOWING BRAIN STIMULATION REWARD John D. Lane, ǂMichael J. Blake and ǂElliot A. Stein, Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107 USA; ǂ Department of Biology, Marquette University, Milwaukee, WI 53233 USA

The apparent rewarding properties of intracranial self stimulation (ICSS) are presumably derived through the activation of specific anatomical and neurochemical pathways which are normally activated when presented with reinforcing stimuli. Furthermore, there is evidence that suggests that the rewarding aspect of ICSS is perceived only through active acquisition of the stimulation and that experimenter-applied or non-contingent stimulation does not possess rewarding qualities and may in fact be aversive. The sites which support this behavior have been well mapped and include areas from prefrontal cortex to the central gray. The results of lesion studies as well as other pharmacological manipulations suggest a role for dopamine in the mediation of this behavior, as well as for several other neurotransmitters and neuropeptides which may have a modulatory role. The aim of this experiment was to determine how several monoamine and amino acid neurotransmitters are involved in ICSS behavior and if a neurochemical distinction could be made between response-contingent and non-contingent ICSS. To achieve this end we have compared the regional turnover of several neurotransmitters (DA, NE, 5-HT, ASP, GLU and GABA) in rats receiving response-contingent ICSS and those receiving either no stimulation or non-contingent electrical stimulation. Male Holtzman rats were implanted with monopolar stimulating electrodes aimed at the ventral tegmental area. Following a one week recovery, rats were placed in stimulating chambers and primed to press for 100Hz, 300msec trains of 0.5msec cathodal electrical pulses. Those animals showing robust ICSS were implanted with chronic jugular catheters. After a three day recovery, the current range supporting ICSS for each rat was determined and used to match animals in the following three groups: 1) an active ICSS group; 2) a yoked group receiving a pulse train at the same time as the active group, at an intensity previously shown to support ICSS; and 3) a sham sedentary group receiving no stimulation. Animals were pulsed at 60 and 90 min prior to sacrifice with neurotransmitter precursors (^{3}H-tyrosine, ^{3}H-tryptophan and ^{14}C-glucose), and were totally frozen in liquid nitrogen. Discrete brain areas were assayed for neurotransmitter content and incorporation of radiolabel using HPLC tecnhiques, and turnover (mol/mg-hour) calculated. As observed by Porrino et al (Science, 224, 306, 1984) with the 2-DG technique, mesolimbic and mesocortical systems are activated by ICSS. Turnover in the nucleus accumbens and medial prefrontal cortex was correlated with the behavioral paradigm. (supported in part by DA-02234 and BRSG)

166.2 PRETREATMENT WITH A PRESYNAPTIC DOSE OF APOMORPHINE ENHANCES THE BEHAVIORAL RESPONSE TO AMPHETAMINE. A.E. Basse, M. Tsutsumi* and G.V. Rebec, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Long-term administration of amphetamine (AMPH) produces a progressive augmentation of locomotion and some components of focused stereotypy in the rat. Recent biochemical (Muller, P. and Seeman, P., Eur. J. Pharmac.,55:149, 1979) and electrophysiological evidence (Kamata, K. and Rebec, G.V., Brain Res.,321:147, 1984; Kamata, K. and Rebec, G.V., Life Sci.,34:2419, 1984) suggests that the augmentation is mediated, in part, by a subsensitivity of dopamine (DA) autoreceptors. If this is the case, then an AMPH challenge administered to rats pretreated with a dose of apomorphine (APO) known to desensitize DA autoreceptors (Rebec, G.V. and Lee, E.H., Brain Res.,250:188, 1982) should produce the same behavioral alterations associated with multiple AMPH injections.

To test this hypothesis, rats were pretreated twice daily with injections of saline, 0.04 mg/kg APO or 1.0 mg/kg d-AMPH for 5 days. Twelve hours after the last injection, each animal received 1.0 mg/kg d-AMPH and individual components of the behavioral response were rated by independent observers.

As expected, pretreatment with 0.04 mg/kg APO caused a profile of sensitization to AMPH similar to that seen in rats pretreated chronically with the drug. Repetitive head movements were significantly increased in both the APO ($p<.05$) and AMPH ($p<.05$) treated groups with respect to controls. Sniffing also was augmented by both drug treatments, but this increase failed to reach significance in the AMPH group. The enhanced behavioral response to AMPH in rats pretreated with a presynaptic dose of APO suggests that subsensitive DA autoreceptors play a major role in the behavioral sensitization to AMPH during long-term treatment.

This research was supported, in part, by USPHS Grants DA 02451 and RR 7031.

166.3 REGIONAL MONOAMINE ACTIVITY, SENSITIVITY TO AMPHETAMINE AND AGGRESSIVE BEHAVIOR IN MICE. K. Noda*, K.A. Miczek and R. Kream*. Dept. of Psychology, Tufts Univ., Medford, MA 02155, and Dept. Anesthesiology, N.E.M.C., Boston, MA 02111.

We have observed that the environmental conditions that modulate aggressive behavior in mice and the performance of aggression itself are associated with changes in levels of brain monoamines and their metabolites. These changes are also reflected in differential sensitivities of animals to sympathomimetic drugs. However, it is unclear whether or not these neurochemical changes are specific to aggressive behavior or of a more general nature. We have manipulated (1) the housing conditions which are thought to be important in the development of aggressive behavior, and (2) the amount of experience with aggressive behavior. Subsequently, either regional levels of brain monoamines or, alternatively, behavioral sensitivity to amphetamine was measured.

Mice were housed in different conditions for at least three weeks, and then sacrificed immediately after meeting an intruder in a resident- intruder confrontation. Dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) concentrations were measured by HPLC-electrochemical detection in punched-out brain tissues. DA turnover as expressed by DOPAC/DA ratios in nucleus accumbens and amygdala of group-housed mice was increased compared to pair-housed counterparts. However, no change in turnover was observed in corpus striatum or tuberculum olfactorium.

The behavioral effects of d-amphetamine (0, 0.1, 1.0, 10 mg/kg i.p.) were assessed in three groups of male mice. Isolated or pair-housed mice with no prior fighting experience, as well as pair-housed mice with ten consecutive days of fighting experience, were given amphetamine and then confronted with an intruder. Various social, aggressive, and motor behaviors were analyzed. Isolated and pair-housed mice showed similar levels of attack behavior in the control condition; this behavior was enhanced slightly at the lower amphetamine doses and suppressed by the 10 mg/kg dose. The animals with prior daily fighting experience showed a higher level of fighting when given saline, with no enhancement by low doses of drug. Their fighting behavior was suppressed by all doses of amphetamine.

166.4 **LONG TERM SINGLE AND MULTIPLE UNIT RECORDINGS FROM BRAINSTEM REGION OF THE LOCUS COERULEUS IN AWAKE, BEHAVING NON-HUMAN PRIMATES.** **S.J. Grant, J.R. Taylor*, D.E. Redmond, Jr. Neurobehavior Lab., Yale Univ. Sch. Med., New Haven, CT 06510.**

Bundles of fine (32-64 um) wires have been successfully used in a number of species (mainly rodents and cats) to record single and multiple unit neuronal activity for extended periods (e.g. hours to days) from unanesthetized, freely moving subjects. This technique has not been used in primates, even though studies of awake, behaving primates may have advantages for investigations of cognitive and emotional functions. On the other hand, the technical difficulties and limitations (e.g. electrode deviation, sampling biases, limited penetrations) associated with fine wire electrodes may be greater in primates than in other species and thus limit the technique's usefulness.

We implanted three stump-tailed macaques (Macaca arctoides) with bilateral movable microdrives aimed at the region of the nucleus locus coeruleus. Bundles consisted of three 32 um diameter and three 64 um diameter wires. Some wires were etched to a micro-electrode type tip (>1 MegOhm), while others had low impedence blunt cut tips. Differential recordings through pairs of wires were amplified by a custom 6-channel FET headstage mounted on the subject's skull.

A variety of neurons were recorded through both the etched and blunt wires. Unit activity was tested for responsiveness to naturalistic stimuli, simple sensory stimuli, and tones paired with either orange juice delivery or mild air puffs directed at the face. Most activity remained stable for many hours, and in some cases days, even though the monkey's head and body were free to move within the constraints of a primate chair. Such long term stability permitted recordings to be maintained throughout the full time course of centrally acting agents, e.g., the alpha-2 adrenergic agonist clonidine (20-40 ug/kg, i.m.).

Compared with recordings from the same region of the brain stem using conventional glass coated tungsten microelectrodes, fine wires exhibited a lower signal to noise ratio, and a lower yield of neurons per penetration. On the other hand, stable recordings could be maintained longer and more consistently with fine wires than with microelectrodes. Fine wire electrodes have the potential to provide longer term recording from specific populations of neurons in awake, behaving primates with less restraint than is currently possible with other techniques.

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166.5 REGIONAL EFFECTS OF AMPHETAMINE IN THE NEOSTRIATUM: SINGLE-UNIT RESPONSES IN FREELY-MOVING RATS. G.V. Rebec and T.W. Gardiner* (SPON: S.D. Curtis). Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

The neuronal response to amphetamine (AMPH) in immobilized (IMM) rats is both dose-dependent and regionally specific. Low doses ($\leq$ 2.5 mg/kg d-AMPH) typically suppress unit activity in the anteromedial neostriatum, whereas high doses ($\geq$ 5.0 mg/kg) produce the opposite effect. Neurons in the ventrolateral neostriatum, on the other hand, generally are excited by low and inhibited by high doses of d-AMPH. Multiple-unit recordings obtained from freely-moving (FM) rats, however, have indicated that both low and high doses of AMPH uniformly produce excitations in neostriatal activity. We have begun to examine these differences using single-unit recording techniques to determine the AMPH response in FM rats on a region-specific basis.

Differential recording of single-unit activity was obtained using a tungsten microelectrode, with the input signal fed into a lightweight headstage amplifier. This arrangement permitted free and unrestricted movement by the animal, without significant movement artifact. Thus far, in 7 different animals, the mean baseline firing rate (3.0 spikes/sec $\pm$ 1.8) of neurons in the anteromedial neostriatum was comparable to that previously obtained for IMM rats. No large variations were noted in the firing rates of individual neurons, although many cells (4 of 7) showed clear increases in activity associated with movement. Each rat then was challenged with an ip injection of 1.0 mg/kg d-AMPH. Within 20 minutes, the firing rate of 3 of 7 neurons increased by more than 300%; this response peaked at approximately the same time as the behavior. The remaining 4 neurons were clearly depressed by AMPH (by 50-80%). Although preliminary, these results contrast with both the single-unit recordings obtained from IMM rats and the multiple-unit recordings of FM animals. They suggest, however, that like the responses observed for IMM rats, AMPH may exert complex alterations in neostriatal unit activity, rather than uniform excitatory responses. Further work will extend these observations to other doses of AMPH and to other regions of the neostriatum.

Support by USPHS Grants DA 02451 and RR 7031.

166.6 ACTIONS OF d-AMPHETAMINE ON CEREBELLAR PURKINJE CELLS IN FREELY MOVING RATS. A.J. Michael, M.O. West, J.K. Chapin, B.D. Waterhouse, D.J. Woodward, Dept. Cell Biology, UTHSCD, Dallas, Texas 75235.

The present study was conducted to investigate the actions of d-amphetamine (d-A) on spontaneous discharge and GABA-induced inhibitory responses of cerebellar Purkinje (P) cells in the freely moving rat. Adult Long-Evans rats were prepared for chronic recording with a detachable microdrive positioned over the anterior cerebellum (midway between lambda and the occipital crest, 1 mm lateral to midline). Extracellular activity of P cells was recorded with multibarrel glass micropipettes during periods when the animal was motionless but alert. Drug response histograms collected before, during, and after d-A iontophoresis were used to quantitatively evaluate d-A effects on spontaneous discharge and inhibitory responses to microiontophoretic pulses (10 sec. duration, 40 sec. intervals) of gamma-aminobutyric acid (GABA). In 7 of 8 (88%) neurons, microiontophoretic application of d-A suppressed spontaneous discharge at currents ranging from 3 to 100 nA (mean=25 nA). In 6 of the 7 (86%) cells in which inhibition was observed, spontaneous firing rate returned toward control levels within minutes after cessation of d-A administration. In 2 of 3 units, d-A augmented GABA-mediated suppression of P cell discharge relative to changes in spontaneous activity. In both of these neurons, d-A caused a potentiation of GABA-induced inhibition at microiontophoretic doses which caused little or no change in spontaneous discharge.

These data support and extend our previous findings in anesthetized animals that iontophoretic doses of d-A can suppress spontaneous discharge of P cells, and that d-A can produce an augmentation of GABA-mediated inhibition of P cell activity. Previous investigations have demonstrated that norepinephrine iontophoresis and locus coeruleus stimulation can also potentiate GABA action in the cerebellum.

Thus, the data presented here provide a crucial link between studies in acute, anesthetized animals and studies in chronic, awake animals. Furthermore, these experiments serve as a foundation for the further evaluation of the actions of d-A and other drug substances on the physiology of neuronal circuits during behavior.

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166.7 SEX DIFFERENCES IN AN ANIMAL MODEL OF AMPHETAMINE PSYCHOSIS Dianne M. Camp* and Terry E. Robinson (SPON: E. S. Valenstein). Department of Psychology and Neuroscience Laboratory, The University of Michigan, Ann Arbor, MI 48104-1687

The "behavioral sensitization" produced by the repeated intermittent administration of amphetamine (AMP) is thought to be an animal analogue of AMP psychosis. In studying the factors that influence the development of behavioral sensitization we recently found that female rats show a much more robust sensitization of AMP-induced rotational behavior than males. The present experiment was conducted to determine: (1) whether there are similar sex differences in the sensitization of other AMP-induced behaviors; and (2) whether there are sex differences in forebrain dopamine (DA) utilization in sensitized animals.

Adult Holtzman rats received an i.p. injection of AMP every 4 days, for a total of 10 injections. To compensate for sex differences in the metabolism of AMP male and female rats received different doses of AMP. Male rats received 3.0 mg/kg. Female rats received either 2.6 mg/kg (which produces brain levels equivalent to males receiving 3.0 mg/kg), or a lower challenge dose (1.78 mg/kg) that did not behaviorally distinguish males and females following the initial AMP treatment. AMP-induced stereotypy was quantified using a rating scale, and locomotor activity (distance traveled) was continuously monitored in automated activity monitors. All animals were killed 8-13 days following the last AMP injection and the medial frontal cortex and striatum assayed for DA and dihydroxyphenylacetic acid (DOPAC) using HPLC-EC. The ratio of DOPAC/DA was used as an index of DA utilization.

Both males and females showed a progressive enhancement in stereotyped behavior over the 10 test sessions. However, there were also significant sex differences. As predicted, female rats showed a greater sensitization of stereotyped behavior than males. The quality of stereotypy also differed in sensitized males and females, with females displaying a greater hyperactive component. There were sex differences in the sensitization of locomotor activity as well. Both female groups showed a significant change in locomotor activity across the 10 test sessions, whereas the activity of males did not.

Interestingly, there were also sex differences in DA utilization in sensitized animals. In female rats AMP pretreatment produced a significant increase in striatal DA utilization ($p=.02$), and a trend for enhanced frontal cortex DA utilization ($p=.075$), relative to non-AMP treated controls. In contrast, AMP sensitization had no measurable effect on striatal or frontal cortex DA utilization in male rats. We conclude that sex has a major influence on the development of behavioral sensitization and suggest that the neural correlates of behavioral sensitization may be easier to identify and study in female animals.

166.8 SIMULTANEOUS RECORDING OF DOPAMINE-CONTAINING NEURONS IN THE SUBSTANTIA NIGRA AND VOLTAMMETRIC RELEASE OF DOPAMINE IN THE CAUDATE NUCLEUS OF BEHAVING CATS. V.M. Trulson and M.E. Trulson. Department of Anatomy, College of Medicine, Texas A&M University, College Station, Texas 77843.

Electrophysiological recordings of single dopamine (DA)-containing neurons in the substantia nigra (SN) of anesthetized and/or immobilized animals have long been used as an index of functional activity of the central DA system. These neurons appear to possess autoreceptors which are sensitive to DA as well as DA agonists. More recently, a technique was developed for recording the electrophysiological activity of single DA-containing neurons in the SN of behaving cats. Drug manipulations under these conditions were similar to those observed in anesthetized and/or immobilized rats. However, examining the activity of DA-containing neurons in behaving cats revealed that, unlike other neurons in the brain, these cells showed no significant change in activity during sleep. Furthermore, these studies revealed that the activity of DA neurons increased by only a small amount (i.e., 15-20%) when the animal engaged in active movement. These latter data seem somewhat surprising in view of the postulated role of the nigral striatal DA system in movement. Since the introduction of the technique of *in vivo* voltammetry for measuring DA release it has become possible to examine the neurochemical turnover of DA in freely moving animals. Unit recordings were made using Formvar coated 32 micron diameter nichrome wires. DA release was measured using linear sweep voltammetry with semi-differentiation using electrodes that separate dopamine from ascorbic acid. Ag/AgCl electrodes and 27-gauge stainless steel needles were used as reference and auxiliary electrodes, respectively. The working electrodes were scanned at a rate of 10 mV/s over the range of -0.1 to +0.5 V every 5 min using a BAS CV37 voltammograph. *In vitro* recording using standards of DA revealed that peak for DA occurred at 0.28 V. The data revealed that, while unit activity was not changed across the sleep/waking cycle, the voltammetric release of DA was significantly decreased by approximately 35% during REM sleep. In addition, during episodes of active movement the activity of single DA-containing SN neurons increased only 15-20%, while the voltammetric release of dopamine in the caudate nucleus was increased by 40-50%. These data suggest that the measurement of single unit activity of DA-containing neurons does not accurately reflect the functional activity of central DA system under all conditions. Therefore, one must be extremly cautious of extrapolating from electrophysiological recordings of single DA neurons to the functional state of the central dopaminergic system.

166.9 BILATERAL 6-OHDA LESIONS OF MESOLIMBIC TERMINALS SELECTIVELY ENHANCE SPONTANEOUS AND SCOPOLAMINE-INDUCED LOCOMOTOR BEHAVIOR. M.R.Lynch and R.J.Carey. S.U.N.Y. Upstate Medical Ctr. and V.A. Medical Ctr., Syracuse, New York 13210.

A substantial amount of evidence exists to support a mesolimbic substrate for DA agonist-induced hyperactivity. However, bilateral 6-OHDA lesions of the nucleus accumbens (following DMI pretreatment) have produced both hyper- and hypoactivity. Most of these studies have employed only photocell measurement of spontaneous motor activity and yield overall session totals which are not useful for characterizing the post-lesion behavioral profile. Also, many reports are confounded by DA depletions in striatal areas as well. Therefore, the present study examined the habituation pattern of discrete locomotor responses in an open field both before and after bilateral 6-OHDA infusions made at deGroot coordinates AP +3.0, L +2.5 and DV -8.0 from dura. Subjects were six male Sprague-Dawley rats and all received 25 mg/kg DMI pretreatment. Cross-overs were recorded for five 2-min intervals over a 10-min observation session. Rearings were monitored in the same manner and total sec. grooming time was also recorded. Rats were tested at 48 hr intervals, between 1500 and 1800 hr. Further, as both dopaminergic and cholinergic systems appear to be intimately involved in the control of motor behavior, all animals were also tested with 1.0 mg/kg d-amphetamine, 0.1 mg/kg apomorphine and 0.5 mg/kg scopolamine-HCl, both prior to surgery and at 13-21 days post-lesion. All drugs were administered i.p. 30 min before testing, and return-to-baseline sessions were conducted between consecutive drug administrations.

Rats were allowed to recover for one week. From day 7 to 11 post-surgery, activity measures were not significantly altered from pre-surgery levels. From day 13 to 21 however, session totals for crossings revealed a significant increase over pre-lesion values. Examination of the within-session data indicated a significant habituation pattern, which was not apparent during pre-lesion testing. Pre- and post-lesion comparisons for the drug effects revealed differences between the three behaviors. Thus, both pre-lesion amphetamine-induced increases in rearing and decreases in grooming were abolished by the surgical manipulation. Apomorphine produced significantly greater decreases in grooming and scopolamine significantly increased crossings, (whereas only a slight increase was produced by this anticholinergic drug prior to the lesion), with no effect on the other two measures.

These findings indicate that, contrary to previous suggestions, the hyperactivity produced by bilateral 6-OHDA lesions of the nucleus accumbens is not due to a failure to habituate to the test environment. They also provide further indication of a dopaminergic-cholinergic interaction at the mesolimbic level which merits further investigation.

166.10 QUIPAZINE MALEATE, A 5HT AGONIST, POTENTIATES AMPHETAMINE-INDUCED LOCOMOTION IN THE MOUSE. E. Wanek and W.H. Riffee, Div. of Pharmacology, College of Pharmacy, Univ. of Texas, Austin, TX 78712

There is increasing evidence of a serotonergic component in the neural control of locomotion. Neurons with cell bodies in the raphe nuclei innervate higher dopaminergic brain areas concerned with locomotion, such as frontal cortex, nucleus accumbens and neostriatum (caudate-putamen). Alteration of central 5HT levels by l-tryptophan or parachlorophenylalanine, decreases or increases, respectively, spontaneous locomotor activity in rats. The aim of this study was to determine whether quipazine maleate, a putative serotonergic receptor agonist, has an effect on locomotion induced by a moderate (2.5 mg/kg) dose of amphetamine in the male CD-1 mouse. Mice were food-deprived for 24 hours prior to experimentation. Drugs were dissolved in saline and were given in a dose of 0.01ml/gram body weight. Doses were calculated and given as the salt. After a saline preinjection and a 60 minute habituation in the testing environment, a plexiglass cage monitored by infrared detectors, quipazine (2.5 mg/kg) was administered intraperitoneally, and the mouse replaced into the test cage. After 15 minutes, saline or amphetamine was given and activity monitored for the next 60 minutes. The parameter of activity measured was total distance moved per 5 minute periods. Activity was analyzed by a microprocessor which permitted only horizontal activity resulting from interruption of two successive infrared beams to be recorded. When quipazine was administered, there was a large, significant and sustained increase in amphetamine-induced locomotor activity compared to animals receiving a saline control injection. The apparent discrepancy between the results mentioned above using spontaneous locomotor activity in the rat and those of this study can be explained based on the habituated murine model utilized in this study. Earlier studies in our laboratory have shown that habituation in the test environment eliminates the exploratory (spontaneous) locomotor activity and permits the observation of direct effects of drugs on motoric behavior rather than including confounds such as handling and novel environment. The ability of quipazine to potentiate the amphetamine-induced locomotor activity shown in this study suggests that there may be direct or indirect serotonergic influences on dopaminergic systems underlying motor activity. These results also imply that the murine model utilizing habituation permits a clearer view of these drug interactions than those using non-habituated animals. (Supported by grant MH 33442 to W.H.R.)

166.11 EFFECTS OF DIETARY PROTEIN AND TYROSINE SUPPLEMENTATION ON STEREOTYPY AND BRAIN CATECHOLAMINES IN STREPTOZOTOCIN-DIABETIC RATS. L. L. Bellush and N. Rowland. Department of Psychology, University of Florida, Gainesville, Florida 32611.

Diabetic rats have been shown to have reductions of striatal dopamine synthesis (Trulson & Himmel, 1983) as well as increased dopamine receptor number (Lozovsky, Saller & Kopin, 1981). Attenuated responses to the dopaminergic agonists apomorphine and amphetamine have also been found (Marshall, 1978; Rowland, Joyce & Bellush, 1985). These alterations may, in part, be a consequence of reductions in brain concentrations of tyrosine, the amino acid precursor of dopamine known to occur in diabetes. In the present studies, we have examined whether dietary protein enrichment (exp. 1) or selective enrichment of synthetic diets (exp. 2) or powdered chow (exp. 3) with tyrosine will alleviate some of these abnormalities in diabetics. In all 3 experiments, behavioral stereotypy induced by amphetamine (3 mg/kg and 5 mg/kg) and apomorphine (1 mg/kg) were compared in diabetic and control rats on the various diets. In experiment 2, L-DOPA concentrations were measured in nucleus accumbens, hypothalamus and striatum following decarboxylase inhibition.

In experiment 1, high levels of dietary protein (46%) in combination with either carbohydrate or fat as the other major macronutrient, failed to restore the behavioral response of diabetic rats. In experiment 2, addition of 1% tyrosine (w/w) to synthetic diets containing casein (1% tyrosine) led to elevated L-DOPA concentrations in all three brain regions of diabetics, although only the elevation in nucleus accumbens was statistically significant. However, stereotypy was not modified by diet, nor were there correlations between L-DOPA concentrations and individual summed stereotypy scores. In experiment 3, enrichment of powdered chow (.68% tyrosine) with 1.36% or 4.08% additional tyrosine produced no modulations in the stereotypy of diabetics. The general conclusion from these findings seems to be that while dietary supplementation with tyrosine may enhance dopamine synthesis in some brain regions, this may not lead to enhanced release of transmitter which could actually correct performance deficits in diabetic rats.

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166.12 THE EFFECTS OF INTRAVENTRICULAR INFUSIONS OF 5-HT AND TRYPTAMINE ON SELF-STIMULATION. J. Broadbent* and A.J. Greenshaw* (SPON: S. Richardson). Psychiat. Res. Div., Sask. Health, Univ, Sask., Saskatoon, Sask., Canada.

Previous reports have suggested that 5-HT may inhibit self-stimulation behaviour maintained by stimulation of the lateral hypothalamus. The effects of the structurally related 'trace amine' tryptamine has never been assessed in this paradigm. Tryptamine has recently been shown to be active in the rat CNS. Tryptamine binding sites which are distinctive from those of 5-HT have also been described. This study therefore assessed the effects of 5-HT and tryptamine infusions on lateral hypothalamic self-stimulation using rate-free measures of reinforcement to separate performance effects from specific reinforcement effects.

Male Wistar rats were implanted with 3rd ventricular cannulae and lateral hypothalamic twisted bipolar stimulating electrodes. Subjects were trained to lever press (60 HZ sine wave, 36-106 µA) on a fixed interval 3 sec. schedule. Duration of stimulation trains was determined by the length of time the lever was depressed. Rate of responses and duration of stimulation trains during the non-reinforced interval and during reinforcement were measured by an on-line micro-computer. After stable lever pressing was established subjects were tested with different current intensities and a working current chosen to allow changes in the duration of stimulation. Subjects were given daily 60 min. training sessions until less than 20% variation in baseline performance over 3 consecutive training sessions was observed. Clorgyline, a monoamine-oxidase inhibitor was given (5 mg/kg IP) 2 hours before the test session. Drug infusions of 10 µl of CSF, 5-HT (6.25 - 100 µg free base) or tryptamine (50 - 400 µg) were controlled by a Harvard infusion pump. Subjects were only used for one injection.

5-HT given intraventricularly induced a 50% inhibition of responding at doses of 12.5µg and above. An increase in the duration of non-reinforced lever presses was also observed, however, there was no change in the duration of stimulation trains. Tryptamine at doses of 200 µg and above also resulted in decreases in response rate . Again, non-reinforced duration was elevated but stimulation duration did not change.

As 5-HT did not affect the duration of stimulation trains, these changes were interpreted as non-specific performance effects. The results of tryptamine infusions were similarly interpreted. 5-HT and tryptamine did however result in similar effects on behaviour, although, a much higher dose of tryptamine was necessary.

It is concluded, therefore, that both 5-HT and tryptamine induce non-specific decreases in lateral hypothalamic self-stimulation on this schedule when infused into the third ventricle.

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166.13 TRYPTAMINE INDUCES CONDITIONED TASTE AVERSION LEARNING IN THE RAT. P.J. Fletcher* (SPON: J.D. McQueen) . Psychiat. Res. Div., Sask. Health, Univ, Sask., Saskatoon, Sask., Canada.

It has been suggested that duration of action may be an important determinant of the formation of a drug induced CTA (Goudie and Dickins, 1978, Pharmac. Biochem. Behav. 9, 587). Several short acting compounds (eg., heroin, cocaine and phenylethylamine) are weak elicitors of a conditioned taste aversion (CTA) whereas longer acting related compounds (eg., morphine, amphetamine) reliably induce a CTA. Experiments in this report investigate the possible aversive stimulus properties of the short acting trace amine tryptamine (T). In addition since T may act at 5-HT receptor sites (see Jones, 1982, Prog. Neurobiol. 19, 117) the effects of T were compared to those induced by the 5-HT agonist quipazine.

Male Wistar rats were water deprived and presented with a 0.1% saccharin solution, followed by injection of T (20, 40, 60 or 80 mg/kg) or saline. A second group of rats were treated identically except that they received quipazine (2.5, 3.5, 5.0 or 7.1 mg/kg) or saline. On 4 retention trials rats were presented with saccharin and water, and the percentage preference for saccharin was calculated for each rat. On trial 1 all doses of T, except 20 mg/kg, induced an aversion to saccharin. By trial 4 this aversion was evident only in rats treated with 80 mg/kg T. These results differed from those obtained with quipazine. All doses of quipazine except 2.5 mg/kg produced a CTA which persisted over the 4 retention trials.

The effects of T were then examined in a single bottle, repeated injection paradigm. Water deprived rats were presented with saccharin and injected immediately afterwards with T (20, 40, 60 or 80 mg/kg) or saline. The saccharin-T pairings were administered 3 times at 4 day intervals. Although all doses of T except 20 mg/kg induced significant aversions to saccharin on 3 retention trials only rats treated with 80 mg/kg T exhibited a progressive decline in saccharin consumption across the retention trials.

The results show that T, like other agents altering indoleaminergic neurotransmission, produces a CTA. However, unlike these agents, T appears to be a relatively weak elicitor of a CTA. Since T is metabolised rapidly in rat brain (Wu and Boulton, 1973, Can. J. Biochem. 51, 1104) this may account for the weak action of T on CTA learning.

166.14 2-DG UPTAKE PATTERNS IN RAT CNS FOLLOWING ADMINISTRATION OF ABUSE POTENTIAL SUBSTANCES: A COMPUTER FACILITATED ANALYSIS. D.J. Woodward, Z.N. Stowe, W.K. Smith, D.L. McEachron, J.K. Chapin, and B.D. Waterhouse. Dept. of Cell Biology, UTHSCD, Dallas, TX 75235.

The ^{14}C-2-deoxyglucose (2-DG) method was used to examine glucose utilization patterns in animals receiving one of three abuse potential drugs; amphetamine, cocaine or ethanol. One week prior to experimental sessions, Long-Evans hooded rats were surgically implanted with stimulating electrodes under the toepad of each forepaw. For each experiment, animals received an i.p. injection of either: d-amphetamine sulphate, high dose = 10 mg/kg; low dose = 1 mg/kg; cocaine-HCl 10 mg/kg; ethanol 1.3g/kg or saline vehicle alone (control) followed 15 min. later by 2-DG (i.p., 14 mCi/100 gr). These drug dosages are known to produce behavioral effects in laboratory rats. The animals were then placed in a plexiglass enclosure for 60 min. prior to sacrifice. During this time both forepaws were continuously (0.25 Hz) stimulated at levels just subthreshold for limb withdrawal. Autoradiographs were prepared from brain sections (20 μm) according to standard procedures and then video digitized, linearized and color enhanced by a computer-based imaging system. Visual examination of autoradiographs indicated that all of the compounds caused increased 2-DG uptake in the cerebellar cortex, thalamus, caudate and cerebral cortex. Unlike amphetamine and cocaine, which caused increased 2-DG uptake in the inferior olive and surrounding brainstem, glucose utilization in these areas was reduced relative to controls after ethanol administration. High dose amphetamine produced a distinct pattern of alternating high and low glucose metabolic patches throughout the cerebellar hemispheres and vermis. Likewise, 2-DG uptake in several thalamic nuclei, particularly the ventral medial, intralaminar and lateral posterior, was clearly increased in autoradiographs from ethanol and cocaine brains and was highest in animals receiving high or low dose amphetamine. Glucose utilization was also increased above control levels in layer IV throughout the neocortex after amphetamine, cocaine and ethanol administration. For all drugs, glucose metabolism was not significantly different from controls in the hippocampus, inferior and superior colliculi and vestibular nuclei. In summary, these findings indicate that abuse potential compounds produce specific changes in glucose metabolism that are localized to discrete anatomical regions and probably reflect drug-induced enhancement or suppression of activity in local neuronal circuits. Overall, it appears that cocaine and amphetamine produced qualitatively similar changes in 2-DG uptake patterns which might be correlated with the similarity of their behavioral actions contrasted with those of ethanol. (Supported by AA3901, DA 02338, the Biological Humanics Foundation to DJW; AFOSR-85-0155 to BDW).

166.15 PHYSIOLOGY OF ABUSE POTENTIAL SUBSTANCES IN CENTRAL NEURONAL CIRCUITS: EFFECTS OF COCAINE ON SPONTANEOUS DISCHARGE AND GABA RESPONSIVENESS OF CEREBELLAR PURKINJE CELLS. B.D. Waterhouse, Z.N. Stowe*, J.-T. Cheng, A.J. Michael, D.J. Woodward, Dept. of Cell Biology, UTHSCD, Dallas, TX 75235.

Previous studies from this laboratory demonstrated that d-amphetamine could potentiate depressant responses of cerebellar Purkinje (P) neurons to microiontophoretic application of GABA as well as suppress P cell spontaneous firing rate. In the present experiments, we have examined the effects of cocaine, another widely abused CNS stimulant, on GABA-mediated inhibition and spontaneous discharge of cerebellar P neurons. Extracellular activity of single P cells was recorded from halothane-anesthetized rats during iontophoretic pulses (10s duration, 40s intervals) of GABA (16-50 nA). Peri-event histograms collected before, during and after microiontophoretic or i.p. administration of cocaine-HCl were used to quantitatively assess cocaine-induced changes in P cell spontaneous firing rate and GABA responsiveness. Parenteral doses of cocaine used here have been shown to produce alerting effects (10 mg/Kg) or increased locomotion (35 mg/kg) in laboratory rats (Scheel-Kruger et al., 1977). Following injection of high dose cocaine, P cell spontaneous discharge was routinely suppressed (n=5). In 4 of these 5 cases, inhibitory responses to GABA were reduced ($\bar{x}$ = 66%) from control levels. The onset of these effects was within 3 min. of cocaine injection and were maximal by 10 min. post-injection. Recovery toward the control level of GABA response was observed by 35-60 min. after cocaine application. Following injection of of low dose cocaine, P cell responses to GABA were consistently augmented an average of 63% (n=4) above control levels, while spontaneous firing rates were decreased (n=2), unchanged (n=1) or increased (n=1) from pre-cocaine levels. These drug-induced effects were observed within 3 min of cocaine injection, reached maximal levels by 25 min. and began to subside 30-40 min. post-injection. Enhancement of GABA-induced inhibitory responses and suppression of P neuron spontaneous activity were also observed with iontophoretic administration of cocaine (7-15nA). In summary, these results indicate that parenterally or iontophoretically applied cocaine has depressant effects on P cell spontaneous discharge. At low doses, both systemic and local administration of cocaine can also produce an enhancement of GABA-mediated inhibition similar to that observed with iontophoretic d-amphetamine. Such enhancement of neuronal responsiveness to GABA within local neuronal networks could facilitate the feature extraction function of brain circuits and might prove to be a common mode of CNS stimulant drug action. (Supported by NIDA DA 02338 to DJW; AFOSR-85-0155, NINCDS 18081, and the Klingenstein Foundation to BDW).

166.16 EFFECTS OF ELEVATED CALCIUM ON LEARNED HELPLESSNESS AND BRAIN SEROTONIN METABOLISM IN RATS. K. Arasteh, D.W. Ray*[1] and M.E. Trulson. Department of Anatomy, College of Medicine, Texas A&M University, College Station, TX 77843, and [1]Department of Psychology, Marshall University, Huntington, WV 25701.

Mineral metabolism in depressive illness has been the subject of investigation for many decades. Calcium metabolism in mood disorders has been studied since the 1940's. Altered calcium homeostasis associated with depressive illness has been reported. However, since these studies are purely correlational in nature, they have not determined whether the altered calcium levels is a cause or a consequence of the disease state. Moreover, most of these studies have measured serum calcium levels, which do not necessarily reflect brain calcium concentrations. In addition to patients with the primary diagnosis of depression, patients with hyperparathyroidism also suffer from mood disturbances. The etiology of at least some of these disorders has been hypothesized to be related to abnormal calcium metabolism. Increased levels of calcium in the cerebral spinal fluid have been reported during the manifestation of depression in these patients, while the reverse occurs during recovery from such symptoms. Recent data from our laboratory revealed that small elevations of calcium ions (10-15%) significantly suppressed the activity of serotonin-containing dorsal raphe neurons by approximately 35% in vitro. Therefore, the abnormal levels of serotonin frequently found in depression may constitute a secondary effect rather than a primary one. In the present study, the effects of elevated calcium on learned helplessness in rats was tested by maintaining animals on either distilled water or water containing 2.5% calcium. Animals that were maintained on drinking water containing high calcium showed elevated levels of brain (+18%) and serum (+22%) calcium. Rats that were maintained on high calcium drinking water showed longer escape latencies (33.0 sec) than their non-calcium counterparts (18.3 sec) after they were pretreated with inescapable electric shocks ($p<0.025$). Lower levels of 5-hydroxyindolacetic acid associated with the state of "depression" were found in the forebrain (-26.3%) and brainstem (29.7%) of these animals. Therefore, elevated levels of calcium enhance learned helplessness and decrease brain serotonin turnover. These data suggest that the primary deficit in certain depressive states may be in calcium homeostasis rather than in serotonin metabolism.

166.17 STUDIES ON LOCOMOTOR ACTIVITY AND ROTATION AFTER LESIONS WITHIN OR LATERAL TO THE MEDIAN RAPHE NUCLEUS. D. Wirtshafter, W. Montana* and K.E. Asin. Dept. Psych., Univ. IL at Chicago, Chicago, IL 60680.

It has been well established that electrolytic lesions of the median raphe nucleus (MR) produce large increases in locomotor activity in most novel environments. The identity of the neural substrates underlying this effect, however, is not clear. Studies employing intra-MR injections of the serotonin (5HT) neurotoxin 5,7-DHT or injections of the excitotoxin ibotenic acid have suggested that MR-lesion induced hyperactivity may reflect damage to both nonserotonergic cells within the MR and to fibers of passage (Asin & Fibiger, Brain Res, 1983).

In the current study we attempted to more precisely characterize the substrate of MR-lesion induced hyperactivity by examing locomotion after placement of small lesions either within, or immediately lateral to, the MR. Lesions were produced by lowering a piece of 22 gauge stainless steel tubing. Midline "punches" passed directly through the MR and produced small, but significant, decreases in hippocampal 5HT. Punches 0.6mm lateral to the midline passed through the so-called predorsal bundles (tectospinal tracts) and failed to alter striatal or hippocampal 5HT levels. Both types of lesions produced equivalent increases in open field and tilt cage activity and equivalent decreases in running wheel activity in a 24 hour test compared to controls. These results demonstrate that it is possible to completely dissociate changes in locomotor activity and 5HT levels following lesions in the paramedian tegmentum. However, it is unclear whether the midline and lateral punches produce hyperactivity by damaging distinct neural systems, or whether they both damage a single system which passes diffusely through the MR and region lateral to it.

Since lesions of the superior colliculus have been reported to result in hyperactivity, we examined the possibility that the increases in activity produced by the lateral punches might have resulted from damage to the crossed component of the tectospinal tract. Therefore, we next examined locomotor activity following sagittal knife cuts through the dorsal tegmental decussation, through which descending tectospinal fibers pass. These cuts neither produced hyperactivity by themselves nor altered the effects of the lateral punches. It is unlikely, therefore, that damage to descending collicular fibers plays a significant role in the hyperactivity produced by electrolytic MR lesions.

It has also been reported that asymmetric MR lesions produce contralateral rotation. To further examine this effect, we placed unilateral punches .3,.6,.9 & 1.2mm lateral to the midline of the MR. On Day 2 post-op, rats with punches at .3 or .6 showed similar rates of rotation; on Day 10 post-op, only the most medial punch still produced significant rotation. These results suggest that the substrates of the rotation lie medial to the tectospinal tract.

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166.18 MUSCARINIC CHOLINERGIC BINDING SITES REVERT DURING EXTINCTION OF CONDITIONED EMOTIONAL RESPONSE (CER) Angela K. Dolce* and John D. Lane (SPON: I.M. Korr), Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107 USA

Rats were classically conditioned to associate a conditioned stimulus (CS) with footshock, so that on test day, CS presentation alone produced suppression of food-reinforced responding and collateral 'emotional' behaviors reminiscent of anxiety (conditioned emotional response, CER). Previous studies (Lane et al., Europ. J. Pharmacol. 83, 183, 1982) demonstrated that this paradigm resulted in total behavioral suppression and a 40% reduction in cortical muscarinic cholinergic (quinuclidinyl benzilate—QNB) binding sites (change in Bmax and not Kd). These experiments were extended to assess the role of repeated CS presentations (extinction) on binding. After CER conditioning, rats were subjected to 15 additional once daily trials of 60 min food-reinforced responding, during which the CS was presented continuously during the final 15 min, but footshock was never delivered. At 2 day intervals groups of rats were sacrificed and cortical QNB binding assessed. Control groups for pre-CS and for no-CS (up to 11 trials) were run to ensure that baseline binding parameters did not vary over the entire course of the experiment. Behavioral suppression extinguished within 13 trials. Cortical QNB binding (changes in Bmax and not Kd) followed a parellel time course and returned to within normal limits. Benzodiazepine binding was also assessed. At trial 1, binding was decreased 25% (change in Bmax and not Kd) but was still decreased 11% after 15 trials of extinction. The operant chamber may represent a pseudo-CS for the animals. These transient phenomena are consistent with other behavioral paradigms, and suggest that the cholinergic system mediates or responds to conditioning-emotion. The effects of cholinergic agonists and antagonists, and AChE inhibitors on the behavior and binding parameters are now being assessed. (supported in part by MH-31835 to JDL).

166.19 BEHAVIORAL SEQUELAE FOLLOWING MPTP ADMINISTRATION IN THE MOUSE J.H. Kordower, S.Y. Felten, D.L. Felten, and D.M. Gash, Dept. of Anatomy, Univ. of Rochester Sch. Med. Den., Rochester, N.Y. 14642.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has received considerable attention with regards to inducing Parkinson's disease in humans and a Parkinsonian-like state in animals. Rats appear to be resistant to the neurotoxic effects of this compound. Systemic injections into mice cause long-lasting depletion of dopamine and its metabolite in the striatum (Neuropharm. 23: 711, 1984). While there have been observations of motor abnormalities reported, there has been little systematic assessment of the behavior in these animals. The present study examines the effects of MPTP upon motor and ingestive processes, two behaviors dependent upon nigrostriatal activity.

Mice were matched into groups based upon stable basal activity levels as measured by automated photocell activity meters. Group 1 (n=8) received a single subcutaneous injection of MPTP (30 mg/kg, methyl ester) daily for 5 days. Group 2 (n=8) received similar injections of vehicle (20% ethanol). Activity measurements were made for 30 min 1, 3, 5, and 7 days after drug treatement. Vehicle injections failed to alter activity levels. While the mice appeared hypoactive immediately following each injection, MPTP produced a significant hyperactivity 3, 5, and 7 days after drug treatment as compared to both baseline levels and vehicle treated mice. Activity levels in response to d-amphetamine and apomorphine were then assessed. Vehicle treated mice were hyperactive to amphetamine (1.5 mg/kg, sc). In contrast, MPTP mice were unaffected by d-amphetamine administration. Apomorphine (.1 mg/kg) produced a similar hypoactivity in both groups.

An additional two groups of mice were matched into groups based upon stable basal food intake and were administered MPTP (n=9) or vehicle (n=7) as before. Food intake, water intake, and body weights were then measured for 2 weeks. MPTP failed to produce any changes in any of these parameters. Following the 2 week period, mice were administered 2-deoxy-D-glucose (0, 300, 600, 900 mg/kg in ascending and descending order) to determine whether MPTP would alter the resulting hyperphagia. MPTP failed to affect the hyperphagia that resulted from the 600 and 900 mg dose.

High performance liquid chromatography is presently being carried out to determine the catecholamine and indolamine levels in the striatum, nucleus accumbens, medial basal hypothalamus, and substantia nigra of these mice.

In the present experiments MPTP produced a significant basal hyperactivity for at least one week following drug treatment. This may be due to increased activity of remaining dopaminergic neurons or due to MPTP's effects upon non-dopaminergic systems. MPTP eliminated d-amphetamine hyperactivity. This latter result suggests the inability of the remaining nigrostriatal fibers to increase their release of dopamine in response to d-amphetamine stimulation. In contrast to other animal models of Parkinson's disease, ingestive processes are unalterd by MPTP. (Supported by AGT32 107 (JHK), NIH NS15109 (DMG)).

167.1 THE EFFECTS OF AP-cmNTS LESIONS OR SUBDIAPHRAGMATIC VAGOTOMY ON SALT APPETITE

S. Uysal*, J. Schulkin* & T.M. Hyde* (SPON. E. Stellar)
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There is evidence that lesions of the area postrema (a circumventricular organ) which in most cases also includes the caudal medial area of the nucleus of the solitary tract (AP-cmNTS) provoke changes in sodium homeostasis. Many abdominal vagal afferents terminate in this region; vagotomy at various levels is also known to provoke changes in sodium homeostasis. In the following study, the behavioral responsiveness to a salt appetite arousing treatment was studied in AP-cmNTS lesioned, or subdiaphragmatically vagotomized rats.

Adult male Sprague-Dawley rats were used. Five rats received AP-cmNTS aspiration lesions, 5 were subdiaphragmatically vagotomized and 10 were sham lesioned controls. Testing was performed one month after surgery. At testing the AP-cmNTS lesion group weighed 375 grams, the vagotomized group 330 and the control group 412 grams. Rats were adapted to a test chamber for 2 hrs. a day and given access to 3% NaCl and distilled water. Intakes in all 3 groups were negligible at this time. They were rendered salt hungry by being placed on a sodium deficient diet for 2 days; after the first 24 hrs. they were injected IP with 5 mg of furosemide to promote sodium loss. Twenty-four hours later the rats were placed in a test cage and saline and water intakes were noted for 30 minutes.

The results showed that all three groups increased their ingestion of the saline when rendered salt hungry. The AP-cmNTS group ingested a mean of 11.3 ± 4.8, vagotomized group ingested 5.8 ± 2.6 and the control group ingested 7.1 ± 2.5. On another trial (one week later) during a ten minute pre-ingestion access to the saline, the AP-cmNTS lesion group ingested 7.6 ± 3.6, the vagotomized group 4.5 ± 1.7 and the intact-control group 7.2 ± 2.5. When subsequently given access to the saline for an additional 30 minutes they ingested 6.5 ± 2.1 (AP-cmNTS) 2.6 ± 0.7 (vagotomized) and 2.3 ± 1.3 (intact-control)respectively.

The results revealed that AP-cmNTS and vagotomized rats increased their saline consumption in response to the salt appetite arousing treatment. However, the AP-cmNTS group tended to ingest more salt; the vagotomized group tended to ingest less salt. These results are consistent with other reports of elevated consumption of fluids in AP-cmNTS lesioned rats and decreased ingestion of subdiaphragmatic vagotomized rats.

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167.2 NEONATAL SPONTANEOUSLY HYPERTENSIVE RATS SHOW AN EXAGGERATED NaCl PREFERENCE. K. E. Moe. Department of Psychology, Washington State University, Pullman, WA 99164-4830.

Spontaneously hypertensive rats (SHRs) of the Okamoto-Aoki strain show an exaggerated NaCl preference relative to normotensive Wistar-Kyoto (WKY) rats. In contrast, other animal models of arterial hypertension are associated with a reduced NaCl preference. It is not clear whether this higher NaCl preference precedes, accompanies or follows the development of SHR hypertension. Therefore, NaCl preference was assessed in SHR and WKY pups at 12-13 days of postnatal age (prehypertensive stage; Lais et al, Blood Vessels, 14:277,1977).

On the day of testing, pups were removed from their dams, implanted with an anterior mouth catheter, and then adapted to a warm, moist incubator. They were offered 2, 5 or 7% NaCl (0.34, 0.86, 1.20 M) or distilled water by pulsatile infusion through the mouth catheter for 30 minutes. Change of weight (to the nearest 0.01 g) over the course of the infusion provided the measure of intake.

As shown in the table below, the increased NaCl preference characteristic of adult SHRs is also demonstrated by prehypertensive SHR pups, relative to WKY pups. No differences were observed for water intake by pups of the two strains.

It has been hypothesized that chronically elevated activity or sensitivity of the brain renin-angiotensin system (BRAS) contributes to the development of hypertension in the SHR model. Such a perturbation might also explain the enhanced NaCl preference of SHRs, as (1) the brain renin-angiotensin system has been shown to be important for the arousal of NaCl appetite in normotensive rats (Moe et al, Am. J. Physiol., 247: R356, 1984) and (2) central administration of an angiotensin converting enzyme inhibitor reduces the exaggerated NaCl preference of adult SHRs (DiNicolantonio et al, Nature, 298: 846, 1982). The results reported here may be a behavioral indicator of BRAS perturbations in advance of the development of SHR hypertension.

	SHR pups	WKY pups
Water*	0.74 ± 0.09	0.74 ± 0.13
NaCl		
2% (0.34 M)	2.31 ± 0.26	1.09 ± 0.10
5% (0.86 M)	1.19 ± 0.08	0.58 ± 0.11
7% (1.20 M)	1.03 ± 0.09	0.17 ± 0.08

*Fluid intake is reported as mean ± SEM grams of fluid ingested per 100 grams body weight. Equal numbers of male and female pups were used; each value is based on at least 10 pups from at least 5 different litters.

167.3 CAPTOPRIL-INDUCED SODIUM APPETITE IN RATS: BRAIN SITES OF ACTION. J.Salisbury, N.E.Rowland, Z.King-Allen*and M.J.Fregly*(SPON: G.M.Hope). Departments of Psychology and Physiology, Univ. of Florida, Gainesville, FL 32611.

Captopril (CAP) is an orally active inhibitor of the enzyme which converts angiotensin I (AI) to angiotensin II (AII). Doses of CAP which inhibit the enzyme in the periphery also induce appetite for NaCl solution in rats. This may be due to increased AI in the periphery which enters the brain where it may still be converted to AII. Elfont et al (J.Physiol. 354: 11, 1984) found that this NaCl appetite could be inhibited not only by cerebroventricular (icv) infusion of CAP but also by implanting an empty cannula into the 3rd ventricle. In the first part of the present study we have confirmed that CAP, administered in food (1 mg/g food x 6 days), induces a robust appetite for 0.3M NaCl solution (given in a 2 bottle ad libitum choice with water) in intact rats. A similar magnitude of NaCl appetite was found in rats implanted 6 days beforehand with an indwelling 23 gauge stainless steel cannula into the lateral cerebral ventricle. A third group of rats similarly received an indwelling cannula, aimed obliquely to end in the anterior 3rd ventricle but missing midline structures. These rats increased NaCl intake relative to a no CAP control group, although their 3x increase in NaCl intake above their own no CAP baseline was not statistically significant. We thus find no marked impairment of CAP-induced NaCl appetite and believe that damage to the blood brain barrier cannot account for the previous failure (Elfont et al) to induce NaCl appetite soon after brain cannulation. Instead, specific tissue damage may occur with the implants, and recovery from such damage may underlie any return of NaCl appetite.

In a second study, rats received electrolytic lesions aimed at the subfornical organ (SFO, a central receptor site for AII), and rats with effective lesions were identified by reduced or absent drinking response (mean water intake 2.0 ml/h) to peripheral AII (200ug/kg, sc) relative to those with ineffective lesions (7.8 ml/h). When CAP was added to their food, as in the first study, the rats with effective lesions drank 11.4 ml 0.45M NaCl /24h after 5 days CAP, and the ineffective lesion group drank 15.1 ml (not significantly different). It thus appears that SFO lesions may impair, but not abolish CAP-induced NaCl appetite. These animals are still being tested in other NaCl appetite paradigms, the results of which will be reported. Supported by NIH AM31837-03.

167.4 MINERALOCORTICOID-INDUCED SALT APPETITE: CENTRAL SITE OF ACTION? J. Buggy, J. Valentine*, N.E. Rowland, J. Carlton, M.J. Fregly, and W.G. Luttge. Departments of Physiology, Psychology, and Neuroscience, University of South Carolina, Columbia, SC 29208 and University of Florida, Gainesville, FL 32611.

Both angiotensin and mineralocorticoids stimulate salt appetite and recently a synergy of these hormones in brain has been hypothesized. However, our results indicate that salt appetite induced in rats by systemic administration of deoxycorticosterone (DOC) does not depend on central angiotensin mechanisms. Intracerebroventricular (ICVT) injections of the angiotensin receptor antagonist saralasin block central responses to angiotensin and significantly inhibit salt appetite induced by sodium depletion but had no effect on intake of 1.8% NaCl aroused by subcutaneous DOC treatment (2.5 mg/day for 5 days).

Since many hormones act specifically in brain and since aldosterone binding sites have been described in brain, we next assessed whether mineralocorticoids administered directly to the brain rather than systemically would stimulate salt appetite in rats. One series of studies used microcrystalline implants of DOC tamped into 27 gauge inner cannula and bilaterally implanted into either perifornical hypothalamus or dorsal hippocampus, regions reported to contain aldosterone receptors; there was no increase compared to baseline in ad lib intake of 0.9% or 1.8% NaCl for up to 7 days of treatment. Another series of experiments used aldosterone tamped into 24 gauge inner cannula and bilaterally implanted into either posterior hypothalamus or ventral hippocampus; again, there were no immediate or delayed effects of the implants on ad lib NaCl intake. Another approach used DOC suspended in propylene glycol and physiological saline for ICVT injections at the level of the anteroventral third ventricle, an effective injection site for stimulation of salt appetite by angiotensin; compared to baseline intake, 2 or 4 ug/day of DOC for up to 5 consecutive days failed to stimulate intake of 1.8% NaCl. We cannot be sure that the site(s) or doses of mineralocorticoids delivered are appropriate but given the efficacy of intracerebral steroids to modify other brain functions and behavior, these may be taken as preliminary findings against these loci as central sites for action of mineralocorticoids in salt appetite.

In summary, our data indicate that salt appetite induced in rats by mineralocorticoids does not depend on central angiotensin mechanisms. Furthermore, we have failed to identify a central site of action for mineralocorticoids that stimulates salt appetite. Although brain regions not tested here may mediate salt appetite by mineralocorticoids, the possibility that salt appetite results from a peripheral effect of mineralocorticoids, perhaps on salivary sodium concentration, should be considered. Supported by NIH-AM 31837-03.

167.5 DIURESIS AND SUPPRESSION OF SALT APPETITE BY LATERAL VENTRICULAR INFUSIONS OF ATRIOPEPTIN II. R.L.Thunhorst, D.A.Fitts* and J.B. Simpson. Dept. of Psychology NI-25, Univ. of Washington, Seattle, WA 98195

Experiments were performed to determine the central nervous effects on hydromineral balance for atriopeptin II (APII), a peptide known to possess natriuretic and kaliuretic properties when injected intravenously. During tests, solutions were infused via a lateral ventricular cannula and water and 0.3M NaCl solutions were available from calibrated burettes. Urine was collected into tubes below the cages for measurement of volume as well as concentration of Na^+, K^+ and mOsm. Normally hydrated rats (n=9) fed Purina lab chow received isotonic saline vehicle (ISO), 30 pmol and 60 pmol/2 ul/hr APII on separate days. Neither dose of APII affected spontaneous water intake. However, APII infusions increased U_V(ISO=2.8±1.2, 30 pmol=4.0±1.5, 60=pmol 4.5±1.8 ml) and decreased U_{Osm}(ISO= 760±23, 30 pmol=559±150, 60 pmol=521±127 mOsm/kg) and U_{Na}(ISO=100±49, 30 pmol=66±33, 60 pmol=64±32 mmol/L). Rats (n=14) made sodium deplete following combined treatment with furosemide diuresis (25mg/kg) and low sodium diet overnight received 3hr infusions with either ISO, 6pmol or 60 pmol/hr APII. Control rats drank saline with a median latency of 7.7 min and consumed 7.7±3.7 ml in the first hr. The 60 pmol dose increased the latency to 44.3 min and suppressed intake in all 3hrs with less than 5.0 ml total drunk. Water intake was not affected. The 60 pmol group increased U_V in hr. 1(3.7±0.8 vs 1.8±1.1 ml for ISO) and had the lowest total osm over 3 hrs (442±175 vs 746±262 mosm/kg for Iso). Rats infused i.v. (n=19) with ISO, 60 or 600 pmol APII for 1 hr showed no differences on any variable measured, including U_{Osm}, U_{Na}, U_K, as well as total Na^+, K^+ and solute excretions. Fluids were not available for consumption here. Normally hydrated rats (n=15) were given a 7 hr infusion of either ISO, 120 pmol/ul/hr angiotensin II (AII) or AII in combination with 120 pmol/hr APII. The peptide infused groups drank both fluids with similar latencies but there was a suppression of total water and saline intakes in the combined infusion group vs the AII-alone infused group. We conclude that APII, when infused centrally, stimulates urine flow without increasing solute excretion and reduces salt appetite in sodium depleted and AII-stimulated rats.

167.6 DRINKING RESPONSES TO INTRACEREBROVENTRICULARLY (ICV) APPLIED DIPSOGENS AFTER AN AREA POSTREMA/CAUDAL MEDIAL NUCLEUS OF THE SOLITARY TRACT LESION M. Weiss, T. Hyde and R. Miselis. Dept of Biology, Animal Biology, School of Veterinary Medicine, Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

The Area Postrema/ caudal medial Nucleus of the Solitary Tract lesion (AP-cmNTSX) causes a number of distrubances in rats' food and water intake: they become transiently hypophagic; chronically polydipsic, polyuric, and natriuretic; and develop an enhanced sodium intake (Hyde and Miselis,'83 & '84). It has been reported (Edwards and Ritter,'82) that rats over-respond to peripheral angiotensin II injection after AP/cmNTSX. We further characterized the AP/cmNTSX phenomenon by analyzing the effect of the lesion on the response to three different dipsogens injected directly into the third ventricle. We examined the drinking response induced by a pulse 1 ul ICV injection of either a low or high dose of angiotensin II (Ang II; 6 and 60 ng); the acetylcholine mimetic, carbachol (CARB, 40 ng); 3.4 % NaCl; or a control injection (0.9% sterile NaCl). The order of presentation was randomized and the animals received two injections of the low dose of Ang II and the control substance and one injection of the others. After the last injection the rats were given aspiration AP/cmNTS lesions and allowed to recover for two weeks. All animals recovered from the acute effects of the lesion, as determined from a daily body weight curve. Following re-adaptation to the cages, their drinking responses to the same treatments as stated above were measured. After AP/cmNTS lesion the animals drank more in response to CARB: 22.9 ± 4.7 ml/kg pre, 37.1 ± 6.7 ml/kg post. The intakes induced by other dipsogens were not affected by the lesion: Ang II 6 ng- 33.2 ± 4.9 ml/kg pre, 32.3 ± 3.7 ml/kg post; Ang II 60 ng- 39.5 ± 3.7 ml/kg pre, 47.1 ± 9.0 ml/kg post; 3.4% NaCl-4.3 ± 2.0 ml/kg pre, 2.6 ± 2.4 ml/kg post (intakes expressed as average water intake/ kg body weight ± S.E.; the control intakes have been subtracted out). Enhancement of drinking to peripheral but not centrally administered Ang II after this lesion suggests that there is a disinhibition of drinking by a peripheral mechanism, possibly of baroreceptors afferents (Robinson and Evered,'83). Supported by GM 277739 and MH 15092.

167.7 NEW AFFERENT AND EFFERENT NEURAL CONNECTIONS OF THE SUBFORNICAL ORGAN. R. W. Lind and L.W. Swanson. The Salk Institute, La Jolla, CA 92037.

Following the emergence of the subfornical organ (SFO) as the principal central site of action of circulating angiotensin II, attention was soon focused on the organization and functions of its neural connections. These early studies described a heavy efferent projection to the hypothalamus, with few reciprocating afferents, and these pathways are thought to be important for the central actions of circulating angiotensin II. Recent work using new and highly sensitive anterograde and retrograde tracing techniques suggests that (1) the subfornical organ sends efferent fibers to a number of targets that lie outside of the hypothalamus and which may be important for the arousal of behavioral responses to angiotensin II; and (2) the SFO receives direct and heavy neural inputs from circumscribed cell groups in the hypothalamus, limbic region, and pons.

Injections of the anterograde marker, PHA-L, into the SFO verified its known efferent projections to the hypothalamus, and injections of the retrogradely transported fluorescent dyes, true blue and fast blue, into these terminal fields, established the topographical organization of the cells of origin of these pathways. Employing a similar combination of anterograde and retrograde tracing techniques, the SFO was found to send additional fibers to the prefrontal cortex (infralimbic area), the rostral and ventral parts of the bed nucleus of the stria terminalis, the substantia innominata, the anteroventral periventricular nucleus of the preoptic region (Terasawa's nucleus), the arcuate nucleus, the lateral hypothalamic area, the dorsomedial hypothalamic nucleus, the zona incerta, the midline thalamus, and the dorsal and median nuclei of the midbrain raphe system.

Injections of retrogradely transported markers into the SFO resulted in the labeling of cell bodies in a number of locations that appear to receive SFO efferents. These include the median preoptic nucleus, the OVLT, the bed nucleus of the stria terminalis, Terasawa's nucleus, the lateral hypothalamic area, the dorsomedial hypothalamic nucleus, the zona incerta, the nucleus reuniens of the thalamus, and the dorsal and median raphe nuclei. Additionally, a few retrogradely labeled cells were found in the laterodorsal tegmental nucleus and in the lateral parabrachial nucleus.

These findings suggest that the current view of the SFO may underestimate its true importance. Thus, the SFO appears not to be simply a humoral sensory device in essentially one-way communication with the hypothalamus. Rather, there appear to be numerous routes for the input of neural information, as well as a rich array of efferent pathways allowing for the direct stimulation of arousal, somatomotor, and cognitive aspects of thirst.

167.8 SUPPRESSION OF FOOD INTAKE BY OPIOID ANTAGONISTS IN THE SHR/N-CORPULENT RAT. J.R. Andrade*, D. Padmore*, C.T. Hansen*, and M.J. Lewis (SPON: L. Hicks). Department of Psychology, Howard University, Washington D.C. 20059, and Veterinary Resources Branch, DRS, NIH, Bethesda, Maryland, 20205.

Endogenous opioids have been strongly implicated in the regulation of consumatory behavior in several animal models of obesity. In the Zucker rat and ob/ob mouse, very low doses of the opioid antagonists naloxone and naltrexone significantly suppress over-eating in obese animals, but not lean littermates. The purpose of this research was to investigate the effects on the opioid antagonists naloxone and naltrexone on food consumption in a newly developed cogenic strain of genetically obese rat, SHR/N-corpulent (cp).

The cogenic strain SHR/N-cp was developed by mating a Koletsky rat which was heterozygous for the cp gene to a spontaneously hypertensive rat (SHR). The homozygous (cp/cp) animal displays hyperphagia and manifests a hyperglycemic/hyperinsulinemic syndrome resembling insulin-dependent diabetes. SHR/N-cp and lean littermates were maintained on a six hour feeding schedule of ground Purina rat chow. Water was available ad lib. Following baseline measures of food intake, animals received acute injections (ip) of naloxone HCL (2.0 mg/kg saline). Analysis of the data revealed that the imposed six hour restricted feeding schedule resulted in no significant difference in baseline consumption between lean and obese rats. However, despite equal baseline consumption, both naloxone and naltrexone significantly suppressed feeding in obese rats but not lean littermates. These results suggest that the genetic obesity observed in the SHR/N-cp rat may be mediated by a similar endogenous opioid system which is believed to mediate excessive feeding and obesity in the Zucker obese rat and ob/ob mouse.

167.9 THE ANORECTIC EFFECT OF NALTREXONE: A LACK OF PHYSIOLOGICAL OR CONTINGENT TOLERANCE. M. Ferguson-Segall* and D.L. Margules. Dept. of Psychology, Temple Univ., Philadelphia, PA, 19144.

Food restricted rats develop tolerance to the anorectic effects of amphetamine (Carlton,P.L.,Phys. & Behav.,7:221, 1971), cocaine (Woolverton,W.R.,NIDA Res. Mono.,18:127, 1978), phenobarbital (Tang,M.,Ibid.,142, 1978), and quipazine (Rowland,N., Psychopharmacology,81:155, 1983) if they received the drug before, but not after, an opportunity to either drink a palatable solution (i.e. sweetened condensed milk) or eat lab chow. Carlton (1971) called this contingent tolerance to signify the importance of the timing between the drug injection and food presentation. Does contingent tolerance occur to the anorectic effects of opiate antagonists? The anorectic effects of naltrexone were tested for tolerance in ad lib female rats (Zucker). Thirty individually housed rats, 6 months old, were equally divided into three groups: naltrexone before (NB), naltrexone after (NA), and saline (S). All animals were injected twice, thirty minutes before and after a 10 minute presentation of apple juice (diluted with tap water 1:1). The NB group received .33 mg/kg of naltrexone before and saline after the apple juice, the NA group received saline before the apple juice and .33 mg/kg naltrexone after, and the S group was given two saline injections. After 2 sets of 6 daily injections, the NB group did not exhibit tolerance to the anorectic effects of naltrexone; during the last 6 days the NA and S groups were drinking an average of 5.8 ml while the NB group drank 2.7 ml. The drinking on the last day for each group was (mean+S.E.); NB - 2.7+.4, NA - 5.4+.7, and S - 5.9+.9. By the student t-test, the NB group was significantly different from both NA ($t=10.6$, $p<.001$) and S ($t=10.3$, $p<.001$). NA and S were not significantly different ($t=1.39$, $p>.05$). Because there is neither physiological nor contingent tolerance to naltrexone's anorectic effects, the mechanism by which naltrexone causes anorexia must be very different from the mechanisms by which amphetamine, cocaine, phenobarbital and quipazine cause anorexia.

167.10 TEMPERATURE-DEPENDENT DRINKING IN RATS WITH SEPTAL LESIONS. T. L. Steele and J. C. Mitchell. Dept. of Psychology, Kansas State Univ., Manhattan, KS 66506.

Research has shown that animals with septal lesions overrespond to sweet and bitter tastes. Yet no studies have established the effect of water temperature on water intake in such animals. The present experiments were performed to compare the drinking behavior of normal rats (n=12) to rats with septal lesions (n=6) when presented with water of different temperatures. Subjects were first given 35 days of ad libitum access to ambient temperature water in order to establish drinking baselines. They were next presented with cold (2°c), ambient (22°c), or warm (37°c), water in a 1-bottle, 6 hr access test (15 days), followed by a 2-bottle, 6 hr access test (9 days), a 1-bottle 30 min access test (25 days), and a 2-bottle, 30 min access test (9 days). Animals with septal lesions drank more water than normal animals when given ad libitum water access, but not when placed on the restricted water schedules. Both normal and septal animals drank more warm water than cold water in all four experiments, suggesting that warm water was less satiating than cold water. Septal lesions did not appear to have an effect on temperature-dependent drinking.

During the two 30 min studies, tongue contacts with the drinking tubes were recorded and each 1 min segment was analyzed according to whether or not the animals made contact for at least 10 sec. The data showed that normal animals generally confined warm water drinking to the beginning of the sessions. Drinking tube contacts dropped rapidly after the first 5-6 min of access, and little warm water was ingested thereafter. The majority of cold water was also consumed during the initial portion of the session, although cold water sampling decreased more gradually. Animals sampled from the cold water bottles more frequently than from the warm water bottles through the remainder of the session. The persistent sampling of cold water suggests that cold water was not aversive and may have been preferred to warm water during the last 2/3 of the sessions. Animals with septal lesions displayed similar drinking patterns, but differences between cold and warm water sampling were small. It appears that normal animals suppress warm water sampling during the final portion of the sessions while animals with septal lesions sample water of all temperatures at the same rate.

167.11 CONCURRENT REDUCTIONS IN APPETITIVE AND CARDIOVASCULAR VARIABLES T.A. McCaffrey*, J.A. Czaja, and E.A. Baronowsky*. Dept. Pathology, Cornell Univ. Medical School, New York, NY 10021 and Dept. Psychology, Miami University, Oxford, OH 45056

Previous experience in our laboratory has demonstrated that estradiol treated guinea pigs exhibit reduced drinking that is independent of a simultaneous decrease in food intake (Czaja, Butera, & McCaffrey, Behav. Neurosci. 97: 210-220, 1983). The reduced feeding and drinking are accompanied by a reversible decrease in carotid artery blood pressure and pressor response to exogenous norepinephrine (NE) measured in conscious, unrestrained animals. In addition, the 24 hour changes in blood pressure appear to be significantly correlated with the concurrent changes in water intake (McCaffrey & Czaja, Soc. Neurosci. Abst. 9: 544, 1983). We therefore designed 3 experiments to evaluate 1) additional parameters relevant to fluid regulation in estradiol treated females, 2) the cardiovascular effects of imposed changes in food and water intake similar to those observed during estradiol treatment, and 3) the potential role of changes in vascular smooth muscle as a factor in the reduced cardiovascular variables. In the first experiment 24, ovariectomized females guinea pigs were treated with 0, 3, or 30 µg of estradiol benzoate (EB) for 4 days. The estradiol treatments produced significant reductions (ANOV) in food intake, water intake, urine output, body weight, resting blood pressure, and the blood pressure response to NE. To simulate the effects of estradiol treatment, 7 subjects in experiment 2 were restricted to 70% of their normal food and water intake. By itself, this procedure produced no significant changes in the cardiovascular variables. In experiment 3, aortas of females pretreated for 4 days with either estradiol benzoate or the oil vehicle were tested for their responsiveness to NE in an isolated smooth muscle preparation. Relative to tissue from the oil (control) subjects, aortas of EB treated females were significantly less responsive to the contractile effects of NE. Altogether, results from these 3 experiments lead us to conclude that direct actions of estradiol on vascular tissue may mediate some of the cardiovascular changes produced by this hormone. If so, it is also possible that neural or hormonal changes resulting from these vascular alterations may provide stimuli for modification of thirst or other appetitive states.

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167.12 SOYBEAN TRYPSIN INHIBITOR INCREASES PLASMA CHOLECYSTOKININ BUT DOES NOT DECREASE GASTRIC EMPTYING IN THE RAT. J.D.Falasco*, A.A.Avilion*, D.Greenberg*, G.P.Smith, J.Gibbs, R.A.Liddle*, J.A.Williams* (SPON: A.Strohmayer). Dept. of Psychiatry,Cornell University Medical College, Eating Disorders Institute and the E.W.Bourne Behavioral Research Laboratory, New York Hospital, White Plains, N.Y.,10605, and Cell Biology Laboratory, Mt. Zion Hospital and Dept. of Medicine, Univ. of California, San Francisco, CA, 94120.

Circulating levels of cholecystokinin (CCK) have been shown to increase following either intragastric (i.g.) preload of Soybean Trypsin Inhibitor (STI; Liddle, Goldfine and Williams, Gastroenterol., 87:542,1984), or intraperitoneal (i.p.) injection of CCK-8 (see abstract by G.P.Smith et al., this meeting). Gastric emptying has been shown to decrease following i.p. injection of CCK-8 (J.D.Falasco et al., Neurosc., 10:532,1984 abstract, Scarpignato et al., Archs. Int. Pharmacodynam., 246:286, 1980). To determine if endogenous release of CCK was sufficient for this effect, we measured gastric emptying following a preload of STI or injection of CCK-8 under conditions identical to those used by D.Greenberg et al. (see abstract this meeting) and G.P.Smith et al. (ibid).

Method Sprague-Dawley male rats (n=6), equipped with gastric cannulas were deprived of food overnight. Ten min prior to a gastric emptying test they were either: a) injected i.p. with CCK-8 (4 mcg/kg) or 0.15 M NaCl, or b) infused i.g. with 5 or 10 ml STI (20 mg/ml) or equivolumetric 0.15 M NaCl. Gastric emptying of 5 ml saline was measured over the subsequent 10 min period using a phenol red technique. The results are in the table:

Condition:	Saline inject	CCK-8 inject 4mcg/kg	Saline preload	STI preload 100mg	STI preload 200mg
Blood levels (pM equiv CCK)	---	3.2 ± 0.6	1.2 ± 0.2	9.5 ± 1.5	---
Gastric Emptying (ml/10 min)	4.56 ± 0.16	1.72 ± 0.16	4.30 ± 1.9	4.30 ± 0.19	4.28 ± 0.21

Exogenous CCK-8 decreased gastric emptying and increased plasma CCK, but endogenous CCK released by STI did not decrease gastric emptying despite increasing plasma CCK more than exogenous CCK-8 did. This failure of endogenous CCK to decrease gastric emptying parallels the similar failure of endogenous CCK released by STI to decrease real or sham feeding in the rat (see abstract by D.Greenberg et al., this meeting).

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167.13 PLASMA LEVELS OF CHOLECYSTOKININ PRODUCED BY SATIATING DOSES OF EXOGENOUS CCK-8. G.P. Smith, D. Greenberg*, J.D. Falasco*, J. Gibbs, R.A. Liddle*, and J.A. Williams*. Dept. of Psychiatry, Cornell University Medical College, Eating Disorders Institute and E.W. Bourne Behavioral Research Laboratory, New York Hospital White Plains, NY 10605 and Cell Biology Laboratory, Mt. Zion Hospital and Dept. of Medicine University of California, San Francisco, CA 94120.

Administration of exogenous cholecystokinin (CCK) decreases feeding in the rat. It has been suggested that the circulating CCK levels produced by exogenous CCK in doses sufficient to decrease feeding are at pharmacological rather than physiological levels. Circulating levels of CCK in the rat are detectable with a bioassay utilizing pancreatic acini (Liddle, Goldfine & Williams, Gastroenterol. 87: 542, 1984). We used this assay to determine circulating CCK levels in rats administered exogenous CCK intraperitoneally (ip) and compared these levels to levels of endogenous CCK obtained after a test meal.

Plasma levels of CCK were determined in 17 hour food deprived male rats (Charles River) 10 minutes after ip administration of either 2 or 4 μg/kg CCK-8 (Squibb) or after eating a high carbohydrate liquid diet (BioServ, 40% v/v) for 10 minutes. The plasma CCK results are in the table.

Treatment	n	Plasma CCK (pM equiv CCK) Mean ± SEM	range
Test Meal	10	1.2 ± 0.2	(0.5 - 3.2)
CCK-8 (2 μg/kg)	8	0.9 ± 0.1	(0.5 - 1.25)
CCK-8 (4 μg/kg)	4	3.2 ± 0.6	(1.17 - 4.25)

Both doses of exogenous CCK-8 produced statistically significant decreases in food intake in separate experiments in these rats: 2 μg/kg produced a 23.3% inhibition and 4 μg/kg produced a 37.9% inhibition. Since the level of circulating CCK measured after 2 μg/kg of exogenous CCK-8 was not significantly larger than the level of circulating CCK measured near the end of a test meal, these data suggest that the satiating effect of exogenous CCK-8 can be obtained with doses that produce circulating levels of CCK that are within or near the range of circulating CCK produced by the ingestion of food.

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167.14 SOYBEAN TRYPSIN INHIBITOR INCREASES PLASMA CHOLECYSTOKININ (CCK) BUT DOES NOT DECREASE FOOD INTAKE. D. Greenberg*, G.P. Smith, J. Gibbs, J.D. Falasco*, R.A. Liddle* and J.A. Williams* (SPON: J.A. Sechzer). Dept. of Psychiatry, Cornell Univ. Medical College, Eating Disorders Institute and E.W. Bourne Behavioral Research Laboratory, White Plains, NY 10605 and Cell Biology Laboratory, Mt. Zion Hospital and Dept. of Medicine Univ. of California, San Francisco, CA 94120.

Soybean trypsin inhibitor (STI) stimulates the release of endogenous CCK and leads to circulating CCK levels equal to or larger than those measured after the ingestion of a liquid meal in the rat. These plasma levels of CCK in the rat have been measured with a bioassay utilizing pancreatic acini (Liddle, Goldfine & Williams, Gastroent. 87:542, 1984). Thus STI can be used to increase circulating levels of endogenous CCK and therefore to determine the satiating effect of endogenous CCK.

STI (100mg/rat in 5 ml of 0.15M saline) was administered as an intragastric (ig) preload to 13 male Sprague Dawley rats (Charles River) 5 min prior to ingestion of a high-carbohydrate liquid diet (BioServ diluted to 40% v/v) for 30 min. The same dose of STI was also administered as an intraduodenal (id) preload to 7 rats 5 min prior to sham feeding the same liquid diet for 30 min. (Sham feeding was accomplished by having liquid diet drain out an open gastric fistula.) Equivolumetric preloads of 0.15M saline were used as controls in both conditions. After the effects of STI on real and sham feeding were measured, the effect of ig and id preloads of STI on plasma CCK were measured in a terminal experiment in most of these same rats.

The preloads of STI produced significant increases of plasma CCK, but STI failed to decrease either real feeding or sham feeding (see table).

Treatment	n	30 minute intakes (ml) mean ± SEM	n	Plasma CCK (pM equiv-CCK) mean ± SEM
Real feeding				
STI (ig)	13	25.7 ± 2.7	10	9.5 ± 1.5*
saline (ig)	13	24.1 ± 1.4	10	1.2 ± 0.2
Sham feeding				
STI (id)	7	57.3 ± 3.8	4	6.4 ± 0.9*
saline (id)	7	57.1 ± 3.1	4	0.6 ± 0.1

* $p < .001$ compared to saline preload

These results do not provide evidence for a satiating effect of endogenous CCK. Furthermore, they are puzzling because the levels of endogenous CCK produced by STI are significantly larger than the plasma levels of CCK produced by satiating doses of exogenous CCK-8 (see abstract by G.P. Smith et al. this meeting).

Supported by NIH Grants MH40010 (GPS), AM33248 (JG), and RSA MH00149 (GPS), RDSA MH70874 (JG), and the General Foods Fund, Inc.

167.15 INTERRELATIONSHIPS BETWEEN CORTICOSTERONE AND VASOPRESSION DURING NORADRENERGIC FEEDING ELICITED VIA THE PARAVENTRICULAR NUCLEUS. B.L. Bogart*, Z.P. Pietrzak*, J.K. Nishita, and E.H. Ellinwood, Jr. Dept. of Psychiatry, Duke Univ. Med. Center, Durham, NC 27710.

Feeding behavior elicited by injection of norepinephrine (NE) into the paraventricular nucleus (PVN) of satieted rats has been shown to be dependent upon circulating corticosterone (Leibowitz, Roland, Hor, & Squillari, 1984). In contrast, feeding behavior elicited by injection of opiates into the PVN is not altered by circulating glucocorticoids (McLean & Hoebel, 1982). Recently, Crine (1983) has drawn attention to the possible role played by vasopressin (VP) in PVN-elicited feeding behavior. VP is known to potentiate the effects of CRF on the release of ACTH and is specifically co-localized with CRF in parvocellular neurons of the PVN. In addition, VP immunoreactivity has been shown to increase in parvocellular neurons following adrenalectomy (Kiss, Mezey, & Skirboll, 1984 ; Sawchenko, Swanson, & Vale, 1984). Since the parvocellular neurons project to the external zone of the median eminence, we have begun to study the relationships of VP on PVN-elicited feeding behavior and its possible neurochemical effects on the hypothalamic-pituitary-adrenal axis.

In our preliminary experiments, 12 male Sprague-Dawley rats (370.9 ± 7.25 g) were decapitated in pairs every 4 hrs over 24 hrs for baseline RIA assay of corticosterone (CORT). Beginning at 08:00 (lights on) these values were: 1.77, 1.41, 5.29, 18.96, 26.94, 13.42, and 1.77 ug.%. Adrenalectomy (ADX) reduced CORT to levels <1 ug.%. Subcutaneous 100 mg replacement pellets of 50% CORT restored CORT to 23.58 ug.% after 3 days.

Prior to ADX, experimental rats demonstrated NE-induced feeding (2.25 ± .5 g) after 24-nmole injections into the PVN. Three days after ADX, these same rats ate 2.60 ± .6 g (SHAM), 0.83 ± .09 g (ADX), and 1.17 ± .1 g (ADX + CORT). Adrenal weights and latencies for NE-induced feeding did not differ among the three groups. Immediately following the completion of the behavioral testing (Leibowitz procedure), each rat was decapitated, trunk blood was collected, and whole brains were rapidly frozen. VP and catecholamine levels were assessed from micropunched tissue samples taken from various brain regions using HPLC and RIA assays. Results of brain tissue assays are not yet completed.

Our preliminary results replicate those of Leibowitz et al. (1984) and further emphasize the permissive action of corticosterone on the NE-induced PVN feeding response.

167.16 SOMATIC, ENDOCRINE AND METABOLIC CORRELATES OF SET POINT RECOVERY IN WEANLING RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS (DMNL RATS) AND SHAM-OPERATED CONTROLS (CON) AFTER BODY WEIGHT REDUCTION. L.L. Bernardis, G. McEwen* and M. Kodis*. Neurovisceral Lab. VA Med. Ctr. and Dept. of Med. SUNY at Buffalo, NY 14215.

DMNL rats and CON were food-restricted for 28 days and then refed. After restriction, both DMNL and CON rats had lost comparable weight relative to ad lib-fed DMNL and CON rats. Both formerly restricted (FORE) DMNL and CON rats had comparable reductions in plasma glucose, insulin and triglyceride but there was no difference among the groups in plasma glycerol, cholesterol and free fatty acids. However FORE DMNL rats had lower plasma protein than FORE CON. Restriction caused similar reductions of % carcass fat in DMNL and CON but FORE DMNL had lower % fat than ad lib DMNL. Per cent carcass protein was comparable in FORE and ad lib DMNL rats and FORE CON. However, ad lib CON had lower % protein than all other groups. Among 7 parameters of in vitro intermediary metabolism using U-14C-glucose as a tracer, FORE DMNL rats in general showed more robust responses than ad lib DMNL and CON. Among 8 parameters of in vitro intermediary metabolism of U-14C-glucose of liver, responses were varied but there was no difference among the groups in tracer incorporation into lipid, glycogen and glycogen/protein. FORE DMNL also had more robust tracer incorporation into lipid, and saponifiable lipid than ad lib DMNL rats. On refeeding, FORE DMNL rats ate more than ad lib DMNL rats whereas FORE CON ate as much as ad lib CON. This was still noticeable 9 days after refeeding. Efficiency of food utilization (EFU) was comparable among the groups prior to restriction, was greatly depressed during restriction in both DMNL and CON and was greatly increased in both FORE groups on realimentation; this too was still evident 9 days after refeeding. Body weight recovery paralleled food intake and EFU. After 4 days of refeeding, plasma glucose, insulin and triglyceride showed comparable increases over restriction levels and plasma protein in FORE DMNL normalized. Nine days after realimentation, EFU was still greatly increased in both FORE groups as were some of the parameters of epididymal fat pad and liver intermediary metabolism. Indeed, responses of liver parameters became more enhanced at that time. However, tracer incoporation into liver lipid, saponifiable lipid, total glycogen, glycogen/protein and glucose oxidation were comparable among groups at 9 days after refeeding. The data confirm a previous hypothesis that DMNL rats, despite their absolute hypophagia and hypodipsia and reduced ponderal and linear growth, normally defend their lesion-induced lower body weight set point. This set point is a "true" body weight and not a fat set point.

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167.17 Short-term Food Intake Following Glucose in Intact and Vagotomized Rabbits. Paula J. Geiselman, Allon Shiff*, Lisa Culbreth*, and Donald Novin, Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

It previously was shown that oral intake of a small volume of glucose (10 ml of a .3M solution) increased later chow intake in the rabbit. The feeding-enhancement effect was potentiated by bilateral subdiaphragmatic vagotomy. These results have been extended to study the effects of repeated trials of glucose in intact and vagotomized rabbits.

Bilateral subdiaphragmatic vagotomy or sham laparotomy was performed on female New Zealand rabbits. Postoperatively, half of the sham-operated rabbits were fed ad libitum. The other half of the sham-operated rabbits were pair fed with the vagotomized rabbits during their recovery from surgery (1-3 weeks). Following recovery, rabbits were allowed to ingest 30 ml of a .3M glucose solution or were subjected to a mock procedure. The glucose and the mock procedures were repeated for five trials each.

Across trials, rabbits in the sham-ad libitum group ingested more chow following glucose than they ate in the mock condition. (Food ingested in the mock condition for this group was comparable to that observed in a control group given repeated mock procedures only.)

Rabbits in the sham-pair fed group also showed a feeding-enhancement effect following glucose. Further, in sham-pair fed rabbits, food intake increased with repeated trials in both the glucose and the mock conditions. This was not merely an effect of time because a sham-pair fed control group subjected to mock procedures only did not exhibit a change in food intake across trials.

In the vagotomized group, food intake was greater across trials in both the glucose and the mock conditions in comparison to food intake observed in vagotomized rabbits subjected to mock procedures only. Prior to glucose presentation, food intake for these two groups was comparable.

Thus, the feeding-enhancement effects following the oral intake of glucose do not subside, and may even increase, with repeated trials. It also is suggested that the feeding-enhancement effects of glucose may be generalized to other feeding conditions. The present results further have implications for gastrointestinal and metabolic mechanisms that may control feeding.

167.18 GUSTATORY INHIBITION AND OPIATE RECEPTOR UPREGULATION FOLLOWING CHRONIC NALOXONE TREATMENT IN RAT. W.C. Lynch, S. Krall,* B.Q. Fernandez* and C.M. Paden Departments of Psychology and Biology, Montana State Univ., Bozeman, MT 59717.

Chronic opiate receptor blockade can affect both opiate receptor binding (e.g., Tempel, A. et al., PNAS, 81: 3893, 1984) and behavioral sensitivity to opiate drugs (e.g., Snell, D., et al., Pharmacol. Exp. Ther., 221: 444, 1985). Inhibition of feeding and drinking by opiate antagonists is now well established and has been related to taste reactivity in nondeprived, nonstressed animals (Lynch, W.C., et al., Life Sci., 33: 1909, 1983) and to the localization of opiate receptors in CNS taste and feeding areas (Lynch, W.C., et al., Pharmacol. Biochem. Behav., in press).

The present study examined both gustatory and receptor-regulatory effects of chronic naloxone (NAL) in the same animals. Two groups of adult male (Sprague-Dawley) albino rats (N=12 each) initially received repeated access (30 min/day) to highly preferred solutions of sucrose, sucrose + quinine (S+Q), sucrose + HCl (S+H) or NaCl in 2-bottle tests. Once intake had stabilized, a subcutaneous Alzet minipump (Alza Model 2002) was implanted in each rat. Pumps contained either naloxone-HCl (150 mg/ml) or 0.9% NaCl. Intake tests continued for 2 weeks, after which pumps were removed; 24 hrs later autoradiographic assays for μ-receptors were performed (Modified from Herkenham, M. and Pert, C.B., J. Neurosci., 2:1129, 1982). Horizontal sections (20μ) of fresh frozen brain (N=3/grp) were cut, mounted (3/slide), and incubated with 1.5 nM [^{3}H]-dihydromorphine (DHM, 87.7 Ci/mM) in 0.17 M Tris (pH 7.4) for 30 min at 25°C. Every third section was incubated as above in the presence of excess levalorphan (1.0 uM) as nonspecific control. Slides were firmly apposed to LKB Ultrofilm for 45 days before developing.

Chronic NAL inhibited intake of sucrose ($p<.05$) and S+Q ($p<.01$) but neither S+H ($p>.10$) nor NaCl, although the latter effect approached significance ($p<.10$). Developed films were analyzed by quantitative densitometry using an Eye Com II image processor (Spatial Data Systems) and Amersham [^{3}H] Micro-scales standards. Highest receptor densities were found in areas previously reported for [^{3}H]-DHM binding. Chronic NAL produced a significant upregulation of receptors in most high-density areas, although this effect was not uniform. Largest increases in binding were seen in lateral N. amygdala, parts of the medial thalamus, N. accumbens and frontal cortex. Smaller increases were seen in interpenduncular nucleus, presubiculum, caudate/putamen, and inferior colliculus. These gustatory and receptor-regulatory effects of chronic NAL in the same animals, suggest a further possible link between opiate system function and gustatory/feeding behavior. (Supported by a grant from Procter and Gamble Co.)

167.19 EFFECTS OF HYPOTHALAMIC INJECTION OF PHENYLEPHRINE AND CLONIDINE ON WATER INTAKE CAUSED BY CENTRAL ANGIOTENSIN II AND WATER DEPRIVATION. L.A. DE LUCA JR.*; A. RENZI*; W.A. SAAD; L.A.A. CAMARGO* and J. V. MENANI*. Departamento de Fisiologia e Patologia, Faculdade de Odontologia de Araraquara-UNESP., Araraquara, SP, Brasil.

Norephinephrine and epinephrine injected into the lateral hypothalamus (LH) inhibit drinking in 24 hour water deprived rats (Grossmann, Am. J. Physiol. 202(5): 872, 1962). Fregly et al.(Brain. Res. Bull. vol. 7:661, 1981) showed that clonidine inhibited drinking caused by systemically injected angiotensin II (AII) and by water deprivation in rats. The purpose of this study was to test the effect of clonidine (α_2 agonist)and phenylephrine (α_1 agonist) injected into the LH (ILH) on drinking induced by AII-ILH and in water deprived rats. Exp. 1: Holtzman male rats were inplanted with one cannulae directed to the LH. Water intake was recorded 1 hour after injection of AII or isotonic saline. Isotonic saline (1 ul) ILH alone induced no drinking but 8.8±0.5 ml of water was ingested when AII (8 ng/ul) was injected into the LH 20 min after isotonic saline. When clonidine (5, 10, 20 and 40 nmol/ul) and phenylephrine (20, 40 and 80 nmol/ul) were injected into the LH 20 min before AII-ILH (8 ng/ul) the following dose response curves were obtained : 7.6 ± 1.3, 3.6 ± 0.8, 1.7 ± 0.4, 1.6 ± 0.4 ml and 7.4 ± 0.5, 3.9 ± 0.8, 0.6± 0.5 ml respectively. The results were significant for $P < 0.05$. Exp. 2: Other groups of rats were injected with clonidine, phenylephrine or isotonic saline (1 ul) into the LH after 30 h of water deprivation, and water intake was recorded for 1 h after injection. The results obtained were 10.4 ± 0.9 ml for isotonic saline only, 9.0 ± 0.9 ml and 9.6 ± 0.7 ml for phenylephryne (80 and 160 nmol/ul), respectively, and 2.3 ± 0,8 ml for clonidine (40 nmol/ul). The result for clonidine was significant for $P < 0.05$. In conclusion, Exp. 1 and Exp. 2 showed that both clonidine and phenylephrine were centrally effective in blocking centrally AII - induced drinking and that clonidine but not phenylephrine, was also centrally effective in blocking thirst in water deprived rats.Our data agree with those obtained by Rowland and Fregly (neurosc.Abstr. pg. 1011. 1984) and Fregly et al.(Brain. Res. 298, 1984).

167.20 BEHAVIORAL CHARACTERISTICS OF INBRED HYPERACTIVE RATS. R.E. Musty, L.H. Conti*, D.J. Wessel* and E.D. Hendley. Dept. of Psychology and Dept. of Physiology and Biophysics, University of Vermont Burlington, VT 05405

Hyperactive Rats (HA) were tested on several tasks to assess their behavioral characteristics. The HA rats were derived from recombinant breeding of the Spontaneously Hypertensive rat (SHR) and the Wistar-Kyoto rat (WKY), followed by brother sister mating of the offspring. These HA hybrids were selected for high spontaneous activity and low blood pressure. The F8 and F9 generations were tested on the following tasks: spontaneous activity, habituation of spontaneous activity, activity in an automated open field, habituation to novel (distracting) stimuli in the open field, learning and retention of a spatial maze (shock-motivated plus maze), retention of a passive avoidance response, and intra-specific aggressive behavior.

Spontaneous activity and activity in the open field was high in the HA rat relative to the WKY and was not different from the SHR. HA females were more active than HA males. Habituation of spontaneous activity in the HA was not different from that of the WKY, while the SHR displayed retarded habituation. In response to a novel stimulus in the open field, both the HA and the SHR were more active than the WKY, suggesting increased distractibility in the HA and the SHR. HA rats were not different from WKY or Wistar (Charles-River) rats in the acquisition and the retention of a spatial maze task (shock-motivated). When tested for the retention of a step-through passive avoidance response, the HA rat showed high retention, characterized by freezing in the start compartment, the WKY rats had normal retention scores, similar to those obtained from other strains of rats, and the SHR rats showed low retention of the avoidance response. In tests in intraspecific aggressive behavior, HA rats were paired with WKY rats in a testing arena. HA rats were more aggressive than WKY rats in this context, in terms of number of attacks and aggressive postures displayed.

The HA rat is similar to the SHR rat in terms of activity. The HA displays normal habituation of spontaneous activity, is hyperreactive to novel stimuli, and is highly emotional and aggressive. Further research is needed to clarify these differences. Supported by PHS 07125-118 and the Sugar Association, Inc.

167.21 SUCROSE FEEDING AND BEHAVIOR IN HYPERACTIVE RATS. E.D. Hendley, D.J. Wessel*, L.H. Conti* and R.E. Musty. Dept. of Physiol. & Biophys., and Dept. Psychol., Univ. VT., Burlington, VT 05405.

A newly developed inbred strain of hyperactive rats (HA) was used as subjects in behavioral studies designed to examine the effects of sucrose feeding on hyperactivity. HA rats were derived from a cross between Wistar-Kyoto Spontaneously Hypertensive Rats (SHR) and their normotensive control strain (WKY). The hybrids of this cross were inbred using selected brother/-sister matings, and breeding pairs were selected for high spontaneous activity and low blood pressure. The F8 and F9 inbred populations of HA rats were subjected to ad lib. feeding with sucrose-supplemented rat chow, or with chow alone in controls, either in acute (overnight) or chronic (14-18 d) feeding of the diets prior to behavioral testing. HA rats that are hyperactive without hypertension were compared with the parental strains: SHR that are hyperactive and hypertensive, and WKY that are neither.

Spontaneous activity, as measured in either an activity cage or in the open field test, was high in HA and SHR as compared with WKY, and sugar-feeding, either acute or chronic, had no effects on spontaneous activity in any of the strains. In a learning and memory task using the plus-maze and electric shock avoidance, no differences were observed in either learning acquisition or memory retention in any of the strains as a result of sucrose-feeding. Female HA and WKY controls paired in an arena for aggressiveness-testing similarly showed no changes in aggressive behaviors as a result of sugar-ingestion. In female HA rats and no other groups, we found a significant impairment in habituation to the small bare activity cage during 4 repeated 15-min trials, one hour interval between trials, as a result of sugar-feeding. Similarly, young (7-17 wk) HA females were impaired in habituation to a novel environment, the open field, whose features had been altered with distractors prior to a second exposure of the rats to the open field.

We concluded that sugar-feeding had no effects on a wide range of behaviors in hyperactive and non-hyperactive rats, including spontaneous activity, exploratory behavior, active avoidance learning, memory retention, and aggressiveness. However sugar did influence two habituative tasks, with and without distracting stimuli, in HA females alone, the most hyperactive group among these strains. The findings suggest that attentional/distractibility responding showed impairment with sucrose-feeding in this selected population of hyperactive rats. Supported by a grant from The Sugar Association, Inc.

SUBCORTICAL SOMATOSENSORY PATHWAYS II

168.1 ULTRASTRUCTURE OF RICIN INDUCED TRANSGANGLIONIC DEGENERATION IN FELINE DENTAL AFFERENTS. L.E. Westrum, M.A. Henry*, L.R. Johnson*, and R.C. Canfield* (SPON: A.H. Bunt-Milam). Depts. of Neurological Surgery, Biological Structure, and Restorative Dentistry, Univ. of Wash., Seattle, WA 98195.

Toxic ricin has recently been shown to be an extremely sensitive and exciting new tool for the demonstration of primary central afferents from peripheral nerves. The action of ricin involves its retrograde transport to the cell body where the ricin exerts its gangliolytic effect by inactivating ribosomal function thus causing the cell and its central processes to degenerate. An earlier light microscopic study was able to demonstrate with the use of ricin, the combined dorsal and ventral dental terminal fields that were previously seen only with either transganglionic horseradish peroxidase (HRP) transport or transganglionic degeneration following dental lesions.

The purpose of the present study was to characterize, using electron microscopy (EM), the type and the temporal sequence of degeneration. Also, the location and organization of primary dental afferents within partes caudalis (PC) and interpolaris (PI) of the trigeminal brain stem nuclear complex was investigated.

Toxic ricin (RCA II(60), E-Y Labs) was injected (1-3 µl/tooth) into the pulpal chambers of unilateral maxillary and mandibular posterior teeth and cuspids in adult cats. Following 7 days survival, typical electron dense degenerating terminals and axons were identified with ease, but were lucent enough so that the morphology of the affected terminal or axon could still be determined. Degenerating terminals were found in both the ventral and the dorsal regions of PC and PI. The terminals contained round synaptic vesicles, were presynaptic to small/medium dendrites and postsynaptic to other terminals with flat vesicles (F terminals). Small (0.5-2.0 µm) thinly myelinated axons were degenerating in both PC and PI. Other findings occasionally seen, included; 1) atypical forms of degeneration (vesicle depletion, increased glycogen content and/or neurofilamentous hyperplasia) which may represent early forms of typical dense degeneration or a unique form of degeneration and 2) degenerating F terminals, possibly representing selective transsynaptic degeneration. The results show; a) electron dense terminal and axonal degeneration occurs and is the EM correlate of argyrophilia seen with Fink-Heimer preparations, b) the types of profiles involved correspond to other EM studies of primary dental afferents but also involve structures not previously seen (F terminals) and c) with the use of a single technique, it is possible to reveal the dual dental projection that had previously only been shown by combining the results of both HRP and dental lesion studies.

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168.2 THE MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED HRP-FILLED VIBRISSA PRIMARY AFFERENT TERMINALS IN SUBNUCLEI PRINCIPALIS AND ORALIS OF THE RAT. W.E. Renehan, M.F. Jacquin, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, Univ. of Med. and Dent. of N.J.-SOM and RMS, Piscataway, N.J., 08854.

Standard intra-axonal recording and HRP injection techniques were used to study the terminal arborizations of 15 mystacial vibrissa primary afferents in trigeminal subnuclei principalis (PrV) and oralis (SpVo) of the normal adult rat. Labelled parent fibers did not bifurcate into an ascending and descending branch and no collaterals were observed in rostral PrV. In the caudal three-quarters of PrV and throughout SpVo, however, functionally distinct afferents gave rise to similarly shaped arbors (rapidly adapting, N=6; slowly adapting type I, N=6; slowly adapting type II, N=3). Multiple collaterals emanated from each fiber (2.75 ± 0.9 in PrV, 3.5 ± 2.12 in SpVo) and terminated in each subnucleus as a series of discontinuous, highly localized aggregates. The average diameter (in the widest dimension) of the terminal arbors in PrV was 125.5 ± 53.3 µm as compared to 112.7 ± 57.2 µm in SpVo. Collaterals terminated in a manner consistent with the topography recently demonstrated by HRP bulk labeling (Arvidsson J., JCN, 1982, 211:84-92).

Surprisingly, the morphology of a given vibrissa afferent terminal in PrV was not significantly different from the structure of the central arbors of the same fiber in SpVo. Cytochrome oxidase and succinic dehydrogenase staining of PrV in adult rodents (Ma and Woolsey, Brain Res., 1984, 306:374-379) both reveal a pattern (presumably reflecting primary afferent terminations) consistent with our single fiber data. No such pattern has been shown for SpVo. It is possible that the massive oral and perioral input to SpVo (Azerad, J., et al., Brain Res., 1982, 246:7-21) coupled with the relatively small size of this subnucleus (Jacquin, M.F. and R.W. Rhoades, JCN, 1985, 235:129-143) may "mask" the vibrissa terminations when this region is stained with these techniques. Supported by DE06528, EY04170, the March of Dimes, the UMDNJ Foundation (RWR) and NRSA NS07444 (WER).

WER and MFJ are currently at the Dept. of Neuroscience, NYCOM, Old Westbury, N.Y. 11568.

168.3 COMPARISONS OF THE DISTRIBUTION OF TRIGEMINOTHALAMIC AND TRIGEMINOTECTAL NEURONS STUDIED WITH FLUORESCENT DYES IN RAT, HAMSTER, AND MOUSE. L.L. Bruce, J.G. McHaffie* and B.E. Stein. Dept. Physiology, Medical College of VA, Richmond, VA 23298.

Comparisons of the distributions of neurons that project to superior colliculus (SC) and thalamus were made to determine if similar projection and collateralization patterns are present in different rodents. The retrogradely transported fluorescent dyes, fast blue and diamidino-dihydrochloride yellow were used. In each anesthetized animal, one dye was injected into the SC and the other into the thalamus in topographically congruent areas. Counts of yellow, blue, and double-labeled cells were made throughout the trigeminal complex: principalis (VP), pars oralis (Vo), pars interpolaris (Vi), and pars caudalis (Vc).

Trigeminothalamic projections were similar in each species. The densest concentration of retrogradely labeled cells was in Vp, with substantially fewer cells in Vi, and fewer still in Vo and Vc. A common pattern was also noted for trigeminotectal cells among for three species. Most trigeminotectal projections originated from cells in Vi, somewhat fewer from Vo, and least from Vp and Vc. Following paired injections of the tracers, double-labeled cells were scattered throughout the sensory trigeminal complex. Although comparatively few double-labeled cells were observed, most were in Vi. In mouse and rat, nearly all double-labeled cells were in Vi (80-95%), whereas in hamster only about 50% were in Vi. Double-labeled cells within Vi were restricted to the ventrolateral portion, corresponding to the representation of the vibrissae. These cells could be further distinguished morphologically because they were among the largest cells in the region of Vi. In hamster and mouse, only about 10% of all cells projecting to SC also projected to thalamus. In the rat, however, the proportion was doubled; about 20% of all cells projecting to SC also projected to thalamus.

These data indicate that distribution of trigeminotectal and trigeminothalamic cells are similar among different rodents. The vast majority of labeled trigeminal cells project either to SC or thalamus, and this might be expected on the basis of the very different behavioral roles these structures play. On the other hand, a subpopulation of trigeminal cells (principally in Vi) projects to both SC and thalamus. Since double-labeled cells in Vi correspond roughly with the vibrissae representation, it is likely that these collaterals convey the same information regarding vibrissae displacement. It is not yet clear, however, what specific physiological properties distinguish this subpopulation of double-labled cells from neighboring trigeminofugal cells. Supported by a grant from the Jeffress Foundation and NIH grant EY05612.

168.4 STUDIES OF ASCENDING INTRATRIGEMINAL PATHWAYS USING THE AUTORADIOGRAPHIC TECHNIQUE. W.M. Panneton and J.H. Haring, Dept. of Anatomy, St. Louis Univ. Sch. of Med., St. Louis, MO 63104

Previous studies using horseradish peroxidase as a retrograde marker have shown that numerous neurons within the subnucleus interpolaris, the paratrigeminal nucleus, and the medullary dorsal horn project into the dorsolateral pons, including the principal nucleus of the trigeminal sensory complex, the Kolliker-Fuse nucleus, and the parabrachial nucleus. However, since the effective area of uptake was difficult to determine in the retrograde studies, the present study was undertaken using the anterograde transport of tritiated leucine as a marker to determine the termination of caudal trigeminal neurons in the dorsolateral pons more accurately.

Anesthetized cats were secured in a stereotaxic apparatus, and the location of the trigeminal sensory complex was determined using electrophysiological mapping techniques. Injections of tritiated leucine were then placed within trigeminal and paratrigeminal areas. After 10 days, the animals were sacrificed and their brains processed for autoradiography and exposed for 2 to 6 weeks. Label was transported to the dorsolateral pons in all cases, but there were differences between cases. An injection centered in the dorsal aspect of the spinal trigeminal tract at the level of the obex and including the dorsal part of the paratrigeminal nucleus and the dorsal tip of the subnucleus interpolaris resulted in relatively dense label over neurons clustered in the lateral, rostral one-third of the principal nucleus and the dorsal rim of its dorsomedial subdivision; label over the Kolliker-Fuse and parabrachial nuclei was sparse to moderate in comparison and diffuse. Injections centered at the ventromedial tip of the spinal trigeminal tract and including neurons interstitial to the tract, the ventral medullary dorsal horn and the immediately adjacent reticular formation resulted in silver grains over the ventral tip of the principal nucleus, the Kolliker-Fuse nucleus, and dorsolateral parts of the parabrachial nucleus. Injections centered within the subnucleus interpolaris at the level of obex labeled all parts of the principal nucleus, the Kolliker-Fuse, the lateral parabrachial nucleus, and the medial parabrachial nucleus caudally. These data suggest that neurons found in the paratrigeminal nucleus dorsally have projections different than those found interstitially within the spinal trigeminal tract ventrally and implies that they are not functionally homogeneous. Supported by the American Heart Association, Missouri Affiliate.

168.5 A COMPARISON OF THE DISTRIBUTION AND MORPHOLOGY OF THALAMIC, CEREBELLAR AND SPINAL PROJECTING NEURONS WITHIN TRIGEMINAL NUCLEUS INTERPOLARIS OF THE RAT. K. D. Phelan and W. M. Falls. Department of Anatomy, Michigan State University, East Lansing, MI 48824.

It has been well established that trigeminal nucleus interpolaris (Vi) is the source of a number of secondary trigeminal projections to diverse targets along the neuraxis. Previous studies have suggested that the trigeminothalamic (TT), trigeminocerebellar (TC) and trigeminospinal (TS) projection neurons may represent separate cell populations within Vi. Although detailed analyses of the distribution and morphology of TT, TC and TS neurons have been conducted in the cat, comparable studies in the rat are conspicuously lacking. The present study utilizes the retrograde transport of HRP, following large injections into either the contralateral thalamus, the ipsilateral cerebellum or the ipsilateral upper cervical spinal cord, in order to determine the number, distribution and morphological types of TT, TC and TS neurons throughout the rostrocaudal extent of rat Vi.

As in the cat, TT and TC cell populations in the rat encompass similar size ranges and exhibit similar morphological varieties of neurons. However, TT and TC neurons are distributed along the entire rostrocaudal extent of rat Vi, while in the cat these same neuronal populations exhibit a partial spatial segregation along this axis. Although significant overlap occurs between TT and TC cell populations, some specific regional differences in their size, density, distribution and morphology are evident. For example, the TT neuronal population contains a larger number of smaller ($<$15 um) cells with round/oval-shaped somata distributed in medial and ventral parts of caudal Vi and in the dorsomedial part of rostral Vi. The majority of TC neurons in these same regions are medium-sized ($\approx$15-30 um) cells with oval/multipolar-shaped somata. Furthermore, the two spinal recipient zones, which border the spinal V tract (Soc. Neurosci. Abstr., 10:796, 1984) contain predominantly either TT cells (dorsolateral) or TC cells (ventrolateral). TS neurons, which comprise a smaller population of Vi cells, present a characteristic distribution pattern restricted to ventral and lateral regions of the nucleus. These neurons possess morphologies either similar to or distinctly different from TT and TC neurons depending upon their location within Vi. The results of the present study indicate the existence of several morphological types of TT, TC and TS neurons which exhibit some distinct differences in their regional distribution within Vi of the rat. This pattern of organization is distinctly different from that previously reported in the cat and possibly reflects a species difference in the functional organization of Vi.

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168.6 POST-SYNAPTIC AFFERENT TERMINATIONS IN THE CAT DORSAL COLUMN NUCLEI VISUALIZED BY PHA-L BEAN LECTIN J. Pierce and A. Rustioni, Dept. of Anatomy, The University of North Carolina at Chapel Hill

The dorsal column (DC) system of the spinal cord is known to contain a mixture of fibers; including local spinal interconnections, 1° afferent collaterals, ascending post-synaptic afferents, and descending fibers. The post-synaptic dorsal column (PSDC) component can be differentiated both electrophysiologically and anatomically. The studies of Angaut-Petit (1975) and Brown et al. (1983) demonstrate that the information carried by these fibers displays a high degree of modality and somatotopic convergence. Moreover, they seem to project to brainstem regions that generally do not overlap with the main targets of 1° afferent collaterals. Studies using the successive degeneration technique (Rustioni, 1974), and spinal wheat germ agglutinin-horseradish peroxidase (WGA-HRP) injections following rhizotomy at cervical levels (Pierce et al., 1983) indicate that they probably terminate primarily in the ventral and rostral parts of the cuneate nucleus. However, neither of these approaches allowed one to a) discriminate between cut fibers of passage and terminal varicosities, and b) visualize individual fibers, with their pattern of collateral arborization.

With the use of the red kidney bean lectin phaseolus vulgaris leucoagglutinin (PHA-L) (Gerfen and Sawchenko, 1984) we are able now to address this problem. A laminectomy of the lower cervical region was performed, followed by a unilateral series of six iontophoretic PHA-L injections into the dorsal gray matter, from C6 to C7. 5μ amp pulsed positive current for 15 minutes was used to make each injection. Under these conditions PHA-L is thought to be taken up exclusively by cell bodies, and transported anterogradely. Three weeks later the animal was sacrificed, and the tissue prepared for light microscopy, after PHA-L immunohistochemistry.

This method also allows one to reconstruct cells involved in uptake. A subpopulation of the labelled cells we have seen have characteristics that correspond to those described by Brown et al. (1981) in their intracellular HRP study of PSDC cells. Often an axon could be followed into the DC's where most labelled fibers are observed. Terminal varicosities are seen, usually in "en passant" chains, distributed throughout the ventral and rostral cuneate nucleus. Patches of grouped terminals are also seen sporadically in the "cluster" zone of the cuneate, and in the external cuneate nucleus. In both of these regions WGA-HRP label has been seen in our previous studies (Pierce et al., 1983). Current work is underway to reconstruct individual terminal arbors in each of these target regions.

Supported by 12440.

168.7 BRAINSTEM PROJECTIONS TO AND FROM THE CUNEATE NUCLEUS OF RATS AND CATS. R. J. Weinberg and A. Rustioni, Dept. of Anatomy, University of North Carolina, Chapel Hill, NC 27514.

The existence of GABAergic inhibitory mechanisms in the dorsal column nuclei of rats and cats has been firmly established by a variety of methods. Immunocytochemistry demonstrates that about 25% of neurons in the central core region ("MCd" of Cheema, et al, 1983) of the cat's cuneate nucleus (CUN) stain for glutamic acid decarboxylase (GAD). EM immunocytochemistry reveals numerous GAD-positive terminals of various morphologies. Recalling the electrophysiological demonstration that stimulation of the reticular brain stem core inhibits transmission through CUN (Cesa-Bianchi, et al.,1968, 1969), this finding of morphological heterogeneity raises the question whether GABAergic terminals in CUN originate exclusively from intrinsic neurons, or arise in part from extrinsic GABAergic neurons in the brainstem. The present experiments represent a first step toward answering this question, by investigating whether any significant input to CUN arises from other brainstem regions.

We made small injections of WGA-HRP into CUN of anesthetised rats and cats by iontophoresis (1-5 μA for 5-15 minutes) from fine glass pipettes (tip diameter 5-15 μm). After survival times of 24 hours, the animals were sacrificed. 40 μm frozen sections were processed for HRP using the TMB technique and examined with a Leitz microscope under bright- and dark-field illumination.

Our results to date indicate that small injections strictly confined to the dorsal part of the middle region of CUN in both rats and cats result in labeling of neurons in the vicinity of the injection, and virtually no retrograde labeling outside the nucleus. For cases in which the injection spread rostral to the middle region (in cats) or to the ventral fringe of the nucleus, retrograde labeling in the brainstem was relatively sparse and mainly confined to the rostral medullary tegmentum (n. magnocellularis, n. gigantocellularis, and raphe magnus) and to the contralateral red nucleus. We are now attempting double labeling experiments to investigate whether any of these projections might involve GABAergic neurons.

The sparseness of the retrograde labeling contrasted with the anterograde transport of WGA-HRP. Consistent with the results obtained by other investigators using degeneration techniques and autoradiography, we have observed a projection to the contralateral colliculi, pretectal area, and pontine nuclei. Ipsilateral to the injection site in rats, efferents were traced to the inferior cerebellar peduncle and to the superficial layers of the spinal trigeminal complex and of the dorsal cochlear nucleus, even when the injection was restricted to the central part of CUN.

This work was supported by NIH grants NS 07132 and NS 12440.

168.8 IMMUNOHISTOCHEMICAL DEMONSTRATION OF SEROTONIN-CONTAINING PROCESSES WITHIN THE CAT CUNEATE NUCLEUS. M.V. Trinh*, J.C. Pearson, M.D. Goldfinger, & K.S. Shalala. (SPON:G.Crampton) Depts. of Anatomy, Physiology, and Micro. & Immuno., Sch. Medicine and Col. Science & Engineering, Wright State University Dayton, OH 45435.

The cuneate nucleus is a somatosensory synaptic relay site involved with the transmission of mechanosensory information from forelimb afferent units. In addition to primary afferent input, the nucleus receives relayed projections from both spinal and supraspinal sources. Physiological studies have revealed excitatory and inhibitory influences on the transmission of afferent information. Recently, Goldfinger et al. (1) showed the presence of 5-hydroxyindoleacetic acid (5HIAA) in cuneate push/pull perfusates recovered during peripheral somatosensory stimulation; the presence of 5HIAA suggests the possibility of serotonergic (5HT) elements within the cuneate circuitry (as shown for the rat cuneate:2).

The present work tests for 5HT within the cat cuneate nucleus, using a sequential immunohistochemical procedure (3). Following fixation by perfusion with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), the brainstem tissue is frozen sectioned at 40um and incubated with a solution containing anti-5HT antibody (Polyscience, Warrington, PA). Biotinylated secondary antibody (antiIgG) is added to introduce biotinyl binding sites to the primary antibody. Next, an Avidin-biotinylated HRP complex (Vector Labs, Burlingame, CA) is added to bind to the secondary antibody. Reacted tissue is then incubated in a peroxidase solution. The cuneate nucleus is examined for 5HT reaction product, as defined by observation of reaction result within the Raphe nuclei of the same preparation.

The data reveal 5HT reaction product within thin processes which are axon-like in appearance. Labeled processes show spaced varicosities, and can be followed through the section depth. The spacing of process-contained varicosities suggests distributed contact points. These results are consistent with the recovery of extracellular 5HIAA, which could result from stimulus-elicited release of 5HT and subsequent enzymatic &/or thermal 5HT oxidation. These findings add to other evidence which suggest a role for serotonin in cuneate information processing.

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168.9 RECOVERY AND DETECTION OF AMINO ACIDS RELEASED WITHIN THE CAT CUNEATE NUCLEUS. M.D. Goldfinger, S. Hensley*, and D. Schmalholz*. Dept. of Physiology, Wright State University, Dayton, Ohio 45435.

This work seeks to recover and detect primary amino acids released into the cuneate nucleus, using push-pull perfusion and HPLC (High Performance Liquid Chromatography) analysis, respectively.

Push-pull perfusion: The objective is to continuously sample the extracellular space containing cuneate relay neurons (located within 2 mm beneath the exposed brainstem surface). The push tube consists of a glass pipette drawn to a 100 um tip with a tungsten microelectrode fixed in the tip lumen. The push tube is positioned into the nucleus without engaging surface blood vessels. Artificial CSF vehicle solution is pushed through the tip at 20 ul/min and flows back towards the brainstem surface by capillarity and negative pressure applied via a second glass pipette at the brainstem surface next to the push tube entry site. Fluid is collected via the surface pipette into the negative pressure reservoir for subsequent HPLC analysis. Electrical activity is concurrently recorded from the microelectrode.

HPLC analysis (cf.ref.1): 25 ul of sample fluid is reacted with 100 ul of derivitizing solution (o-phthalaldehyde, mercapto-EtOH, 0.1M NaTetraBorate:1) for 2.5 min to form isoindole derivatives of primary amino acids. 20 ul of the reaction fluid is injected into the HPLC. System configuration parameters are: ODS-5 um reverse-phase column (T=34°C); isocratic mobile phase of 30% MeOH, 70% 0.1 M NaPhosphate; pump flow = 1.6 ml/min; Electrochemical Detector (BAS LC-4B, output filter = 1 Hz, oxidation mode); Electrodes - glassy-carbon working (V=+700 mV) and calomel reference. Testing with standards (L-amino acids in artif. CSF, pH=7.3-7.4) shows detector output with wide-range linearity (eg, 40-5000 pmole) and acceptably low variability (eg, $<$2%-elution times; $<$10%-peak heights). However, 10 of 22 amino acids could not be detected.

Preliminary study with 3 cats (prep.:2) sought samples from 5 minutes of continuous perfusion during periods with or without repetitive ipsilateral forepaw percutaneous electrical stimulation (0.1 ms-duration pulses at 10/sec.) Results showed that stimulation elicited an increase of baseline amino acid levels, with 4 to 6 readily discernible HPLC output peaks. GABA levels did not increase above baseline levels; yet, P-wave electrical activity (picrotoxin-sensitive:3) was concurrently recorded.

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168.10 SENSORY PATHWAY SPECIALIZATION RELATED TO CALCITONIN GENE-RELATED PEPTIDE IMMUNOREACTIVITY IN THE RAT. L. Kruger, N. Brecha, C. Sternini and P. Mantyh. Depts of Anatomy, Anesthesiology and Medicine and Brain Research Institute, Center for Ulcer Research and Education, UCLA School of Medicine, LA, CA 90024 and VA Medical Center, Wadsworth, LA, CA 90073.

Calcitonin gene-related peptide (CGRP) has been localized to a variety of sensory, motor, autonomic and integrative structures (Nature, 304:129,1983). We report the distribution of CGRP immunoreactivity in sensory pathways of the rat using new CGRP antisera generated to [Tyr]-rat CGRP(23-37) coupled to keyhole limpet hemocyanin via glutaraldehyde. Normal and colchicine-treated rats were perfused with a paraformaldehyde solution. Brains were cut in transverse, horizontal or parasagittal axes. Sections were processed by the avidin-biotin peroxidase technique. Specificity was demonstrated in adjacent sections incubated in preimmune serum or in antiserum previously absorbed with 10 uM synthetic rat CGRP. CGRP immunoreactive axons in skin are numerous, especially within glabrous epidermis, in relation to a variety of dermal blood vessels and zones of nociceptor specialization. Small and medium sized sensory ganglion cells emitting thin axons are numerous and give rise to heavy bundles of presumptive nociceptor axons running in Lissauer and spinal V tracts terminating principally in marginal and gelatinosa layers. Small neural somata are present in cord gelatinosa and spinal V, and also unexpectedly, a few lemniscal neurons are labeled. Gustatory and visceral afferent axons are found in selected sectors of the solitary nuclei, medial parabrachial nuclei and the caudomedial thalamic ventral group, usually designated VM or VPMpc. Another fiber component can be traced through a sector of the posterior group and continues rostrad in the medial internal medullary lamina terminating in submedius and paracentralis nuclei, but not in the ventrobasal complex or other regions of the ventral group recipient of spinothalamic projections. Axons in the posterior group run medial to a peripedunclar cluster of labeled somata at the ventrocaudal border of the medial geniculate. Neurons of the thalamic reticular nucleus and sparse arrays of small neurons are labeled at the mesodiencephalic junction extending across the pretectal area and ventrally encompassing parts of periaqueductal gray and parafascicular, centromedial and reuniens nuclei- zones only indirectly implicated in pain mechanisms. A parsimonious principle accounting for the total pattern remains elusive. However, it may be relevant to note that in addition to CGRP in established chemosensory pathways (olfactory, gustatory and visceral) there is label in pathways related to polymodal nociceptor inputs; another class of sense organ for which chemically mediated excitation is suspected.

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168.11 LOCAL CIRCUIT NEURONS IN THE RAT VENTROBASAL THALAMUS.
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Most thalamic nuclei contain a sizeable population of local circuit neurons. These neurons have been found to be inhibitory and to contain the neurotransmitter γ-aminobutyric acid (GABA). In the ventrobasal thalamus of the rat, a simpler circuitry appears to exist and few indications of any local circuit neurons have been found. In order to carry out a direct search for such neurons, an immunocytochemical study was made using a recently available antiserum to GABA.

Standard peroxidase-antiperoxidase techniques were used to show that a small population of cells in the rat ventrobasal complex (VB) does contain GABA. In order to determine if these were local circuit neurons, injections of horseradish peroxidase (HRP) were made into the primary somatosensory cortex. A band of cells in VB was retrogradely labeled. GABAergic cells were immunocytochemically labeled using carbazole as a chromagen, allowing a search for double labeled cells. No double labeled cells were found, indicating that the GABAergic cells did not project to the cortex. These probably represent a small population of local circuit neurons.

The GABA-labeled cells made up 0.4 ± 0.3% of the cells in rat VB. They were distributed throughout VB, with more cells along the lateral boundary of the nucleus and fewer medially. The average cross-sectional area of these cells was somewhat smaller than that of all cells in VB (81 ± 34 μm vs. 105 ± 36 μm). Aside from the number of cells, these properties are similar to local circuit neurons in other thalamic nuclei.

The functional significance of such a small population of local curcuit neurons is not clear. They are not likely to play a major role in this nucleus, indicating that the neuronal circuitry in the rat VB is particularly simple. Perhaps they play a role in processing a particular somatosensory modality, such as noxious stimuli.

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168.12 MORPHOLOGY OF ELECTROPHYSIOLOGICALLY CHARACTERIZED NEURONS AND FIBERS IN THE MEDIAL AND LATERAL VENTROPOSTERIOR NUCLEI OF THE CAT'S THALAMUS. C.Vahle-Hinz*, K.-M.Gottschaldt †, L.M.Pubols, M.J. Friedlander and K.-D.Kniffki* (SPON: R.F.Schmidt). Physiologisches Institut der Univ., D-8700 Würzburg, FRG; Neurological Sciences Inst., Good Samaritan Hosp.& Med. Ctr., Portland, OR 97209 and Dept. Physiol.& Biophys., Univ. Alabama, Birmingham, AL 35294.

Horseradish peroxidase-filled micropipettes were used for intracellular recording and injection of single cells and lemniscal fibers in the thalamic face and body representation areas. Antidromic activation from the corresponding SI cortical fields was used to identify thalamocortical relay neurons; neurons activated transynaptically from the same cortical locations were classified as thalamic interneurons. Ascending fibers were identified by their spike form and unresponsiveness to cortical stimuli. Functional properties of neurons and fibers were determined with mechanical stimulation of fur, skin and sinus hairs. Vibratome sections were reacted with cobalt-intensified diaminobenzidine and the labeled neural elements reconstructed with camera lucida drawings.

Thalamocortical relay neurons with receptive fields on the face (n=5) have round somata of 10-30 μm diameter with 5 primary dendrites regularly spaced around the soma, giving the cell a star-like shape. The dendrites branch in a bushy fashion and cover a spherical volume 370-500 μm in diameter that is uncorrelated to soma size. Relay neurons with receptive fields on the body (n=4) have elongated somata 22-35 μm long and 8-13 μm wide. Three to 5 dendrites arise from the cell poles, two usually being larger, giving the arbor a medio-lateral orientation extending 300-550 μm.

Interneurons with face or body receptive fields (n=2) have large elongated somata, 25 and 33 μm long, and spherical dendritic arbors 560 and 570 μm in diameter, with spiny dendrites and large appendages (which however can also occur on relay neurons). A second type of interneuron (n=2) has been found that has a sinus hair receptive field and a small soma, 11 and 15 μm in diameter, and only a few irregular processes bearing bouton-like structures.

Fibers with face receptive fields (n=6) have one terminal arbor, and those with body receptive fields (n=4) one to three arbors spaced 100-700 μm apart rostro-caudally or medio-laterally. Most fibers' arborizations contain densely packed terminal boutons, but one fiber whose activity was inhibited by vibration was distinctly different; this had a loose terminal arborization with sparse boutons.

Thus, two types of thalamic interneurons have been observed. Both cells and fibers seem to have a distinct morphology in the medial and lateral ventroposterior nuclei. Specific functional properties may be expressed in the pattern of lemniscal fiber terminations.

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168.13 *MULTIVARIATE STATISTICAL ANALYSIS OF NEURONAL STRUCTURE IN VENTRAL POSTERIOR THALAMUS.* *J. C. Pearson, B.L. Mann*, and J. R. Norris. Depts. of Anatomy and of Mathematics and Statistics, Sch of Med. and Col. of Science and Engineering, Wright State University, Dayton, OH 45435.*

Although physiological studies indicate considerable functional diversity among thalamocortical projection neurons in the ventral posterior (VP) nucleus in a variety of species, evidence for corresponding anatomical diversity is contradictory and incomplete. Some reports emphasize the similarity in relay cell structure while others present evidence which suggests grouping of relay cells into subcategories with morphological similarity.

The present study addresses the question of whether morphological variation among VP relay cells is sufficient to warrant subcategorization into anatomical classes by using morphometric procedures and multivariate statistical methods to describe Golgi-impregnated neurons in the VP of opossum, dog, and the lesser bushbaby, (Galago senegalensis). Relay cells are analyzed according to soma cross-sectional area, soma shape, number of major branch points (MBP), dendritic field shape and extent, and the number of appendages (spines) in defined dendritic branch zones. Principal components analysis is used to evaluate structural variation among cells in terms of the weighted sums of all variables. The data indicate that thalamocortical relay cells of opossum VP show some correlation in dendritic field extents, dendritic field shapes, and dendritic branch frequencies, but random variation in soma size and spine content. Thalamocortical relay cells of dog and bushbaby show strong correlation in soma and dendritic field parameters and in the number of spines located in the major branch zones. This pattern suggests that opossum VP cells form a single group of relay neurons in which some variation in structure is present, and that the diversity of relay cell structure in the VP of dog and bushbaby is great enough to acknowledge the presence of anatomical subcategories.

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168.14 **SINGLE UNIT ANALYSIS OF THE FAR FIELD POTENTIAL.** **J.G. Blackburn, M.A. Cordova-Salinas* and S. Trojanowski*. Dept. of Physiology, Med. Univ. S.C., Charleston, SC 29425.**

Far field somatosensory evoked potentials (FFPs) and single unit responses were recorded in cats anesthetized with nitrous oxide. Far field potentials were recorded from a mid-line electrode at stereotaxic AP_O in response to electrical stimulation of the left superficial peroneal nerve (1 pps. 0.1 msec duration and 3 mA intensity). The reference electrode was located on the ipsilateral ear. The evoked potentials were amplified, filtered, summated with a microcomputer and displayed on a digital plotter. Single unit responses were recorded from the nucleus gracilis with tungsten microelectrodes, amplified, filtered and displayed on the digital plotter.

Eight stable components were identified in the FFP in response to superficial peroneal nerve stimulation: 4.80 ± 0.16, 5.66 ± 0.32, 6.39 ± 0.07, 8.35 ± 0.17, 9.15 ± 0.06, 9.61 ± 0.13, 10.41 ± 0.09, and 11.96 ± 0.15 msec. Unit responses appeared with an average latency of 9.04 ± 0.5 msec. The single unit responses appear to correlate in time with the fifth component of the FFP, indicating that this component may be generated in the brain stem. **This investigation was supported by NINCDS grant 2P01 NS 1106610.**

169.1 **DIFFERENT CATABOLIC PATHWAYS FOR THYROTROPIN RELEASING HORMONE (TRH) IN BRAIN AND SPINAL CORD OF NIH:N MICE**

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The catabolism of thyrotropin releasing hormone (TRH) in the hypothalamus and cerebral structures has been well elucidated. However, the biochemical and pharmacological effects of TRH are maximal in animal models of spinal cord disease - spinal cord motor neuron degeneration and spinal cord trauma. We have studied the comparative degradation of 3H-TRH and 125 I-TRH in cerebral cortex and spinal cord from NIH:N mice by reverse phase HPLC. Ten (w/v) percent homgenates were prepared in normal saline or 25 mM potassium phosphate buffer containing 2 mM dithiothreitol and 2 mM EDTA. Degradation of 3H-TRH occurred at a faster rate in fresh cortex homogenates (8.9 $\pm$ 0.1%/min) compared with fresh spinal cord homogenates (3.8 $\pm$ 0.2 %/min). Degradation rates of 125 I-TRH were decreased by 67% in the cortex (3.0 %/min) and by 82% in the spinal cord (0.7 %/min). One cycle of freeze/thawing resulted in a 60% decrease in degradation rate in the cortex and a 90% decrease in degradation rate in the spinal cord. HPLC analysis of degradation products indicated that deamido-TRH (TRH-OH) is the major product in the cortex while His-Pro-OH is the major product in the spinal cord. These results were confirmed by administration of bacitracin which increases His-Pro-OH production in cortex homogenates by inhibiting TRH-OH production but has no effect on the amount of His-Pro-OH produced in spinal cord homogenates. Cyclo-His-Pro production in the spinal cord under optimal buffer conditions is 20% higher than in the cortex. Catabolism of TRH in the spinal cord is slower and proceeds via a different metabolic pathway than in the cortex. These differences may be important in explaining the different biochemical and pharmacological effects of TRH on the spinal cord.

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169.2 ELECTROCHEMICAL DETECTION OF NEUROTENSIN (NT) AND NT FRAGMENTS IN HUMAN CEREBROSPINAL FLUID (CSF) FOLLOWING ON-LINE TRACE ENRICHMENT AND GRADIENT ELUTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC).

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A novel method for detection and chromatographic separation of NT and several of its major fragments has been developed. Gradient elution HPLC coupled with electrochemical detection (EC) was used to detect nanogram ($\geq$ 2 ng) quantities of NT, NT 1-6, NT 1-8, NT 1-10, NT 1-11, NT 9-13, and NT 8-13. EC detection is a more sensitive and specific method than typically used UV detection and allows the detection of NT and its fragments in biological matrices, i.e. CSF and brain tissue extracts. To maximize assay specificity, the significant retention of NT and its fragments by cation exchange and reverse phase HPLC was exploited by combining these two retention mechanisms in an on-line assay. The method yielding the best results involves using a Brownlee 3 cm cation exchange enrichment column with 20 mM potassium acetate (KAc), pH 3.5 and 10% acetonitrile (ACN) to enrich the samples, then back elution with 200 mM KAc, pH 6.4 , 0.75 mM heptane sulfonic acid (HSA) and 10% ACN onto a Waters C18, 10u reversed phase analytical column. Peptides were then resolved using a gradient elution from 10% ACN to 30% ACN in 20 minutes at 1.5 ml/min. Detection was accomplished by EC oxidation at +.850 V. Due to a decline in electrode sensitivity as a result of solvent gradient elution in combination with the matrix assayed and the use of relatively high oxidation potentials, the cell had to be polished daily. The EC oxidation of NT and its fragments is not merely a function of the presence of tyrosine or tryptophan, but apparently varies with the presence and relative position of basic amino acids (e.g., arginine). The chromatographic separation of NT and NT fragments was stable over a period of 6-9 months. When analytical column separation did begin to diminish it could be restored by adding HSA to the gradient mobile phases. Although this method is not as sensitive as most RIAs, it was utilized to specifically determine the nature of NT-like immunoreactivity in human CSF. Efforts to preconcentrate NT and its fragments by lyophilization or Sep-Pak chromatography and solvent evaporation indicated significant peptide degradation by these preparative techniques. The present method, which involves the chromatography of untreated CSF, permitted the specific qualitative determination of NT in human CSF. (Supported by NIMH MH-39415).

169.3 INSULIN-INDUCED HYPOGLYCEMIA ALTERS THE LEVELS OF PROENKEPHALIN A mRNA AND PROENKEPHALIN A RELATED PEPTIDES. T. Kanamatsu*, C.D. Unsworth†, E.J. Diliberto†, O.H. Viveros†, and J.S. Hong . †Laboratory of Behavioral and Neurological Toxicology, NIEHS/NIH, †Department of Medicinal Biochemistry, The Wellcome Research Laboratories, Research Triangle Park, NC 27709

The role of splanchnic innervation in the expression of opioid peptides (OP) in the rat adrenal medulla has been a matter of controversy (1,2). It has been proposed that the increase in OP level observed following both denervation and insulin-induced hypoglycemia results from an initial increase in the rate of splanchnic discharge (2). The measurement of mRNA levels of proenkephalin A ($mRNA^{enk}$) was used in this report as an index of OP biosynthesis and to establish the role of the innervation in OP gene expression.

Male Sprage-Dawley rats weighing 300 g were injected with insulin (IOU/kg, s.c.) to induce hypoglycemia and thus increase splanchnic nerve discharge. Insulin shock was stopped two hr later by oral administration with 1.5 ml 40% sucrose and rats were killed at various times thereafter. The levels of both NOP (native OP, without enzyme digestions) and COP (cryptic OP, with enzyme digestions) were determined by radioreceptor assay. The level of (Met^5)-enkephalin-like immunoreactivity (ME-LI) was determined by radioimmunoassay (RIA). The abundance of $mRNA^{enk}$ was measured by a blot hybridization method using a cDNA clone derived from rat brain.

Two hr of insulin-induced hypoglycemia decreased the level of catecholamines by approximately 55% and produced no significant alteration of the levels of COP and NOP in the adrenal medulla. The same treatment tripled the level of $mRNA^{enk}$. The increase in the level of $mRNA^{enk}$ reached a plateau 26 h after the injection of insulin (15-fold increase compared with controls), then the level declined with an approximate half life of 4 days. Significant increases in the levels of COP and NOP occurred 26 h after insulin treatment (COP, 6-fold; NOP 2-fold) and the increase reached maximal levels after 98 h (COP, 26-fold; NOP 18-fold). Seven days postdose, levels of OP were still higher than the controls (COP, 8-fold; NOP 5-fold). Results from RIA which measures ME-LI were consistent with those measured by radioreceptor assay. Pretreatment with ganglionic blocking agents, chlorosondamine (5 mg/kg, i.p.) plus atropine (1 mg/kg, i.p.) completely abolished insulin-induced increase in $mRNA^{enk}$. These data indicate that splanchnic discharge may stimulate OP biosynthesis, probably at the level of gene expression.

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169.4 LITHIUM INCREASES (Met^5)-ENKEPHALIN LEVELS, PRECURSOR CONTENT AND PREPROENKEPHALIN mRNA ABUNDANCE IN THE RAT STRIATUM. S.P.Sivam and J.S. Hong. Laboratory of Behavioral and Neurological Toxicology, NIEHS/NIH, Research Triangle Park, NC 27709.

Lithium is a well-established drug for the treatment of manic-depressive illnesses though the mechanism of its action remains unknown. This report explores the influence of lithium on (Met^5)-enkephalin (ME) biosynthesis in the rat brain. Male Fischer 344 rats were treated with lithium chloride (4 mEq/kg/day, i.p.) for 1, 2, or 4 days and sacrificed 24 hr after the last dose; another group was sacrificed 2 hr following the dose to study the acute effect. Control animals received saline. The ME levels were determined by radioimmunoassay[1]. Lithium increased the ME level in the striatum in a time-dependent fashion reaching 76% of control value following four doses. No changes were observed in other brain regions studied such as hypothalamus, hippocampus, frontal cortex, brain-stem. In order to characterize the nature of this selective increase of ME level in the striatum, the precursor content as well as the preproenkephalin mRNA abundance were determined. The precursor content as reflected by the cryptic ME content following sequential digestion[2] with trypsin and carboxypeptidase-B was measured by radioimmunoassay. Lithium increased the precursor content in a time-dependent manner and this pattern closely paralleled with the increase in native ME content. The preproenkephalin mRNA abundance was quantitated by blot hybridization of total RNA (isolated by the guanidine thiocyanate extraction procedure[3]) with a ^{32}P labelled cDNA probe derived from rat brain[4]. Lithium increased the mRNA abundance following repeated doses for four days. These results confirm and extent the previous observations[5] in which case a cell-free translation method was used to estimate the preproenkephalin mRNA. The study suggests that repeated injections of lithium increase the biosynthesis of ME in the rat striatum.

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169.5 *DETECTION OF RAT STRIATAL SUBSTANCE P AND SUBSTANCE K mRNA AND EFFECT OF HALOPERIDOL TREATMENT ON THE LEVEL OF SUBSTANCE P AND SUBSTANCE K MESSAGE.* J. Angulo*, K. Wilcox* B. Burkhart*, R. Lampe*, K. Lawrence*, R. Manning*, R. Arentzen*, L. Davis, and G. Christoph.* Central Research and Development Department, E. I. du Pont de Nemours and Co., Experimental Station, Wilmington, DE 19898.

Substance P (SP) is an endogenous brain peptide which has many properties expected of neurotransmitters in the central nervous system. In rat striatum, neuronal perikarya that contain SP project to the substantia nigra. This striatonigral pathway presumably plays a role in basal ganglia function. From published data on the sequence for bovine SP preprohormone mRNA and that of a related tachykinin peptide, substance K (SK), we have synthesized oligodeoxynucleotide probes (39-mers) which specifically hybridize to mRNA for SP and SK. After labeling the probes with 32-P, Northern gel analysis of total RNA from striatum shows one intense band for SP mRNA. The SK probe yields a signal of identical size, but one third as intense as that for SP. This may reflect the relative levels of SP and SK mRNA's or hnRNA's in striatum. No detectable SP or SK hybridization signal was detected with total RNA from cerebellum after 7 days of exposure to film (striatal SP and SK are routinely detected within 4 hours of exposure). Chronic treatment of rats with the dopamine antagonist haloperidol is known to decrease the concentration of SP in the substantia nigra. To determine whether this decrease in peptide concentration was due to reduced synthesis, we compared SP mRNA levels in striatal tissue from control rats and rats chronically treated with haloperidol (delivered by osmotic minipumps at 0.2 mg/Kg/hr for 5 days). The data indicate that chronic haloperidol reduces striatal SP mRNA suggesting that the drug induces a reduction in the expression of the SP gene.

169.6 **NEUROMEDIN B: A NEW BOMBESIN-LIKE PEPTIDE IN MAMMALIAN BRAIN.** **Robert T. Fremeau Jr.[1], Louis Y. Korman[2], Thomas L. O'Donohue[3] and Terry W. Moody[1]. [1]Dept. Biochemistry, George Washington Univ., School of Medicine, Wash., D.C. 20037, [2]VA Medical Center, Wash., D.C. 20901 and [3]Expt. Ther. Branch, NINCDS Bethesda, MD 20205.**

Bombesin, a tetradecapeptide initially isolated from frog skin, induces potent biological (hypothermia, hyperglycemia and satiety) and behavioral (grooming and analgesia) effects after central administration into the rat brain. Some of these actions may be mediated by the endogenous bombesin-like peptides which are present in certain brain regions such as the hypothalamus and spinal cord but not the hippocampus or cerebellum (Moody, T. et al., Peptides 2: 65, 1981). These endogenous bombesin-like peptides may interact with receptors which have been detected in discrete rat brain regions such as the periventricular and suprachiasmatic nuclei of the hypothalamus, dentate gyrus, hippocampus and dorsal horn of the spinal cord (Zarbin, M. et al., J. Neuroscience, 5: 429, 1985). Because receptors but not bombesin-like immunoreactivity are present in the hippocampus it is possible that these receptors are activated by some other peptide. Recently, Minamino, N. et al., (BBRC., 114: 541, 1983) isolated a decapeptide from porcine spinal cord (neuromedin B) which is structurally similar to bombesin (7 of the 10 C-terminal amino acid residues are identical). Here we report that neuromedin B-like peptides are present in certain rat brain regions such as the hippocampus.

A radioimmunoassay was developed for neuromedin B and the antisera did not cross react with bombesin or GRP. Immunoreactive neuromedin B was detected in high density in the spinal cord, hippocampus, hypothalamus and olfactory bulb. Intermediate levels of immunoreactivity were detected in the striatum, cortex, thalamus, midbrain, hindbrain and pituitary. Low levels of immunoreactivity were present in the cerebellum and pineal; the concentration of neuromedin B varied by approximately 2-orders of magnitude in high relative to low areas. Using gel filtration techniques one major peak of immunoreactivity was present which coeluted with neuromedin B. Using HPLC techniques, however, a major and minor peak of immunoreactivity was detected, the former of which eluted with neuromedin B. Also, receptors for bombesin-like peptides bound neuromedin B with high affinity (IC_{50} = 4 nM). Thus in certain brain regions, such as the hippocampus, receptors for bombesin-like peptides may be activated by endogenous neuromedin B-like peptides.

169.7 ETHANOL DECREASES B-ENDORPHIN RELEASE AND PRO-OPIOMELANOCORTIN mRNA LEVELS IN AtT-20 AND RAT PITUITARY CELLS IN CULTURE. J.R. Dave, L.E. Eiden and R.L. Eskay, (SPON: S. Sabol), Laboratory of Clinical Studies, DICBR, NIAAA and Laboratory of Clinical Science, NIMH, Bethesda, MD 20205.

Since ethanol is known to disrupt the hypothalamic-pituitary-adrenal axis, studies were undertaken to determine the effect of ethanol on release and synthesis of pro-opiomelanocortin (POMC)-derived peptides from AtT-20, rat anterior lobe (AP) and rat intermediate lobe (IP) pituitary cells in culture. Cells were preincubated for 24 hr with 0, 0.2 or 0.4% ethanol and basal release of B-endorphin (BE) was determined during an 1 hr release experiment in the presence of either 0.2 or 0.4% ethanol. Incubation of AtT-20 and AP cells with 0.4% ethanol resulted in a consistent 25% reduction in basal release of BE. However, the content of BE did not change in either AP or AtT-20 cells following 24 hr incubation with 0.4% ethanol. To determine the effect of ethanol exposure on POMC biosynthesis, the levels of POMC messenger RNA in AtT-20, AP and IP cells were quantified by Northern-blot and slot-blot techniques. Ethanol produced a dose- and time-dependent decrease in POMC mRNA levels in AtT-20 cells. 24 hr treatment of AtT-20 cells with 0.2, 0.4 or 0.6% ethanol produced approximately 0, 60 or 80% decrease in POMC mRNA levels, respectively. Treatment of AtT-20 cells with 0.4% ethanol for 8, 12 or 24 hr produced a 40, 50 or 60% decrease in POMC mRNA levels, respectively. Similarly, treatment of AP cells with 0.2, 0.4 or 0.6% ethanol for 24 hr produced a 40, 60 or 60% decrease in POMC mRNA levels, respectively. Treatment of primary cultures of IP cells with ethanol produced changes in mRNA levels which were similar in magnitude to those observed with AtT-20 cells. The time- and dose-dependent effect of ethanol on POMC mRNA levels and not on BE release from AtT-20 and rat pituitary cells suggest that the effect of ethanol on BE release may not be related to the decreased POMC mRNA levels. Ethanol may decrease POMC mRNA levels by reducing the transcription of the POMC gene in AtT-20 and rat pituitary cells.

169.8 THE ANTIPSYCHOTIC DRUG HALOPERIDOL DECREASES STRIATONIGRAL SUBSTANCE P, SUBSTANCE K AND PREPROTACHYKININ mRNA. M.J. Bannon, J.-M.Lee*, P. Giraud*, A. Young*, H.-U. Affolter* and T. Bonner*. Dept. of Psychiatry, Yale Univ., School of Medicine, New Haven, CT 06508 and Lab. of Cell Biology, N.I.M.H., Bethesda, MD 20205.

Striatonigral substance P (SP)-containing neurons may influence, and in turn be modulated by, nigrostriatal dopamine (DA) cells. However, in contrast to nonpeptidergic neurotransmitters, measures of the turnover of SP and other peptides have been lacking. Previous studies have shown that repeated administration of DA antagonists decreases substantia nigra SP concentrations. Although the relationship between SP concentrations and SP utilization remains obscure, it has been generally accepted that the net decrease in SP after DA antagonists results from an imbalance between increased SP synthesis and an even greater acceleration of SP release. Recently, the tachykinin substance K (SK) has been identified as a cotransmitter with SP, although the function of this peptide and its response to DA antagonists is unknown. Two mRNAs derived from the preprotachykinin (PPT) gene by alternate splicing encode for either SP (α PPT mRNA) or SP and SK (β PPT mRNA). Quantitating changes in the levels of neuropeptide-encoding mRNA may provide a useful index of changes in brain peptide synthesis and utilization. In the present study, we assessed the effects of acute and chronic administration of the DA antagonist haloperidol (HALO) on the levels of nigral and striatal SP, SK and PPT mRNAs in single rat striata.

Acutely, HALO (1 mg/kg) did not alter either striatal or nigral SP or SK concentrations. 24 hrs. after one dose of HALO, striatal SP and SK were decreased. After 3-10 d of treatment, both striatal and nigral SP and SK were decreased by ~ 20-30%. Thus SK peptide concentrations change in parallel with its co-transmitter SP. Total (α and β)PPT, quantitated using an exon 7-derived genomic probe, decreased significantly 24 hr after one dose of HALO. Further decreases (~30-45%) in PPT mRNA were seen after 3-10 d of HALO. When βPPT was specifically quantitated using an exon 6-containing probe, similar results were obtained. Thus both α PPT (SP encoding) mRNA and β PPT (SP/SK encoding) mRNA decrease in parallel with SP and SK peptides. Contrary to the commonly held hypothesis, chronically administered DA antagonists apparently decrease striatonigral SP/SK synthesis. Quantitation of neuropeptide-encoding mRNA in discrete brain areas may provide a dynamic index of brain peptide synthesis.

169.9 CHLORIDE TRANSPORT AND METABOLIC ENERGY ARE ESSENTIAL FOR COPPER STIMULATION OF LHRH RELEASE FROM MEDIAN EMINENCE EXPLANTS.

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We have previously shown that chelated copper stimulates the release of luteinizing hormone releasing hormone (LHRH) from axonal terminals located in the ME by a mechanism involving a ligand (chelator) specific interaction with a limited number of sites on the LHRH neuron. This interaction leads to release of the peptide by a process that does not require extracellular calcium. In this study, we assessed the Na^+, Cl^- and energy requirements for this release process. ME obtained from 8 weeks old male rats were incubated at 37°C with 100 µM CuHis ($CuCl_2$: Histidine; 1:1) in Krebs Ringer-phosphate buffer, pH 7.4 for 15 min (Phase I) and then in copper-free buffer for 15 min (Phase II). LHRH released into the medium was assayed by RIA. The characteristics of the copper-stimulated release process is such that net stimulated release in Phase I is 9.8 and in Phase II it is 20.8 pg/15 min per ME. Thus, maximal rate of stimulated release is attained after transfer of the ME to copper-free medium. Substitution of Na^+ with $Choline^+$ did not alter the copper-stimulated release of LHRH. In contrast, substitution of Cl^- with isethionate (reducing the Cl^- concentration from 135.6 to 3.6 mM), completely abolished stimulated LHRH release in Phase II but not in Phase I. In addition, when copper-stimulated release was evaluated in the presence of 135.6 mM Cl^- and an anion transport inhibitor (SITS, 4-acetamido-4'-isothiocyano-2,2'disulfonic stilbene), stimulated release was completely inhibited in both Phase I and II. This result is suggestive that stimulated release seen in Phase I, in the presence of 3.6 mM Cl^- and absence of SITS, is due to Cl^- transport. To assess the energy requirement for copper-stimulated release of LHRH, ME were incubated either at 4°C or at 37°C in the presence of a metabolic inhibitor (4 mM NaF). Stimulated release in Phase I and II did not occur at 4°C and it was inhibited by NaF by 50%. In summary, we demonstrate that the process of copper-stimulated release of LHRH from axonal terminals requires metabolic energy and Cl^- but not Na^+. Although Cl^- transport is essential for the accomplishment of the entire release process, the differential Cl^- requirement for Phase I and II is indicative that uptake of copper and initiation of the process of LHRH release requires less Cl^- than the acceleration and attainment of maximal rates of release.

169.10 INTRASTRIATAL INJECTION OF KAINIC ACID INCREASES THE ABUNDANCE OF mRNA CODING FOR PREPROENKEPHALIN A IN RAT HIPPOCAMPUS.

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We have previously reported that intracerebral injection of kainic acid (KA) causes a robust increase in the concentration of (Met^5)-enkephalin-like immunoreactivity (ME-LI) in the rat hippocampus. In order to understand whether KA-induced elevation in ME-LI is due to an increase in biosynthesis or to a decrease in release, we measured the abundance of mRNA coding for preproenkephalin A ($mRNA^{enk}$) as an index of biosynthesis in different brain regions. Male Fischer-344 rats (Wilmington, MA) between 12-14 weeks of age were used. The coordinates for intrastriatal injection were: A, 7.4; L, 2.8; V, 0. Rats were injected with KA (1 mg/ml dissolved in 0.9% NaCl) at constant rate (1 ml min^{-1}) for one min. Concentrations of native (without enzyme digestions) and cryptic (after enzyme digestions) ME-LI were measured by RIA. The abundance of $mRNA^{enk}$ was measured using a cDNA clone coding for preproenkephalin A derived from rat brain. A single striatal injection of KA caused recurrent seizure activities lasting 3-6 h. During this period, there was a 30-40% reduction in the level of native ME-LI in the hippocampus. The ME-LI returned to control value 12 h postdosing and then showed a large rebound 24 h after postdosing (200% of control values); there was a three fold increase in ME-LI 72 h postdosing. The reduction in ME-LI during the convulsing period suggests an increase in the release of this peptide and the rebound in ME-LI in later phase may be due to an overproduction of ME. This possibility was supported by a sharp increase in the abundance of $mRNA^{enk}$ during the phase of recurrent seizures. The level of $mRNA^{enk}$ reached a plateau (4 fold increase) 6 h postdosing then declined linearly and returned to normal value 72 h after injection. The increase in the level of $mRNA^{enk}$ suggests an increase in the biosynthetic rate of enkephalin-containing neurons triggered by the seizure activities. This idea was further supported by the increase of cryptic ME-LI in the hippocampus during the convulsing phase when there was a decrease in native ME-LI. The level of cryptic ME-LI reached a maximum 24 h postdosing then gradually declined, presumably being processed to give rise to native ME-LI. KA-induced alterations in the enkephalin system appeared limited only to the hippocampus and the entorhinal cortex (the location of cell bodies of enkephalin-containing neurons which project to the dentate gyrus), but not in the striatum or hypothalamus. The present demonstration of a robust increase in the metabolic activity of entorhinal-hippocampal enkephalin-containing neurons may be related to several KA-induced behavioral alterations, such as wet dog shake or post-ictal depression.

169.11 KAINIC ACID HAS A BIPHASIC EFFECT ON ENKEPHALIN AND DYNORPHIN IMMUNOREACTIVITY IN RAT HIPPOCAMPUS. J. Obie*, T. Kanamatsu*, J.F. McGinty and J.-S. Hong (SPON: E.M. Lieberman) Lab. Behav. Neurol. Toxicol. NIEHS/NIH Research Triangle Park, NC 27709 and Dept. of Anatomy, East Carolina Univ. Sch. Med. Greenville, NC 27834.

We have previously reported that intracerebroventricular or intrastriatal injections of the excitatory amino acid, kainic acid (KA), cause an increase in met^5- or leu^5-enkephalin immunoreactivity (IR) in the hippocampus (Hong, et al., Nature 258:231, 1980; McGinty, et al., PNAS 80:589, 1983). The maximal increase occurs by 3 days and is sustained for at least two weeks after a single KA injection. Immunocytochemistry (ICC) revealed that the increase in enkephalin (IR) was most apparent in hippocampal mossy fibers but was also noticeable in the perforant-temperoammonic pathway of the hippocampus. Four days post injection, no change in dynorphin A (1-17) (IR) was apparent by radioimmunoassay (RIA) or ICC. In the present study, we conducted a more detailed investigation of the time course of changes in enkephalin (IR) and dynorphin A (IR) in rat hippocampus after intrastriatal injections of kainic acid. Male Fischer 344 rats were anesthetized with Equithesin and injected stereotactically with KA (1 mg in 1 ml of 0.9% NaCl) at a constant flow rate (1 µl min^{-1}) for one minute. Recurrent motor seizures lasted for 3-6 h. during which time met-enkephalin (IR) decreased 30-40% and dynorphin A (1-8) (IR) decreased 78% in the hippocampus. Twelve hours after KA, enkephalin (IR) had returned to 87% of the control value whereas dynorphin A (IR) was still depressed by 63%. Immunocytochemistry performed on 6 h. samples revealed a marked depletion in mossy fiber dynorphin A (IR) and a less severe decrease in hippocampal enkephalin (IR) at this time point. No changes in enkephalin (IR) or dynorphin (IR) were apparent by RIA or ICC in hypothalamus or striatum (uninjected side). By 48 h., hippocampal enkephalin (IR) had increased to 270% whereas dynorphin (IR) had increased to 171% of control values. Both mossy fiber dynorphin immunostaining and enkephalin immunostaining were visibly increased at this time. An increase in enkephalin (IR) in the perforant-temperoammonic pathway, especially in the ventral hippocampal formation, was also apparent. By 72 h., dynorphin A (IR) had returned to control values but enkephalin (IR) was still elevated at 300% of control values. The biphasic changes in enkephalin and dynorphin (IR) subsequent to a single intracerebral KA injection suggest that an increase in the metabolic rate of the perforant path-mossy fiber circuitry during KA-induced seizures results in an initial release of opioid peptides followed by an overproduction of each opioid peptide, a response which is greater for enkephalin than for dynorphin. A rapid, four-fold increase in met-enkephalin mRNA in the hippocampus and entorhinal cortex within 6 h. after KA administration (see Hong, et al. this volume) supports the idea of an increase in biosynthetic activity in enkephalinergic neurons in the perforant pathway and in mossy fibers triggered by KA-induced seizures. Supported by NS 20451.

169.12 **EVIDENCE FOR A POSITIVE FEEDBACK OF GROWTH HORMONE ON HYPOTHALAMIC SOMATOSTATIN BIOSYNTHESIS.** K. D. Stewart and J. F. McKelvy, Dept. of Neurobiology and Behavior, SUNY Stony Brook, NY 11794

Somatostatin is known to inhibit growth hormone (GH) release from the anterior pituitary and physiological evidence has suggested that GH may act via a short loop feedback to regulate its own release. Thus, it is possible that GH may influence the expression of somatostatin in hypophysiotropic neurons. We have investigated this possibility by carrying out direct studies in vivo on the de novo biosynthesis of somatostatin in control, hypophysectomized (HYPOX), and HYPOX-GH-replaced rats.

We developed methods to examine the in vivo biosynthesis of three peptides generated from the somatostatin precursor: somatostatin-14 (SS-14), somatostatin-28 (SS-28), and somatostatin-28(1-12) (SS-28(1-12)). The peptides are radiolabeled by stereotaxic infusion of ^{35}S-cysteine and ^{3}H-proline into the periventricular area of the hypothalamus and transported to the median eminence from which the labeled peptides are extracted and purified to constant specific activity by sequential HPLC and chemical modification. Using this technique we have found that following hypophysectomy radiolabeled SS-28(1-12) was decreased by 56%, SS-28 by 25%, and SS-14 by 32% (compared to control rats). When GH was administered to HYPOX rats somatostatin was stimulated compared to HYPOX levels: SS-28(1-12) by 1.50 fold, SS-28 by 1.90 fold, and SS-14 by 1.72 fold. This stimulation resulted in a partial restoration of the biosynthetic values observed in control animals; such that when compared to control animals SS-28(1-12) was decreased by 84%, SS-28 by 43%, and SS-14 by 55%. The biosynthesis and transport to the median eminence of oxytocin and vasopressin were also assessed in the same experiments (by ^{35}S-cysteine incorporation) and showed no consistent response to the experimental manipulations.

Thus, the removal of GH causes a decrease in somatostatin biosynthesis while GH replacement partially restores somatostatin levels. Somatostatin may therefore be one factor by which GH regulates its own levels.

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169.13 BIOSYNTHESIS OF POMC-DERIVED PEPTIDES IN A DIFFERENTIATED EMBRYONAL CARCINOMA CELL LINE. J.A. Fuller*, J.M. Levine* and J. F. McKelvy. Dept of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, New York, 11794

The murine embryonal carcinoma cell line, O1A1, responds to retinoic acid by differentiating into neuron-like cells (Edwards and McBurney, Dev. Bio., 98:187, 1983). This differentiation process has recently been shown to resemble the terminal differentiation of neuroectodermal cells (Levine and Flynn, this volume). To characterize the phenotype of the differentiated neurons, we have investigated the possible expression of neuropeptides by these cells. Pro-opiomelanocortin (POMC)-like material was detected in differentiated O1A1 cells by indirect immunoflouresence. To validate the expression of POMC peptides by this cell line, and to explore it's possible utility in studying POMC biosynthesis and processing and the induction of POMC gene expression, the de novo synthesis of POMC-derived peptides was studied.

Cells were induced to differentiate by treatment with retinoic acid and grown for 3-5 days in monolayer. They were then starved for 2 hrs in serum-free DME without methionine, and then 70 uCi/ml ^{35}S-methionine was added to the media. After a 6 hr. incubation, 50 ug of carrier peptides were added, the media harvested and the cells lysed in 5N formic acid. The acid-soluble lysate was added to the media, desalted and lyophilized.The material was then trypsin digested, oxidized, and subjected to sequential HPLC purification. After four HPLC separations the peptides were generally purified to constant specific radioactivity.

Both trypsin-digested Beta-Endorphin and acetylated and non-acetylated MSH's were detected. If all three peptides are digested with trypsin, then MSH- and Beta-Endorphin-like material is approximately equimolar, implying that a large amount of the material is in the precursor form. MSH and des-acetyl-MSH material occur in a ratio of 3:1. Non-differentiated cells produce some POMC related material, but show backround levels of immunoflouresence.

Thus, it appears possible to use an in vitro system to study the synthesis and processing of POMC related peptides resembling that found in the CNS and intermediate lobe. Studies are under way to determine this cell line's responsiveness to signals thought to regulate POMC expression, synthesis, and processing in neurons. (Supported by NIH NS 20372 and NS 21198)

169.14 MULTIPLE PRODUCTS DERIVED FROM OPIOID PEPTIDES. E.A. Young* and H. Akil. (SPON: S.Watson) Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109

The presence of multiple active cores within the same peptide is well known. For example, the sequence of alpha-MSH is contained within the sequence of ACTH, the sequence of beta-endorphin is contained in beta-lipotropin, the sequence of dynorphin A 8 is contained in dynorphin A, and the enkephalin sequences are contained in all opioid peptides. Tissue-specific and species specific processing of precursor hormones to differing end products is well described. For instance, beta-lipotropin and ACTH are the preferred end products of pro-opiomelanocortin (POMC) processing in the anterior lobe of the pituitary, while beta-endorphin and alpha-MSH are the predominant products of POMC processing in the neurointermediate pituitary. Likewise, in pituitary and many regions of brain, dynorpin A is further processed to dynorphin A 8. Whether dynorphin A can be further processed to leu-enkephalin is still a matter of speculation. In addition to differences in forms released across tissues, there may be sub-populations of releasable pools within any particular tissue, so that the overall ratio of material stored does not necessarily correspond to what is released. Thus, tissue specific processing and different releasable pools are two different ways to encode multiple messages in the some precursor. Still another strategy exists, that is the conversion of one active peptide to another after release. Such post-release conversion has been seen in non-opioid systems such as the conversion of angiotensinogen to angiotensin I, and subsequently to angiotensin II and III in its target organ. Such post release conversion has not been well explored in opioid peptide systems. Using dynorphin A as a model system, we characterized the bio-transformation of dynorphin A to other biologically active peptide fragments in brain.

Radiolabelled ^{3}H dynorphin A was incubated with membrane fragments of rat whole brain at 0°C for varying time periods from 15 minutes to one hour. After incubation, the suspensions were microfuged and the supernatant pooled and lyophilized for subsequent application to HPLC. Using the radioactivity as a marker, as well as radioimmunoassay of the HPLC fractions, we were able to demonstrate the production of ^{3}H tyrosine from dynorphin A . In addition, a smal peak of radioactivity co-migrated with leu-enkephalin. *In vivo* injection of ^{3}H dynorphin A into the periaqueductal grey (PAG) in cannulated rats demonstrated extremely rapid conversion of dynorphin A to ^{3}H tyrosine and des-tyrosine dynorphin A (demonstrated by RIA of the HPLC fractions). Again, a small peak of radioactivity co-migrated with leu-enkephalin. We are currently seeking further indentification of this possible leu-enkephalin peak. Since both des-tyrosine dynorphin A and leu-enkephalin are biologically active peptides, it does appear that post-release conversion of one active peptide to another occurs in brain. We are also exploring another model system, that is the peripheral conversion in plasma of circulating opioid peptides to other biologically active peptides.

169.15 **MOLECULAR FORMS OF IMMUNOACTIVE ATRIAL NATRIURETIC PEPTIDE IN THE RAT HYPOTHALAMUS AND ATRIUM.** **G.M. Wildey*, T.R. Gibson*, and C.C. Glembotski.** Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Recent studies have demonstrated that mammalian atria contain peptides that are important diuretic, natriuretic, and vasodilatory agents. Several laboratories have isolated various forms of these atrial natriuretic peptides (ANP) which range in length from 21 to 125 amino acids. At the present time there exists little agreement on the major molecular forms of ANP that are present in the atrium or the plasma. All of these peptides possess a common COOH-terminal sequence, and are likely derived from a common precursor which has been sequenced in rats, mice and humans. The atrial peptides exert their effects on well-characterized receptors localized to the vascular smooth muscle cells, kidney, and adrenals. Extra-cardiac locations of the atrial peptides have been identified by immunohistochemistry; among these locations is rat hypothalamus. Receptors for ANP have also been localized to several brain regions and a role for ANP-related peptides in the CNS regulation of cardiovascular function has been implicated. Since the atrial peptides have potentially important endocrine and neurotransmitter activities, a study was undertaken to compare the molecular forms of these peptides derived from the heart and the hypothalamus. Acetic acid extracts of rat hypothalamus and atrium were prepared by a procedure previously shown to minimize proteolytic degradation of peptides. An antisera crossreactive with the COOH-terminal regions of ANP-related peptides was utilized to characterize molecular forms. The hypothalamic extracts contained about 13 ng of ANP per animal, whereas the atrial extracts contained about 30 µg of ANP per animal. The majority of the immunoactive material in the atrial extracts had a molecular weight of approximately 9,000 to 15,000 daltons when analyzed by HPSEC or Sephadex G-75 gel filtration. This suggests that few cleavages of pro-ANP (19,000 daltons) take place in the rat atrium. (These large molecular weight forms of ANP have been previously shown to possess full biological activity.) In hypothalamic extracts the major immunoactive species was about 1,500 to 1,800 daltons. The major molecular weight forms of ANP from each extract were further differentiated by RP-HPLC in a TFA/acetonitrile solvent system. The hypothalamic material eluted at 42% acetonitrile, while the atrial material eluted at 55% acetonitrile. These results suggest that the hypothalamus and atrium may differentially process pro-ANP to form tissue-specific product peptides. Supported by NIH Grant NS20396 and American Heart Grant-in-Aid 84 653

169.16 INFLUENCE OF MICROWAVE IRRADIATION ON ENDOGENOUS RAT BRAIN BRADYKININ LEVELS. K.E. Eason*, H. Okamoto*, L.M. Greenbaum* and J.J. Buccafusco. (SPON: D.S. Feldman). Dept. Pharmacology and Toxicology, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

Bradykinin (BK) is a potent vasoactive peptide in the peripheral circulation. There also is considerable evidence to indicate that BK may function as a neurotransmitter in the brain. Current methods of measuring endogenous brain BK involve the aortic perfusion of a peptidase containing medium in anesthetized rats to 1) kill the animal and 2) prevent BK metabolism in brain tissue and cerebral blood. The tissue is removed and frozen in liquid N_2, followed by a multi-step extraction and chromatographic procedure. Levels of BK have been reported to be ~0.6 pmoles/g tissue (J. Neurochem. 43, 1072, 1984). The purpose of this study was to determine whether focused microwave irradiation could be employed to stabilize BK levels in postmortem tissue. Kallikrein and kininase activities were measured to assure complete inactivation of enzymes involved in release and destruction, respectively, of blood and brain BK. Male, Wistar rats were subjected to focused microwave irradiation (2450 MHz, 3.8 KW) directed at the skull for periods of 1.6, 1.8, 2.1, 2.45 and 2.9 sec. Whole brains were then removed and homogenized in 1M Tris-HCl, pH 8.0 containing 0.15M NaCl. Analysis of enzyme activity revealed that both enzymes were inactive when the irradiation period was at least 2.45 sec. Shorter periods and/or incomplete irradiation resulted in significant enzyme activity. Kininase was more heat-resistant than kallikrein, requiring longer and more thorough irradiation to be inactivated. Therefore, for the BK assay, animals were subjected to at least 2.45 sec of irradiation and the brains removed and homogenized in buffer containing ^{3}H-BK (to calculate recovery). NaCl was added to the supernatants obtained after centrifugation to salt out lipids prior to application to a reverse phase C_{18} extraction column and elution with 50%ACN : 1%TFA. After lyophillization, aliquots of reconstituted extracts were removed to determine % recovery and to measure levels of BK by radioimmunoassay. BK levels from individual brain samples were 0.7±0.075 pmoles/g with values ranging from 0.2 to 2 pmoles/g. Percent recovery of ^{3}H-BK was ~64%. These findings suggest that 1) adequate microwave irradiation of the brain allows the determination of values for BK in brain samples consistent with those obtained by employing more complex perfusion techniques, and avoids the use of anesthetics; and 2) the variability in BK levels obtained from individual animals is not related to incomplete inactivation of kininase or kallikrein. The nature of this variability is under investigation. Supported by HL30046 and the Veterans Administration.

169.17 CHOLECYSTOKININ GENE-RELATED PEPTIDES: THEIR SUBCELLULAR DISTRIBUTION IN RAT BRAIN. L.R. Allard* and M.C. Beinfeld* (SPON: M.H. Cooper). Department of Pharmacology, St. Louis University Medical Center, St. Louis, MO 63104.

Antisera raised against synthetic peptides D-10-Y comprising the carboxy-terminal extension of cholecystokinin (CCK) and V-9-M specific for the amino-terminus of pro-CCK were used in specific radioimmunoassays to study the subcellular distribution of such immunoreactive peptides in rat brain. Subcellular fractionation of whole rat brain determined what cellular component was enriched in these peptides. The molecular weights of D-10-Y-like and V-9-M-like immunoreactive peptides enriched in various subcellular fractions were determined by Sephadex G-50 chromatography.

Primary subcellular fractionation yielded a mitochondrial (P2) and microsomal (P3) fraction, both of which were enriched in D-10-Y and V-9-M-like peptides. Furthermore, a slight enrichment of V-9-M-like immunoreactivity was observed in the soluble fraction (S3). Further purification of the P2 fraction demonstrated an increase of D-10-Y-like and V-9-M-like immunoreactivity in purified synaptosomes. With the exception of the enrichment in the soluble fraction, V-9-M-like peptides as well as D-10-Y-like peptides follow a similar distribution to that of CCK 8-like peptides.

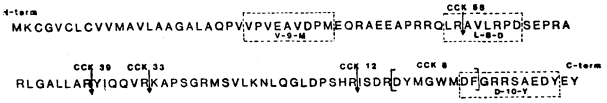
Sephadex chromatography of P2 and P3 fractions indicated the major forms of D-10-Y and V-9-M present. Whole rat brain contains two major molecular forms of D-10-Y-like immunoreactivity, one similar in size to CCK 33 and one slightly larger than CCK 8. In the P2 and P3 fractions, most of the D-10-Y-like immunoreactivity resembled the molecular form slightly larger than CCK 8. Rat brain contains three major V-9-M-like peptides with molecular weights of about 13,000, 8,000 and 2,700. The latter form predominates. The major form of V-9-M present in P2 and P3 fractions has a molecular weight of about 2,700.

It is likely that the D-10-Y-like immunoreactive peptide is an intermediate in the processing of CCK 8, and its enrichment in nerve endings is consistent with the final cleavage and amidation taking place in nerve terminals. The V-9-M-like peptide may represent an intermediate in the processing of CCK, and its presence in synaptosomes may indicate that the proteolytic cleavage of pro-CCK into CCK 58 and the 2,700 form takes place in synaptic vesicles. It is also possible that this amino-terminal portion of pro-CCK may be released along with CCK and play a role in synaptic transmission.

Supported by NS18335 and NS18667 (N.I.H.).

169.18 CHOLECYSTOKININ GENE-RELATED PEPTIDES: THEIR DISTRIBUTION, IMMUNOLOGIC, AND CHROMATOGRAPHIC CHARACTERIZATION IN RAT BRAIN AND SMALL INTESTINE. M.C. Beinfeld* and L.R. Allard* (SPON: N.A. Connors). Department of Pharmacology, St. Louis University Medical Center, St. Louis, MO 63104.

Utilizing the sequence of rat pre-pro cholecystokinin (CCK), synthetic peptides were produced which correspond to different portions of the pre-pro sequence. These peptides (illustrated below) have been used to develop specific radioimmunoassays (RIA) to detect CCK gene-related peptides (CGRP). These antisera detect substantial amounts of CGRP in both rat brain and small intestine. The molecular forms of these immunoreactive peptides have been characterized by Sephadex gel filtration chromatography and their distribution has been mapped with the peptide RIAs.



D-10-Y-like peptides: Antiserum against the fragment D-10-Y (which does not cross react with CCK 8 or related peptides) detects moderate amounts of immunoreactivity in brain (13.8 ng/g wet wt.) and intestine (53.8 mucosa, 15.8 muscle). Brain D-10-Y-like immunoreactivity emerged as two major peaks on Sephadex G-50 chromatography, one similar in size to CCK 33 and one slightly larger than CCK 8. It is likely that these D-10-Y-like peptides represent carboxyl-terminally extended forms of CCK 33 and CCK 8.

L-8-D-like peptides: Antiserum against L-8-D detects small amounts of immunoreactivity in brain (6.3), but larger amounts in intestine (in progress). In brain a single peak of immunoreactivity with molecular weight of about 1750 daltons was obtained, which probably represents the amino-terminal portion of CCK 58 remaining when CCK 39 is removed.

V-9-M-like peptides: Antiserum against V-9-M detects large amounts of peptide immunoreactivity in brain (104) and intestine (203 mucosa, 83 muscle). Several V-9-M-like immunoreactive peptides were detected in rat brain with molecular weights of about 13,000, 8,000, 2,700, and 1,700. The high molecular weight peaks probably represent different forms of pro-CCK, while the smaller forms are probably amino-terminal fragments of pro-CCK.

The CCK-gene-related peptides are present in both the brain and duodenum, and probably represent intermediates in the processing of CCK. Differences observed in molecular forms of CGRP in brain and intestine are consistent with differential processing of pro-CCK in brain and gut.

Supported by NS18335 and NS18667 (N.I.H.).

169.19 AN EVALUATION OF EXTRACTION TECHNIQUES FOR NEUROPEPTIDES IN HUMAN BRAIN. David W. Ellison, M. Flint Beal, Michael F. Mazurek and Joseph B. Martin. Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Several studies have shown alterations in neuropeptides in degenerative brain disorders. However, there has been no systematic study of the extraction techniques used in determining levels of neuropeptides. We measured the immunoreactivity of somatostatin (SS), neuropeptide Y (NPY) and vasopressin (AVP) in human parietal cortex after processing with one of five solvents: water, 90% methanol, 0.1 N hydrochloric acid (HCL), 0.1 N perchloric acid (PCA) and 2N acetic acid (HAc). The following procedures were evaluated alone or in combination: boiling, sonication, homgenization with mortar and pestle, freeze-thaw and polytron homogenization. Data are expressed per mg protein.

SS was extracted most efficiently by boiling in 0.1 N HCl (1.1 $\pm$ 0.09 ng) followed by boiling in 2N HAC (0.7 $\pm$ 0.07 ng) which both gave significantly better ($p<0.01$) results than boiling in water (0.1 $\pm$ 0.02 ng), homogenizing in 90% MEOH (0.1 $\pm$ 0.03 ng) or boiling in PCA (0.03 $\pm$ 0.001 ng). A similar pattern was seen for NPY, although 2N HAC (5.5 $\pm$ 0.34 ng) gave the best yield followed by 0.1 N HCl (4.6 $\pm$ 1.4 ng). When both these peptides were sonicated after boiling there was a marked and significant ($p<.001$) reduction in immunoreactivity (SS: 0.70 $\pm$ .07 to 0.32 $\pm$ 0.03 ng; NPY 5.5 $\pm$ .3 to 3.1 $\pm$ .2 ng). Increases in duration and intensity of sonication caused step-wise reductions in SS. HPLC characterization of somatostatin immunoreactivity showed that there was no alteration in the molecular weight species. Boiling followed by sonication had no effect on AVP. This peptide was extracted well in 0.1N PCA (0.21 $\pm$ 0.02 pg for boiling alone) in addition to 2N HAC and 0.1 N HCl; the best yield was produced by boiling and sonication in 0.1 N HCL (0.36 $\pm$ 0.04 pg). Extraction of protein, a frequent denominator in expressions of neuropeptide levels, varied relative to initial weight of tissue. Typically, extraction in HAc, HCl and PCA gave values of 8%, 10% and 12% mg prot/gm wet weight respectively.

The results suggest that careful evaluation of extraction procedures is critical in the determination of neuropeptide concentrations in human brain tissue. Sonication appears to be detrimental to extraction of several neuropeptides. The best solvents were 0.1N HCl and 2N HAC and simply boiling was as effective as any other procedure.

Dr. Ellison is a Wellcome Trust research fellow. Supported by NINCDS grant NS16367 and NIA grant IP50AG05134.

169.20 ISOLATION OF ADIPOKINETIC HORMONE-LIKE IMMUNOREACTIVE PEPTIDES FROM THE RAT MEDIAN EMINENCE. P.A. Schueler* and R. Elde. Dept. of Anatomy, University of Minnesota, Minneapolis, MN 55455

Adipokinetic hormone (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) was originally isolated from extracts of locust corpora cardiaca. In order to characterize the occurrence of neurons that produce this and similar peptides, we produced an antiserum against the Tyr^o analog of adipokinetic hormone. In addition to staining neurons in several arthropods, the antiserum stained a unique population of neuronal elements in rat median eminence, substantia gelantinosa and sacral parasympathetic nucleus of the spinal cord, and cerebral cortex, as well as certain cells of the pancreatic islet (Sasek, et al., Brain Res., in press). The antiserum also reacted with some cells in human pancreatic islets.

In order to characterize the molecular nature of the immunoreactive substance, 175 rat median eminences were extracted in 0.1% trifluoroacetic acid: 40% 1-propanol and chromatographed using reversed-phase high performance liquid chromatography (HPLC). Using the antiserum as the detection system in an immunoblotting paradigm, the HPLC fractions were screened for immunoreactivity. After repeated HPLC runs and high voltage electrophoresis the immunoreactive peaks were purified to homogenity. The amino acid compositions from each of the two peaks appeared to represent new neuropeptides, heretofore unidentified in the rat. Immunoreactive material with an identical retention time to that of the rat median eminence peptides was found in rat neonatal pancreas, and 2 cell lines; a rat medullary thyroid carcinoma (CA-77) and a clonal hamster beta cell line (HIT). This work further validates the approach of using antisera directed against invertebrate peptides to identify new neuropeptides in higher vertebrates.

Supported by the Graduate School, University of Minnesota, the Minnesota Medical Foundation and ImmunoNuclear Corporation.

170.1 SEROTONERGIC MODULATION OF DOPAMINE SYSTEM IN THE RAT NUCLEUS ACCUMBENS. H.S. Kim* and P.L. Wood, Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ 07901.

Electrolytic lesions of the dorsal (Neurosci. Lett. 15:127, 1979) and medial (Brain Res. 216:422, 1981) raphe have been reported to selectively elevate dihydroxyphenylacetic acid (DOPAC) in the rat nucleus accumbens. These data would suggest that the nucleus accumbens receives a tonic inhibitory input from the raphe. To test this hypothesis, we have evaluated the actions of the S-2 antagonist cinanserin on dopamine metabolism in the rat striatum and nucleus accumbens. The levels of dopamine, DOPAC, homovanillic acid and 3-methoxytyramine (3-MT) in tissue punches from 1 mm slices of microwaved rat brain were measured by gas chromatography-mass fragmentography (Biomed. Mass Spectrom. 9:302, 1982).

DOPAC levels were elevated (130 - 150% of control) 30 to 120 min after cinanserin (5 - 10 mg/kg i.p.). Homovanillic acid (140 - 160% of control) and 3-MT levels (200% of control) also were increased, with no change in dopamine steady-state levels. Using pargyline (75 mg/kg i.p.) to monitor the rate of accumulation of 3-MT and the decline in DOPAC, a 38% increase in the fractional rate constant for DOPAC was measured. Dopamine metabolites in the striatum were unaltered.

In summary, our data further support the hypothesis that the nucleus accumbens receives a tonic serotonergic inhibitory input to the dopamine cells innervating this forebrain nucleus. Furthermore, our data clearly indicate that when this input is disrupted both dopamine synthesis and release are augmented in the rat nucleus accumbens.

170.2 INTERACTION BETWEEN 5-HYDROXYTRYPTAMINE AND THYROTROPIN RELEASING HORMONE IN THE RAT SPINAL CORD. Louis E. Tremblay*, P.J. Bédard and R. Maheux* (SPON: C. Radouco-Thomas). Laboratory of Neurobiology, Hôpital de l'Enfant-Jésus, Québec, G1J 1Z4, Department of Anatomy, Laval University, QUEBEC, CANADA, G1K 7P4.

Thyrotropin releasing hormone (TRH) and 5-hydroxytryptamine (5-HT) coexist in descending bulbospinal pathways in the rat. (Hökfelt et al. Neural peptides and neural communication, 1-23, 1980). This coexistence suggests a functional relationship and indeed we have shown (Barbeau and Bédard, Neuropharmacology, 20: 477, 1981) that 5-HTP and TRH have a similar excitatory action on motoneuronal discharges and that the action of both compounds is enhanced by previous denervation by 5-7 Dihydroxytryptamine (5-7 DHT) and blocked by the 5-HT antagonist cyproheptadine. In the present work we have studied the effect of a single dose of TRH (10 mg/kg i.p.) or of chronic administration of the tripeptide (20 µg/2.5 µl/hour) with an intrathecal cannula and osmotic minipump on the subsequent response to 5-HTP 100 mg/kg i.p. The response studied and quantified was the spontaneous EMG activity of the hindlimb extensor muscles in rats spinalized twenty four hours before but pretreated or not twenty one days before with 5-7 DHT. Such denervation, by itself increased the response to 5-HTP to 160% of control. However, chronic treatment with TRH further increased the response to 5-HTP to 297% of controls. Animals treated chronically with vehicle showed no such increase. In such a denervated preparation, a single injection of 10 mg/kg i.p. of TRH had no potentiating effect on the response to 5-HTP one hour or twenty four hours later. Thus, in a chronically denervated spinal cord, chronic, but not acute treatment with TRH can increase the sensitivity of spinal neurons to 5-HT. The present results may help understand the functional reason for the coexistence of 5-HT and TRH in the same pathways. Supported by MRC of Canada, MT-5750.

170.3 REDUCTION AND RECOVERY OF THE CAT VISUAL EVOKED RESPONSE FOLLOWING ACETYLCHOLINESTERASE INHIBITION: INVOLVEMENT OF PUTATIVE NEUROTRANSMITTERS OTHER THAN ACETYLCHOLINE. A.W. Kirby and T.H. Harding* U.S. Army Aeromedical Research Laboratory, Ft. Rucker, AL 36362.

We previously have demonstrated a preferential loss in the VER to low spatial frequency stimulation with relative sparing of responses to higher spatial frequencies following physostigmine or diisopropyl fluorophosphate (DFP - an irreversible AChE inhibitor). Following DFP, the VER recovers to within a standard deviation of baseline measures within 10 to 20 hours without significant recovery of AChE activity. A second dose of DFP no longer reduces the VER, although administration of picrotoxin does. There is evidence in the literature that dopamine, GABA, and possibly other neurotransmitter systems might alter their activity to compensate for excess ACh. It also is likely that some neurotransmitter systems do not compensate for cholinergic hyperactivity, but are altered by increased activity in cholinergic interneurons. We report here results from HPLC analysis of retina and visual cortex following DFP administration.

Anesthetized and paralyzed adult cats were held in a stereotaxic headholder and fitted with appropriate corneal and auxiliary lenses to focus one eye onto a cathode ray tube. The other eye and a small sample of visual cortex were removed for determination of baseline neurotransmitter levels. VERs were recorded from stainless steel bone screws over visual and parietal cortex using phase alternated square wave luminance gratings as a stimulus. Following baseline response measures and initial determination of AChE activity, 4 mg/kg DFP was given i.v. over 1 minute. After a variable period of survival, animals were sacrificed and the other eye and additional visual cortex removed and prepared for HPLC analysis. Tissue was homogenized in 0.1M $HClO_4^-$ containing 0.1 mg/ml cysteine. The sample was centrifuged at 5000 rpm for 3 minutes after which the supernatant was removed, filtered through 0.45 micron filters, and injected through an HPLC system using electrochemical detection for catecholamines and indoleamines and precolumn derivitization with fluorescence detection for amino acids. The tissue pellet was resuspended in an equal volume of 1.0N NaOH and assayed for protein.

Our results demonstrate tremendous variation in catecholamines, indoleamines, and amino acids between different animals. However, using each animal as its own control, our results demonstrate a consistent increase in cortical GABA, a reduction in cortical serotonin (up to 4 and 5 times control levels, respectively), and little or no change in dopamine, following DFP. Our evidence suggests that the decrease in serotonin may be stress related. The increase in GABA certainly would explain our earlier results with picrotoxin. In retina there is a consistent decrease in dopamine (as much as 8 times), GABA (30%), and glycine (22%).

170.4 α-ADRENERGIC AND $GABA_B$ RECEPTOR-MEDIATED POTENTIATION OF cAMP ACCUMULATION IN RAT BRAIN SLICES: POSSIBLE INVOLVEMENT OF PHOSPHOLIPASE A_2. R.S Duman*, E.W. Karbon* and S.J. Enna (SPON: S.J. Strada). Depts. Pharmacol. and of Neurobiol. and Anat., Univ. Texas Med. School, Houston, Texas 77025.

Cyclic AMP production in brain slices appears to be regulated by at least two different receptor mechanisms. Receptors for isoproterenol (ISO), adenosine and VIP appear to be directly associated with adenylate cyclase through a guanine nucleotide binding protein. Other agents, such as the α-adrenergic agonist 6-fluoronorepinephrine (6-FNE) and the $GABA_B$ receptor agonist baclofen, appear to act at sites that are indirectly linked to this second messenger system. Thus, neither 6-FNE nor baclofen alone have any significant effect on cAMP production, although both greatly potentiate the responses to ISO, adenosine and VIP. Experiments were undertaken to define the mechanism whereby 6-FNE and baclofen facilitate cAMP formation. For the study, rat brain cerebral cortical slices were prelabeled with ^{3}H-adenine and ^{3}H-cAMP formation measured using a double column technique. Experiments with EGTA revealed that the ability of 6-FNE or baclofen to potentiate the cAMP response to ISO was totally dependent upon the presence of extracellular Ca++. Additional studies indicated that quinacrine, an inhibitor of the calcium-dependent enzyme phospholipase A_2 (PLA_2), abolished the potentiating responses to 6-FNE and baclofen in a noncompetitive manner. Systemic administration of corticosterone, a hormone known to reduce PLA_2 activity, significantly attenuated the cAMP potentiating action of 6-FNE in rat brain slices. Neither the lipoxygenase inhibitor nordihydroguaiaretic acid, nor a variety of cyclooxygenase inhibitors had any significant effect on the potentiating actions of 6-FNE and baclofen. The results suggest that α-adrenergic and $GABA_B$ receptors may influence neurotransmitter-stimulated cAMP formation by activating PLA_2 and enhancing fatty acid production. The negative results with the lipoxygenase and cyclooxygenase inhibitors suggests that fatty acid metabolites (e.g. prostaglandins and leukotrienes) may not be involved in this response. This implies that arachidonic acid itself may mediate the potentiating action of these substances on cAMP formation. (Supported in part by USPHS grants MH-35945 and MH-00501, and by grants from the National Science Foundation and Bristol-Myers, Inc.).

170.5 PHARMACOLOGICAL DIFFERENTIATION BETWEEN RECEPTORS COUPLED TO PHOSPHOINOSITIDE METABOLISM AND cAMP PRODUCTION IN BRAIN TISSUE. C. Chiappetta*, D.A. Kendall*, A. Pilc*, R.S. Duman* and S.J. Enna. (SPON: M.J. Schmidt). Depts. Pharmacol. and of Neurobiology and Anat., Univ. of Texas Med. School, Houston, Texas 77025, and Dept. Pharmacol., Univ. Leicester, Leicester, England.

Activation of α-adrenergic receptors by norepinephrine (NE) stimulates phospholipase C which catalizes the conversion of phosphoinositides to inositol phosphates (IP) and diacyglycerol. These latter agents serve as second messengers in a variety of tissues, including brain. Selective α-adrenergic receptor agonists such as 6-fluoronorepinephrine (6-FNE) increase IP formation and potentiate transmitter-stimulated cAMP production although they have little effect on cyclic nucleotide accumulation themselves. Experiments were undertaken to examine whether there may be a functional relationship between effects on phosphoinositide metabolism and cAMP formation in brain. cAMP and IP production were examined in rat brain cerebral cortical slices by prelabeling with either ^{3}H-adenine or ^{3}H-inositol. Concentration -response studies indicated that prazosin, a selective α_1-adrenergic antagonist, completely inhibited NE-stimulated IP formation with an EC_{50} of 30 nM. The α_2-adrenergic receptor antagonist yohimbine was ineffective up to a concentration of 1 μM. Conversely, a saturating concentration (10 μM) of prazosin inhibited only 25% of the α-adrenergic-potentiated cAMP formation in brain slices, whereas yohimbine completely abolished this response without influencing the β-adrenergic receptor component. Like 6-FNE, carbachol and serotonin enhance IP formation in this system but, unlike the α-agonist, neither potentiated the cAMP response to isoproterenol, adenosine or VIP. These results suggest that adrenergic receptors coupled to IP formation in rat brain cortex are prazosin-sensitive, whereas those associated with cAMP production are more sensitive to yohimbine. Furthermore, the lack of correspondence between carbachol and serotonin-stimulated IP production and cAMP formation indicates that either the receptors for these substances are not located on cerebral cortical cells possessing β-adrenergic, adenosine or VIP receptors, or that phosphoinositide metabolism is unrelated to cAMP production under these conditions. (Supported in part by USPHS grants MH-35945 and MH-00501).

170.6 EPINEPHRINE AND NOREPINEPHRINE SELECTIVELY ALTER RESPONSES TO EXCITATORY AMINO ACIDS IN THE FROG SPINAL CORD. C.J. Wohlberg, J.C. Hackman, and R.A. Davidoff. Depts. of Neurology and Pharmacology, Univ. of Miami Sch. of Medicine, and Neurophysiology Laboratory, V.A. Medical Center, Miami, Fl., 33101.

The reported ability of catecholamines (CAs) to modulate reflex transmission within the spinal cord may be caused by affecting neuronal responses to other transmitters. Since the excitatory amino acids (EAAs) L-glutamate (GLU) and L-aspartate (ASP) appear to mediate much of the excitatory synaptic activity in the cord, these compounds may be targets for the modulating actions of CAs. To explore this hypothesis, potentials were recorded from the ventral root (VR) of the in vitro, hemisected frog spinal cord utilizing sucrose gap techniques.

As expected, the bath application of either GLU or ASP (1 mM, 10-30 sec.) depolarized motoneurons (GLU: 6.3 +/- 0.1 mV; ASP 6.6 +/- 0.2 mV). Application of the selective EAA agonists N-methyl-D-aspartate (NMDA), kainate (KA) or quisqualate (QUIS) also resulted in large depolarizations of the membrane potential (NMDA, 100 uM, 8.5 +/- 0.05 mV; KA, 10 uM, 9.9 +/- 0.3 mV; QUIS, 10 uM, 7.9 +/- 0.7 mV; all 10 sec.). In contrast, the addition of NE or Epi (20 uM, 30 sec.) produced a dose dependent hyperpolarization of the membrane potential which was much slower in onset and longer in duration (NE, 0.66 +/- 0.1 mV; Epi, 0.79 +/- 0.1 mV). The difference in amplitude between NE and Epi was significant ($p<0.01$).

Exposure to Epi or NE prior to the application of the EAAs altered the amplitude of the EAA depolarization. Epi (20 uM, 30 sec.) diminished the amplitude of the depolarization resulting from the application of GLU, ASP, KA, and QUIS (25.5 +/- 3; 23.9 +/- 0.6; 17.2 +/- 3.2; 51 +/- 18.9 % reductions, respectively, $p<0.05$; GLU, 17 +/- 2.5; ASP, 7 +/- 0.5% reductions with 20 uM NE). On the other hand, prior addition of 20 uM Epi increased the area of the NMDA-induced depolarization (12.5 +/- 0.1% increase, $p<0.05$). This effect was also seen with the beta agonist isoproterenol (ISO, 10 - 100 uM, 30 sec., 24.6 +/- 0.1 % increase, $p<0.05$)

The use of selective adrenoceptor antagonists allowed us to study the effects of the CAs further. Yohimbine (10 uM,30 min.) or piperoxan (10 uM,30 min), both selective alpha$_2$-antagonists, blocked the Epi and NE induced diminution of the QUIS response, while propranolol, a beta-antagonist, blocked the potentiating effects of Epi and ISO on the NMDA response (10 uM, 30 min. PROP).

Thus, the application of Epi or NE to the cord prior to the EAAs GLU, ASP, KA or QUIS results in a decrease in the amplitude of the EAA-induced depolarization. This effect appears to operate through alpha$_2$-adrenoceptor activation. On the other hand, Epi and ISO potentiate the depolarizing effects of NMDA, apparently through activation of a beta receptor. Supported by NIH grants NS 17577, HL 07188T, and VA Medical Center Funds MRIS 1769 and MRIS 3369.

170.7 TRANSMITTER INTERACTIONS IN THE STRIATUM OF THE RAT STUDIED IN VIVO WITH PUSH-PULL CANNULA. M.T. Ciancone* and W.J. McBride (SPON: J.I. Nurnberger). Dept. Psychiatry, Inst. Psych. Res., Indiana Univ. Sch. Med., Indianapolis, IN 46223.

Studies were undertaken in our laboratory toward the development of a method for examining in vivo transmitter actions and interactions in the CNS of conscious, freely-moving rats. The push-pull cannula technique was employed to perfuse areas of the striatum both for adding test agents and for collecting perfusates for subsequent HPLC analysis of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

Adult male Wistar rats were used in this study. Under pentobarbital anesthesia, a guide cannula was implanted stereotaxically over the left caudate nucleus. After a one-to two-day recovery period, a vented push-pull cannula was introduced via the guide into the caudate. An adjustable feature of the cannula allowed variable depths to be reached, thus renewing the site perfused in successive experiments with the same animal. Krebs-Ringer medium, equilibrated with 95% O_2/5% CO_2, was circulated through the system at a flow rate of 20 μl/min using a peristaltic pump. Experiments began after an initial 20-minute washout period. First, two 20-minute fractions were collected for baseline values. Initial baseline values for DA were 10-50 fmol/min. Initial baseline values for DOPAC and HVA were 200-700 fmol/min. Throughout the course of an experiment (4 or more hours) the baseline release of DA usually did not increase but did sometimes decrease below detectable levels. On the other hand, DOPAC and HVA efflux had a tendency to increase with time, sometimes reaching values of 1000 fmol/min and higher. At these later times, the efflux of DOPAC was usually 200-400 fmol/min higher than the efflux of HVA. In order to provide evidence that the site being perfused was viable, and to mimic certain in vitro conditions, 35 mM K^+ was added to the perfusion media (with a proportionate decrease in Na^+ concentration). Elevated K^+ markedly stimulated DA release to 50-140 fmol/min without causing a similar action on DOPAC and HVA efflux. Within an experiment, the amount of DA released by 35 mM K^+ was variable but not necessarily decremental with successive stimulations. In 3 out of 4 cases, 100 μM carbachol appeared to enhance the 35 mM K^+-stimulated release of DA. However, in the normal perfusion medium, 100 μM carbachol did not stimulate DA release. GABA (either 100 μM or 1 mM) did not alter the 35 mM K^+-induced release of DA. The efflux of DOPAC and HVA was not changed by GABA or carbachol. The data indicate that a qualitatively reproducible release of DA can be obtained with 35 mM K^+, both within the same experiment and with the same animal on successive days. However, additional data are needed to establish that this technique is useful for studying transmitter interactions in vivo. (Supported in part by MH 17107).

170.8 EFFECTS OF LY163502, A HIGHLY SELECTIVE D_2-DOPAMINERGIC RECEPTOR AGONIST, ON ACETYLCHOLINE AND DOPAMINE RELEASE FROM SUPERFUSED RAT CAUDATE SLICES. B. D. Sawyer* and M. M. Foreman (SPON:M. D. Hynes). Dept. of CNS and Endocrine Research, The Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN. 46285.

Stimulation of D_2 dopaminergic receptors has been shown to inhibit the postsynaptic release of acetylcholine (ACh) and the presynaptic release of dopamine (DA) from caudate tissue. The only known compound that appears to be selective for these D_2 receptors is LY171555, trans-(-)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-2H-pyrazol<3,4-g>quinoline monohydrochloride. However, this compound is much less potent than the less selective ergoline type dopaminergic agonists, such as pergolide. LY163502, trans-(-)-5,5a,6,7,8,9,9a,10-octahydro-6-propylpyrimido<4,5-g>quinolin-2-amine dihydrochloride, is a new, highly selective D_2 type agonist.

We have evaluated the effects of LY163502 on the potassium-induced release of tritium from rat caudate slices prelabeled with either ^{3}H-choline or ^{3}H-DA. Anterior caudate fragments were sliced to 300 μM thickness and subsequently incubated with either 100 nM ^{3}H-choline or ^{3}H-DA for 30 min at 37° C. Single slices were placed in 100 μl custom-made superfusion chambers and superfused at a rate of 400 μl/min at 37° C. Basal release buffer contained 118 mM NaCl, 4.2 mM KCl, 25 mM $NaHCO_3$, 1.0 mM $NaHPO_4$, 1.2 mM $MgCl_2$, 1.3 mM $CaCl_2$ and 11.1 mM β-D-glucose and was adjusted to pH 7.4 by gassing at 37° C with 95/5 percent O_2/CO_2. The stimulus buffer was identical to the basal release buffer except for the NaCl and KCl concentrations which were 102.2 mM and 20.0 mM, respectively. Tissues were superfused for 45 min with basal release buffer and then exposed to stimulus buffer for 1 min. The tissues were exposed to basal release buffer with drug starting at t=65 min and exposed to stimulus buffer with drug for 1 min at t=95 min. Superfusion was terminated at t=125 min. The concentration of LY163502 that elicited approximately 50 percent of maximal suppression of ^{3}H-DA or ^{3}H-ACh release was 3×10^{-9} M. The potency of LY163502 was greater than that observed with LY171555 in other experiments. The effects of LY171555 and LY163502 were found to be stereoselective and were blocked by sulpiride, a D_2 antagonist.

170.9 TONIC INHIBITORY INFLUENCE OF HABENULO-INTERPEDUNCULAR PATHWAYS ON ASCENDING DOPAMINERGIC NEURONS IN THE RAT BRAIN. T. NISHIKAWA*, D. FAGE* and B. SCATTON. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), Bagneux 92220, France.

Habenular nuclei receive afferents from several dopamine (DA)-rich forebrain areas via the stria medullaris and have (direct or indirect) connections with DA cell bodies located in the ventral tegmental area and substantia nigra through the habenulo-interpeduncular pathways. These anatomical relationships suggest a potential role for the habenula in the feed-back control of the activity of ascending dopaminergic systems. To explore this possibility we have studied firstly the effect of an acute interruption of impulse traffic into the habenulo-interpeduncular pathways (by means of a local infusion of tetrodotoxin into the fasciculus retroflexus). Secondly, we studied the effect of local injections of cholinergic, opioid and substance P ergic drugs into the interpeduncular nucleus (IPN) on DA metabolism in several DA-rich brain areas in the rat because the transmitter systems modulated by these drugs are known to exist within the habenulo-interpeduncular tract.

The bilateral infusion of tetrodotoxin (50 ng) into the fasciculus retroflexus of conscious rats (via indwelling cannulae) caused a marked increase in homovanillic acid levels, DA synthesis and utilization in the medial prefrontal cortex, nucleus accumbens, olfactory tubercle and striatum. Similar changes in DA metabolism were observed in these areas after bilateral infusion of tetrodotoxin (25 ng) into the stria medullaris. Infusion of atropine (0.4-1 µg) into the IPN increased homovanillic acid concentrations and DA utilization in the medial prefrontal cortex and nucleus accumbens but not in the olfactory tubercle and striatum. Intra-IPN injection of oxotremorine (17 µg) antagonized the increase in DA utilization in the nucleus accumbens (but not in the olfactory tubercle) induced by an intra-fasciculus retroflexus infusion of tetrodotoxin. Local infusion of naloxone (20 µg) into the IPN increased homovanillic acid concentrations in the nucleus accumbens and olfactory tubercle but not in the medial prefrontal cortex and striatum. In contrast, intra-IPN infusion of the substance P antagonist D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11} or of substance P antiserum failed to alter homovanillic acid levels in the four DA-rich areas investigated. The present findings suggest that the habenulo-interpeduncular pathways exert a tonic inhibitory influence on meso-cortical, meso-limbic and meso-striatal dopaminergic neurons. Cholinergic and/or opioid peptidergic neurons coursing through the fasciculus retroflexus could take part in the inhibitory control of meso-cortico-limbic DA neurons.

170.10 ULTRASTRUCTURE AND SYNAPTIC INTERACTIONS OF ADRENERGIC AND GABA-ERGIC NEURONS IN THE RAT ROSTRAL VENTROLATERAL MEDULLA. T.A. Milner, J. Chan*, V.J. Massari[1], W.H. Oertel[2], D.H. Park, T.H. Joh, D.J. Reis and V.M. Pickel. Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021, Dept. of Pharm., Howard Univ. Med. Coll., Washington, DC[1], and Technische Universitaet, Muenchen, Germany[2].

We sought to determine: (1) the ultrastructure of adrenergic neurons and (2) the synaptic interactions between adrenergic neurons and between adrenergic neurons and GABA-ergic terminals in the rostral ventrolateral medulla of the adult rat. The adrenergic and GABA-ergic neurons were identified by immunocytochemical labeling of antisera against their respective synthesizing enzymes, phenylethanolamine-N-methyl transferase (PNMT) and L-glutamate decarboxylase (GAD). The antisera were single labeled by the peroxidase anti-peroxidase (PAP) technique and dual labeled by combined PAP and immunogold methods.

Selective immunoreactivity for PNMT was detected throughout the cytoplasm of neuronal perikarya, dendrites, myelinated and unmyelinated axons and axon terminals. The perikarya were large (18-24µm), had a prominent nucleolus and a slightly infolded nuclear membrane. The most notable cytoplasmic organelles included numerous mitochondria, ribosomes and rough endoplasmic reticulum. The dendrites (0.8-2.0µm) received symmetric and asymmetric synapses from both PNMT-labeled and unlabeled terminals. The PNMT-labeled terminals (0.4-1.2µm) contained many small clear and a few dense core vesicles. Immunoreactivity for GAD was exclusively localized in axon terminals (0.4-2.0µm). The GAD labeled terminals formed primarily symmetric synapses with dendrites containing immunoreactivity for PNMT and with other unlabeled dendrites. We conclude that the adrenergic neurons have morphological features indicative of high metabolic activity and receive afferents from both adrenergic and GABA-ergic neurons. (Supported by NIH grants HL18974, MH0078, and fellowship NS 07340.)

170.11 SEROTONIN-BINDING PEPTIDES BIND MURAMYL DIPEPTIDE. F.C. Westall and R.S. Root-Bernstein*. Institute for Disease Research, Concord, CA 94518; Neurobiochemistry, V.A. Hospital, Brentwood, CA 90073.

Luteinizing hormone-releasing hormone (LHRH), adrenocorticotropic hormone (ACTH), melanocyte stimulating hormone (MSH), and the tryptophan peptide of myelin basic protein (trp peptide) contain structurally similar serotonin binding sites (Root-Bernstein, J. Theor. Biol. 100:373, 1983; Root-Bernstein, FEBS Lett. 168:208, 1984; Root-Bernstein and Westall, Brain Res. Bull. 12:425, 1984). The adjuvant peptide muramyl dipeptide has been found to have serotonin-like properties (Krueger, et al. PNAS 79: 6102, 1982; Masek and Kadlecova, Lancet 1:1277, 1983) and is known to cause experimental allergic encephalomyelitis (EAE) when injected with trp peptide (Nagai, et al. Cell. Immunol. 35:158, 1978) and experimental autoimmune castration when injected with LHRH (Carelli, et al. PNAS 79:5392, 1982). These observations led to the hypothesis that muramyl dipeptide might bind to serotonin binding sites (Westall and Root-Bernstein, Mol. Immunol. 20:169, 1983; Root-Bernstein and Westall, Lancet 1:653, 1983). We report here evidence from nuclear magnetic resonance spectroscopy and other physico-chemical methods demonstrating that muramyl dipeptide binds to the aforementioned serotonin-binding peptides. Muramyl dipeptide binds to LHRH, but not to LHRH 1-3 or 4-10 (indicating that the main binding site is located in the 2-5 region); and it binds to MSH-ACTH 4-10 but not ACTH 1-4. These data suggest that the binding site is composed of a "molecular sandwich" comprised of a Trp paired with a Phe or Tyr and accompanied by Ser or Glu and Arg. We also report data concerning the binding of various muramyl dipeptide analogues and control peptides, which demonstrate that the binding is relatively specific. In addition, we report results of experiments suggesting that muramyl dipeptide, but not control peptides, interferes with ACTH-induced release of corticosteroids from cultured adrenals. These results may be of importance to understanding both the mechanism of induction of autoimmune disease and some of the symptoms caused by bacterial infections, such as drowsiness. Perhaps most importantly, these results suggest a mechanism for the induction of post-vaccinal encephalopathies. It has long been known that vaccines should not be administered if the patient has a fever; perhaps the vaccines interact with bacterial breakdown products (such as muramyl dipeptide) to produce an autoimmune reaction similar to that produced by adjuvants with LHRH and trp peptide.

170.12 OPIATE PEPTIDES BIND CATECHOLAMINES. R.S. Root-Bernstein* and F.C. Westall (SPON: A. Yuwiler). Neurobiochemistry, V.A. Hospital, Brentwood, CA 90073; Institute for Disease Research, Concord, CA 94518.

Opiate actions appear to involve the catecholamines dopamine (DA) and norepinephrine (NE) (reviewed in Oliverio, et al., Int. Rev. Neurobiology 25: 276, 1984) and the endogenous opiate met-enkephalin is often co-stored and co-released with these amines (reviewed in Costa and Trobucchi, eds., Neural Peptides and Neural Communication, 1980). Based upon earlier research demonstrating that amines may bind specifically to peptides (Root-Bernstein and Westall, Brain Res. Bull. 12: 425, 1984) and to aromatic drugs (Root-Bernstein and Westall, Brain Res. Bull. 12:17, 1984), we hypothesized that both endogenous and exogenous opiates might specifically bind DA and NE. We recently presented evidence that morphine (but not naloxone, desipramine, pargyline, fenfluramine, etc.) binds NE and DA, but not serotonin, histamine, tyrosine, etc. (Root-Bernstein and Westall, Neurology 35 (Suppl. 1): 311, 1985). We report here evidence from nuclear magnetic resonance spectroscopy, colligative properties, pH titration studies and chromatography demonstrating that met-enkephalin, leu-enkephalin, and morphiceptin (Tyr-Pro-Phe-Pro-NH2) specifically bind NE and DA, but do not significantly bind serotonin, histamine, acetylcholine, tyrosine, etc. Control peptides, including enkephalin fragments and a number of serotonin binding peptides including LHRH and MSH and the tryptophan peptide of myelin basic protein, did not significantly bind NE or DA. Gastrin tetrapeptide (Trp-Met-Asp-Phe-NH2), however, binds DA, which may be significant since the same sequence occurs in the DA cotransmitter cholecystokinin (Voigt and Wang, Brain Res. 296: 189, 1984). Met-enkephalin and morphiceptin also bind naloxone. The dissociation constant, K, for the binding of both NE and naloxone to met-enkephalin is c. 10-4 per mole. These data suggest that endogenous opiates may act, at least in part, by binding up NE and DA in vivo; and that naloxone, which was found not to bind either DA or NE, may antagonize the opiates by directly binding to the opiate thereby preventing opiate binding to neurotransmitters. Model building also suggests that the opiate-catecholamine complexes may have significantly higher affinity for lipid membranes than either molecule alone. Like other anaesthetics (Franks and Lieb Nature 300: 487. 1982), the opiates may act by incorporating in a DA or NE-dependent manner into the lipid membranes of neurons, interfering with receptor conformations. We also report data from experiments on chronically cultured rat pineals testing for in vitro interactions of the peptide opiates with exogenously administered NE.

170.13 THE EFFECT OF ALTERATIONS IN CENTRAL DOPAMINERGIC ACTIVITY ON TRH CONTENT IN RAT BRAIN. T.M. Engber, M.S. Kreider, D. Lurie*, M. Zweben* and A. Winokur. Depts. of Pharmacology and Psychiatry, Univ. of PA, Philadelphia, PA 19104

Intraventricular administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) to rats results in increases in the TRH content of olfactory bulb, hippocampus and amygdala. Pretreatment with bupropion to prevent destruction of DA terminals abolishes this increase in TRH. However, pretreatment with the NE uptake blocker DMI does not alter the effect of 6-OHDA on the TRH content of these regions. In order to further study the relationship between DA and TRH, the effects of various pharmacologic manipulations of DA systems on regional TRH content were examined.

Male S-D rats received i.p. injections of one of three drugs. Alpha-methyl-para-tyrosine (AMPT), an inhibitor of catecholamine synthesis, was given at a dose of 250 mg/kg once and then 100 mg/kg every 3 hrs; animals were sacrificed either 2 or 24 hrs after the initial injection. Bupropion, a DA uptake blocker, was given at a dose of 50 mg/kg, and animals were sacrificed 2, 24, or 72 hrs. after injection. Apomorphine, a DA receptor agonist, was given at a dose of 15 mg/kg, and animals were sacrificed 2, 24, or 72 hrs. after injection. Control animals received injections of PBS vehicle (The apomorphine vehicle also contained 0.4 mg/ml ascorbate). Following sacrifice, brains were removed and dissected on ice into 10 regions. The TRH content of each region was determined by radioimmunoassay.

The TRH content of all ten brain regions examined was unaffected by AMPT, bupropion, or apomorphine treatment at any of the time points studied. These results suggest that TRH content is not influenced by changes in DA activity alone. The effect of 6-OHDA must be due to loss of DA nerve terminals, not to loss of DA itself. It is possible that a cotransmitter or some other factor contained in DA nerve terminals is responsible for changes in TRH content.

170.14 IVT 6-HYDROXYDOPAMINE ENHANCES TRH IMMUNOREACTIVITY IN RAT DORSOMEDIAL HYPOTHALAMUS. M.S. Kreider, T.M. Engber, K. Cody* and A. Winokur. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

Administration of the catecholaminergic neurotoxin 6-hydroxydopamine has been shown to result in substantial elevations of the TRH content of several regions of the rat CNS. TRH content of regions of the limbic system were particularly influenced by this treatment; however, hypothalamic TRH appeared to be unaffected. Since the distribution of TRH within the various nuclei of the hypothalamus is not uniform and may be regulated by different factors, it was of interest to determine if 6-hydroxydopamine altered TRH localization in individual nuclei of the hypothalamus.

Male Sprague-Dawley rats received IVT injections of 6-hydroxydopamine hydrobromide, 400 ug in 10 ul of 0.9% NaCl containing 0.2 mg/ml ascorbic acid, or vehicle. Three days following 6-hydroxydopamine administration, rats were anesthetized with chloral hydrate, 400 mg/kg, and perfused intracardially with heparinized saline followed by lysine-periodate fixative. Brains were removed and coronal sections of 80 um through the entire hypothalamic region were cut on a cryostat. Sections were incubated for 48 hours at 4 C with a specific antiserum to TRH at a dilution of 1:500 and further processed for immunohistochemistry by the avidin-biotin immunoperoxidase method.

Exposure to 6-hydroxydopamine resulted in a striking increase in the intensity of the immunoperoxidase reaction in TRH containing fibers of the dorsomedial nucleus of the hypothalamus. The density of the TRH positive fibers in the dorsomedial nucleus appeared unchanged. Other nuclei of the hypothalamus known to contain high concentrations of TRH did not exhibit dramatic changes in the density or intensity of TRH staining.

These findings indicate that TRH in the dorsomedial nucleus can be regulated independently of the other TRH containing nuclei of the hypothalamus and may indicate different functions for TRH within the hypothalamus. These findings support the hypothesis that catecholamines play a role in the regulation of TRH systems in brain.

170.15 VASOACTIVE INTESTINAL PEPTIDE EFFECTS ON SEROTONIN TURNOVER IN THE HIPPOCAMPUS. J. Stuckey*, D. Allen* and B. McEwen. (SPON: D. Micco). Lab. of Neuroendocrinology, The Rockefeller University, New York, NY 10021.

Vasoactive intestinal peptide (VIP) is located throughout the brain, including the hippocampus. VIP has been shown to have several biological effects in the hippocampus. One of these is the apparent increase in the binding of serotonin to 5-HT1 receptors in homogenates of hippocampus after pre-incubation with VIP (Rostene et al., 1983). These results suggest that VIP can influence serotonergic neurotransmission in the hippocampus. To further investigate this possibility, we examined the effect of lateral ventricular injection of VIP on hippocampal serotonin (5-HT) turnover.

Male Sprague Dawley rats (275-325g) were anesthetized with Urethane. Twenty three gauge guide cannulas were lowered stereotaxically into each lateral ventricle. The rats then received an intraperitoneal injection of pargyline (75 mg/Kg) and bilateral lateral ventricular injections of 0.15N saline as vehicle control or VIP dissolved in 0.15N saline (75 ng/injection for a total dose of 150 ng). The injection volume was 0.5 ul. The rats were sacrificed immediately, 15 min. or 30 min after injection. The brains were removed and frozen on dry ice and later sliced into 300 um thick sections. Punches were taken from hippocampal areas and the serotonin levels were measured using HPLC.

VIP increased serotonin content in the dorsal subiculum without affecting serotonin content in any other hippocampal area.

Time	0	15	30
5-HT(pg/ug protein)			
Control	3.1±0.5	5.6±1.1	9.8±1.2
VIP	3.7±0.6	8.7±0.5	11.9±0.8

The VIP group differed significantly from the control group by analysis of variance [$F(1,30)=6.59, p<0.05$].

The increase in serotonin content after VIP injection suggests an increase in serotonergic turnover. It is of interest that the effect appears localized to the dorsal subiculum, since that is the area where the effect of VIP on 5-HT binding in the hippocampus is localized (Rostene et al., 1983). The present results provide further evidence that VIP may modulate serotonergic neurotransmission in the hippocampus.

171.1 ADENOSINE AFFECTS SYMPATHETIC NEUROTRANSMISSION AT MULTIPLE SITES IN VIVO. G.E.Evoniuk*, R.W.von Borstel*, and R.J.Wurtman. Lab. of Neuroendocrine Regulation, Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge, MA

We examined the effects of adenosine and its analogs on sympathomimetic responses of pithed rats to electrical stimulation of preganglionic nerves (ES) or to injections of nicotine, phenylephrine (PE), or isoproterenol (ISO). Four physiological indices of sympathetic neurotransmission were measured: blood pressure, heart rate, and contractions of smooth muscle in vas deferens and eyelid. Intravenous infusion of sufficient adenosine to elevate arterial levels from 1.5μM to 2-3μM caused a 2 to 3-fold potentiation of nicotine-induced increases in blood pressure, heart rate, and smooth muscle tension (in both vas deferens and eyelid). Higher adenosine concentrations (3-4μM) produced a smaller potentiation of nicotine's effects. At 2-3μM, adenosine had no effect on sympathomimetic responses to ES or PE. Higher concentrations (3-4μM) attenuated pressor responses to ES and PE and the contractile response of the vas deferens to ES; these levels also potentiated positive chronotropic responses to ISO. The adenosine analogs N-cyclopropylcarboxamidoadenosine (N-CPCA), 2-chloroadenosine (2-CLA) and L- and D-phenylisopropyladenosine (L-PIA, D-PIA) also reduced pressor responses to both ES and PE, exhibiting the potency order N-CPCA > L-PIA > 2-CLA > D-PIA. These analogs exhibited this same potency series in attenuating contractile responses to ES in the vas deferens. However, all four analogs potentiated, at the lower doses tested, the contractile responses of the vas deferens to PE; at higher doses, inhibition predominated. N-CPCA enhanced the chronotropic effects of ISO and ES, while 2-CLA, L-PIA and DPIA decreased heart rate responses to ES. The nonpurinergic vasodilator sodium nitroprusside had no significant effect on the sympathomimetic responses tested, indicating that non-specific vasodilatory actions were not responsible for adenosine's effects. These observations show that circulating adenosine, at levels within or near the physiological range, or synthetic adenosine analogs, can influence sympathetic transmission in vivo by interacting with at least three sites: sympathetic ganglia (where they amplify responses to nicotine), sympathetic nerve terminals (where they reduce evoked release of norepinephrine) and sympathetically-innervated end organs (where they may either reduce or enhance the influence of adrenoceptor agonists, depending on the particular tissue and probably its adenosine receptor subtypes). Adenosine's net effect in vivo represents a complex summation of its multiple actions at anatomically and pharmacologically distinct sites within the efferent sympathetic nervous system.

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171.2 AUTORADIOGRAPHIC EXAMINATION OF ADENOSINE A-2 RECEPTORS IN RAT BRAIN USING [3H]-5'-ETHYL-CARBOXAMIDO ADENOSINE(NECA) E.W.Snowhill* and M.Williams.(SPON:D.J.Pettibone)Neuroscience/Cardiovascular Research, Pharmaceuticals Division,CIBA-GEIGY Corp.,Summit,NJ 07901

Adenosine receptors in mammalian tissues can be classified into A-1 and A-2 subtypes depending on the ability of agonists to inhibit and stimulate adenylate cyclase, respectively. Many adenosine and arylxanthine derivatives exhibit high selectivity for A-1 sites, such as cyclohexyladenosine(CHA),cyclopentyladenosine(CPA) and 1,3-diethyl-8-phenylxanthine(DPX).There are currently no known compounds which can be characterized as purely A-2 selective. This has previously prevented regional localization studies for the A-2 receptor. It was recently shown that the non-selective ligand [3H]-NECA,which has approximately equal affinity for both receptor subtypes in homogenates, when incubated in the presence of a concentration of unlabeled CPA which selectively blocks A-1 receptors, can be used to label A-2 receptors(Bruns et al.(1984)Soc.Neurosci.Abstr.10:957) In the present study, the regional binding characteristics of [3H]-NECA were examined in 20 micron thick sections of rat brain. Non-specific binding was determined in adjacent sections incubated in the presence of 10 uM 2-chloroadenosine(2-CADO) and represented less than 10% of total binding. Specific binding reached equilibrium at room temperature in 120 minutes and was reversible by dilution or by addition of excess 2-CADO. "A-2 specific" binding was determined by incubation of sections with [3H]-NECA and 50nM CPA. Autoradiograms of total, non-specific, and "A-2 specific" binding were generated by apposition of radiolabeled slides to tritium-sensitive film (Ultrofilm;LKB, Bromma) for 3-4 weeks. [3H]-NECA exhibited a heterogeneous distribution throughout the brain. Caudate-putamen showed the greatest density of binding, with quite high amounts in the zonal layer of superior colliculus and the molecular layers of hippocampus and cerebellar cortex. A moderate degree of binding was also apparent in cerebral cortex(minimal laminar distribution), granular layer of cerebellar cortex, and superficial grey layer of superior colliculus. Autoradiograms co-incubated with CPA to selectively displace A-1 binding showed a dramatic decrease in binding (to slightly above non-specific density) in all areas of the brain examined except caudate-putamen. This area retained a density of binding nearly equal to total binding (in the absence of CPA). This is the first quantitative visualization of adenosine A-2 sites in the brain, and emphasizes the importance of autoradiography to the study of receptor function. Studies are ongoing to evaluate the significance of this information on regional adenosine receptor subtype localization and its relevance in regard to physiological function and receptor pharmacology.

171.3 (^{3}H)-N^6-CYCLOPENTYLADENOSINE (CPA), A NEW LIGAND FOR ADENOSINE A-1 RECEPTORS. Michael Williams, Albert Braunwalder* and Thomas J. Erickson*#. Neuroscience Research, Research Dept.,Pharmaceuticals Div.,CIBA-GEIGY Corporation, Summit, NJ 07901 and # DuPont NEN, Boston, MA 02180.

CPA is an adenosine agonist with twice the selectivity of the prototypic ligand, N^6-cyclohexyladenosine (CHA) for adenosine A-1 receptors. In terms of in vitro binding activity, CPA is 700 times more selective for the A-1, as opposed to the A-2, receptor (Bruns et al., Soc.Neurosci.Abstr. 10 (1984) 282.2). High specific radioactivity CPA (46 Ci/mmole) binds with high affinity (Kd = 0.48 nM) to a single site in adenosine deaminase pretreated rat brain membranes. Specific binding was reversible, destroyed by boiling and, at a ligand concentration of 1 nM, represented 90-95% of the total counts bound. Pharmacologically, the specific binding of CPA was consistent with the labeling of an A-1 receptor. CPA, CHA and the R-diastereomer of N^6-phenylisopropyladenosine (PIA) were more active than the non-selective agonist, 5'N-ethylcarboxamidoadenosine (NECA) while the S-enantiomer of PIA was some sixteen times less active than R-PIA. In comparing the binding of (^{3}H)-CPA with that of (^{3}H)-CHA in identical membrane preparations, some discrete differences were observed. R- and S-PIA and the xanthine adenosine antagonist, PACPX (1,3-dipropyl-8-(4-amino-2-chloro)-phenylxanthine were more active in displacing 1 nM CHA than they were in displacing 1 nM CPA while the Kd for CHA was twice that of CPA. In addition, the apparent Bmax for the hexyl analog was some 60% greater than that observed for the pentyl analog. These findings provide further evidence for the possible existence of subclasses of the A-1 receptor and indicate that the increased selectivity of CPA for the A-1 receptor may be useful for the further delineation of such subclasses in vitro.

171.4 EFFECT OF CO-LOCALIZED PEPTIDES ON OXYTOCIN AND VASOPRESSIN RELEASE, IN VITRO. J.A. Schriefer* (Spon. F. Gonzalez-Lima), Dept. of Pharmacol., Ponce School of Medicine, Ponce, P.R. 00732

Recent evidence suggests that a number of other peptides are located in the oxytocin (OT) and vasopressin (VP) containing neurons of the hypothalamo-neurohypophysial system. Such peptides may modulate release of OT and VP from the neurohypophysis. The effects of methionine enkephalin and cholecystokinin, reported to exist in OT neurons, and leucine enkephalin, found in VP neurons, on OT and VP release were examined using the isolated hypothalmo-neurohypophysial preparation (HNS) of the rat.

Adult male rats were decapitated, the HNS removed as a tissue block and incubated under oxygen in a modified Krebs buffer at 37°C in the presence or absence of either D-Ala^2-leucine- (LENK) or D-Ala^2-methionine-enkephalinamide (MENK) or cholecystokinin octapeptide (10^{-10} - 10^{-6} M). OT and VP released into the media during 10 min incubation periods were quantitiated by specific radioimmunoassays. MENK (10^{-6} M) produced a significant decrease in the amount of both OT and VP released into the incubation media. However, LENK (10^{-8} - 10^{-6} M) produced a significant increase in the amount of VP released. OT release was not significantly altered by LENK. Cholecystokinin had no significant effect on OT or VP release at the concentrations tested.

These studies demonstrate that while MENK (present in OT neurons) inhibited both OT and VP release from the isolated HNS, LENK (present in VP neurons) stimulated VP release. This evidence suggests that the various co-localized peptides may have differential effects on neurohypophysial hormone release. Supported by a PMA Foundation Research Starter Grant.

171.5 ADENOSINE INHIBITS SPONTANEOUS FIRING OF RAT LOCUS COERULEUS NEURONS RECORDED INTRACELLULARLY IN BRAIN SLICES. S. A. Shefner and T. H. Chiu*. Dept. of Physiol. and Biophys., Univ. of Illinois at Chicago, Health Sciences Ctr., Chicago, IL 60680

Intracellular recording was used to study the effect of adenosine on rat locus coeruleus (LC) neurons, in a totally submerged brain slice preparation. Known concentrations of adenosine were applied in the bath by switching between control artificial cerebrospinal fluid (aCSF) and aCSF containing adenosine, by means of a valve system. LC neurons in the brain slice preparation show spontaneous firing in the absence of synaptic input; neurons in this study had firing rates ranging from 0.3 - 5 Hz. Concentrations of adenosine from 10 to 200 μM were tested. Adenosine reversibly decreased the spontaneous firing rate in 16 of 17 LC neurons tested. In those cells tested with more than one concentration, the effect was dose-dependent (n=4). Adenosine (100 μM) caused complete block of firing in 80% of cells tested (n=10); firing resumed upon washout, returning to the control rate.

In addition to the inhibition of spontaneous firing, adenosine (100 μM) also caused small hyperpolarizations (2-10 mV) in 10 of 11 cells. The size of the hyperpolarization appeared to be related to the resting membrane potential of the neurons (correlation coefficient = -0.75, n=10, sig. $p<.05$); those cells having the most negative resting potentials showed little or no hyperpolarization with adenosine. When input resistance was measured by passing constant current hyperpolarizing pulses across the cell membrane (n=3) only small reductions in input resistance (7-15%) were noted during the peak of the hyperpolarizing response.

The adenosine receptor antagonist theophylline (200-300 μM) was tested in 7 cells. In most cases theophylline alone increased the basal firing rate somewhat. Theophylline antagonized the adenosine-induced inhibition of firing, hyperpolarization and reduction in input resistance. The degree of antagonism varied from partial to complete, and depended on the respective concentrations of adenosine and theophylline used.

We conclude that adenosine has inhibitory actions when applied to LC neurons in the brain slice preparation. This inhibition may be due to a direct postsynaptic effect. Spontaneous firing of LC neurons in the slice does not seem to depend on synaptic input, therefore, it is unlikely that the adenosine-induced inhibition of this firing is due to inhibition of transmitter release. Adenosine's inhibitory effects on LC neurons appear to be mediated by adenosine receptors since they are antagonized by theophylline.

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171.6 CHARACTERIZATION OF ADENOSINE RECEPTORS IN THE RAT SPINAL CORD. J. I. Choca, H. K. Proudfit, and R. D. Green*. Dept. of Pharmacology, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612.

Adenosine mediates the activity of adenylate cyclase (AC) via two distinct receptors. The adenosine A_1 receptor mediates the inhibition of AC and displays nanomolar affinities for adenosine, whereas the A_2 receptor mediates stimulation of AC and displays micromolar affinities for adenosine. Adenosine A_1 sites have been characterized in both the ventral and dorsal halves of the rat spinal cord using N^6-[^{3}H]-cyclohexyladenosine ([^{3}H]-CHA), a specific ligand for A_1 receptors (Geiger et. al. J. Neurosci. 4: 2303, 1984). The present studies compare the binding characteristics of [3H]-N-ethylcarboxamide adenosine ([^{3}H]-NECA), an agonist with near equal potencies at A1 and at A2 sites, with those of [3H]-N^6-(L-phenylisopropyl)adenosine ([^{3}H]-L-PIA), an agonist with greater potency at the A_1 site than at the A_2 site (Yeung and Green, Naunyn-Schmiedeberg's Arch. Pharmacol. 325: 218, 1984).

Lysed crude mitochondrial fractions of the ventral and dorsal halves of the cervical and lumbar enlargements were utilized to study the binding characteristics of [^{3}H]-NECA and [^{3}H]-L-PIA. In the preliminary saturation studies [^{3}H]-NECA specifically bound more sites than [^{3}H]-L-PIA (VENTRAL: NECA = 251 + 18; L-PIA = 158 + 17 fmol/mg protein; DORSAL: NECA = 322 + 5; L-PIA = 241 + 11 fmol/mg protein). N^6-cyclopentyladenosine (CPA), which demonstrates a 350-fold greater potency at A_1 sites than at A_2 sites (Bruns et. al., Neurosci. Abstr. 10: 957, 1984), biphasically inhibited [^{3}H]-NECA binding with low Hill coefficients (n_H) and high IC_{50}'s in both ventral and dorsal halves (VENTRAL: n_H = 0.47 + 0.10; IC_{50} = 268 + 149 nM; DORSAL: n_H = 0.49 + 0.04; IC_{50} = 156 + 59 nM). In contrast CPA inhibition of [^{3}H]-L-PIA binding was statistically different with lower IC_{50}'s and Hill coefficients closer to 1 (VENTRAL: n_H = 0.84 + 0.07; IC_{50} = 4.97 + 0.15 nM; DORSAL: n_H = 0.79 + 0.04; IC_{50} = 4.23 + 0.13 nM). These saturation and inhibition data suggest the existence of A_1 and A_2 receptors in the rat spinal cord. Ultimate verification of this hypothesis will require the demonstration of adenylate cyclase inhibition and stimulation in the spinal cord, mediated by adenosine analogs. (Supported by PHS grant NS18636.)

171.7 EFFECTS OF SEROTONERGIC ACTING BETA-CARBOLINES UPON HIPPOCAMPAL DENTATE RESPONSES IN UNANESTHETIZED RATS. N.J.Leidenheimer*, J.W.Boja*, L.J.Cauller*, and E.Foldvary* (SPON: S.L.Stuesse), Pharmacology Program, NE Ohio Univ. Col. of Med., Rootstown, OH 44272.

1,2,3,4-tetrahydro-beta-Carbolines (THBC) exist endogenously (Barker et al., Biochem.Pharm.,30:9 1981) and have been shown to inhibit 5-HT reuptake and MAO type A activity (Buckholtz, N-S's Arch.Pharm.,314:215 1980). In addition, the substituted form, 6-MeO-THBC, also binds with high affinity to the 5-HT_1 receptor (Ho, et al., Can.J.Biochem.,51:482 1973; Taylor, et al., J.Pharm.Pharmacol.,36:125 1984).

The hippocampal dentate area receives heavy 5-HT input from midbrain raphe (Moore and Halaris, J.Comp.Neur.,164:171 1975). Stimulation of this 5-HT pathway facilitates the dentate responsiveness to stimulation of its primary afferents, the perforant path (PP), which are thought to be glutaminergic. This study examines the modulatory effects of THBC upon this serotonin-sensitive system.

Rats were permanently implanted with electrodes to stimulate the angular bundle of the PP and record the evoked field potential in the hilus of the dentate gyrus. This preparation provides stable recordings of the dentate response in freely-moving and sleeping rats.

THBC (20mg/kg IP) caused substantial changes in both the population excitatory postsynaptic potential (pEPSP) and population action potential (spike) components of the monosynaptic dentate response. The pEPSP was initially elevated and then fell to sub-baseline levels 1 hr after injection. The spike rose to 150% of the baseline level within 40 min and remained elevated. Both pEPSP and spike returned to baseline level approximately 3 hrs after injection. Dentate excitability was determined by plotting the spike output as a function of pEPSP input. Despite the biphasic nature of the changes in pEPSP, dentate excitability rose monophasically to a maximum 1 hr, and returned to baseline 3 hrs after injection.

This THBC-induced modulation of dentate excitability resembles the facilatory effect of stimulation of the serotonin system. These effects are consistent with a disinhibitory role of serotonin in the dentate where serotonin inhibits the activity of inhibitory interneurons.

171.8 **AGE-RELATED LOSS OF THE CAPACITY OF MUSCARINIC AGONISTS TO INHIBIT THE ACTIVATION OF ADENYLATE CYCLASE IN STRIATUM.** **J. Coupet, C.E. Rauh* and J.A. Joseph. Dept. of CNS Research, Lederle Labs, Pearl River, NY 10965.**

Previous experiments have demonstrated that muscarinic acetylcholine receptor (mAChr) agonists such as oxotremorine (OXO) and carbamylcholine (carbachol) inhibit dopamine (DA) stimulated adenylate cyclase (AC) in rat neostriatal preparations (Olianas et al., Mol. Pharmacol. 23, 393, 1983). Since diminution of striatal functioning seems to be a consistent finding in aged animals, resulting in such changes as declines in psychomotor performance, we sought to determine in the present experiment if the ability of these agonists to inhibit DA stimulated AC would be altered as a function of age. Two concentrations of oxotremorine (50 and 100 μM) and carbachol (0.5 and 1.0 mM) were used to inhibit AC activated by 30 μM DA in neostriatal broken cell preparations from young (3-6 mo) middle aged (13-15 mo) and old (22-26 mo) rats. Results showed that along with an age related decline in the activation of AC by dopamine, oxotremorine and carbachol inhibited DA-stimulated AC to the same extent in young (e.g., oxo at 100 μM inhibited DA stimulated AC by 30 + 0.2%) and middle aged (30 + 0.1%) but not old (9.8 + 0.2%) animals. These agonists had no effect on basal AC activity in any age group. The presence of atropine (10 μM) antagonized the effects of both agonists. For example, atropine reduced the degree of AC inhibition by oxotremorine to 4 + 0.1% in young animals and 3 + .1% in middle aged animals. These findings suggest that there may be specific age-related functional declines in AChr such that they are less responsive to agonist stimulation. It may also be that in the senescent animal mAChr agonists impinge upon uncoupled receptors and subsequent physiological control over other receptor systems (e.g. the striatal D_1 cyclase linked system) is be blunted.

171.9 DO OPIOID PEPTIDES MODULATE TRANSMITTER RELEASE IN THE CAT SUPERIOR CERVICAL GANGLION? D.M. Araujo* and B. Collier (SPON: R. Capek). Dept. Pharmacol. & Ther., McGill University, McIntyre Medical Bldg., Montreal, Quebec, Canada H3G 1Y6.

It has been suggested that enkephalins might have a functional role to modulate transmitter release at a variety of synapses. The present experiments sought to test this idea for acetylcholine (ACh) release in the cat superior cervical ganglion. Measures of ganglionic extracts by radioimmunoassay (RIA) with an antibody to [Met^5]-enkephalin (ME) showed the presence of immunoreactive material, but by HPLC this was separated into two main peaks. Ganglia contained 2209 ± 513 pmol (n = 5) of enkephalin-like immunoreactivity, of which 57.5 ± 11.6% migrated on HPLC as ME and 37.7 ± 13.4% as [Met^5]-enkephalin-Arg^6-Phe^7, the carboxyl terminal heptapeptide of the large preproenkephalin A.

To test whether these peptides efflux from ganglia, tissue was perfused and the effluents collected for RIA. The efflux of immunoreactive material was 700 ± 273 pmol/min (n = 3), of which 75.8 ± 10.1% was ME by HPLC and 24.2 ± 10.3% was MEAP. The addition of thiorphan, an "enkephalinase" inhibitor, to the perfusion medium increased measured efflux of enkephalin by 2.6-fold and MEAP by 1.5-fold.

To determine whether these peptides might modulate transmitter release, ganglia were perfused with medium containing physostigmine. ACh release was evoked by preganglionic nerve stimulation, and the effects of the stable ME analogue D-Ala^2-[Met^5]enkephalin amide (DAEA) or MEAP were tested. The enkephalin analogue, DAEA, did not affect ACh release when presented at 10^{-4}M, but MEAP reduced release in a dose-dependent way. The concentration of MEAP required to reduce ACh release to 50% of control was 8.9 x 10^{-8}M (n = 4).

It is concluded that MEAP may have a modulatory role on cholinergic transmission in the superior cervical ganglion and that this role is unrelated to its ability to serve as a precursor for ME. (Supported by MRC and FCAC, Canada.)

171.10 DIFFERENTIAL EFFECTS OF INTRACRANIAL INJECTIONS OF ADENOSINE ANALOGS ON KINDLED SEIZURES IN RATS. J.B. Rosen, L. Ellison*, and R.F. Berman. Dept. of Psychology, Wayne State University, Detroit, MI 48202.

Adenosine depresses neuronal firing and has both sedative and anticonvulsive properties. We have previously demonstrated that the adenosine agonists L-phenylisopropyladenosine (L-PIA) and N-ethylcarboxamido-adenosine (NECA) inhibit amygdaloid-kindled seizures when injected into the cerebral ventricles of rats. The present study extended these findings by (1) examining the effects of focal injections of adenosine agonists on hippocampal, caudate, and amygdaloid kindled seizures, and (2) comparing the anticonvulsive effects of two adenosine analogs, L-PIA and NECA, which show different receptor subtype specificity.

Chemitrodes were stereotaxically implanted in either the amygdala, hippocampus, or caudate nucleus of male Long-Evans rats. Upon recovery, rats were electrically stimulated via the chemitrode once daily with a current just sufficient to elicit an afterdischarge. Once rats were fully kindled (i.e., 3 consecutive generalized seizures), L-PIA or NECA (0.1 to 5.0 ug/0.5 ul) were injected via the chemitrode 5 min before electrical stimulation and the effects of these agents on amygdala, hippocampus, or caudate kindled seizures were determined.

The results demonstrated that adenosine analogs are potent anticonvulsants when injected intracranially into several kindled seizure foci. When injected into the amygdala, L-PIA and NECA were partially effective in blocking afterdischarges and behavioral seizures. When injected into the hippocampus or caudate nucleus, L-PIA displayed a biphasic effect. Low doses of L-PIA (0.2-0.5 ug) blocked seizures, intermediate doses (1.0-2.0 ug) were ineffective, and higher doses (5.0 ug) again blocked seizures. In contrast, low doses of NECA did not inhibit seizures while higher doses did.

The anticonvulsive effects of L-PIA and NECA suggest that adenosine receptors at the focus of kindled seizures can modulate both focal afterdischarge and behavioral seizures. This effect is more prominent in brain regions with high levels of adenosine receptors (i.e., hippocampus and caudate nucleus) than in a region with low levels (i.e., amygdala). In addition, the differences in the effects of L-PIA and NECA agree with other findings which suggest that L-PIA and NECA display differential adenosine receptor subtype activity.
(Supported by grant NIH RR8167)

171.11 MELATONIN-INDUCED CHANGES IN GABA AND MUSCIMOL BINDING IN RAT BRAIN. F.M. Coloma* and L.P. Niles. Department of Neurosciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Similarities in the psychopharmacological actions of the benzodiazepines, barbiturates and the pineal hormone, melatonin, prompted an investigation of whether melatonin, like these drugs, acts by modulating the central activity of γ-aminobutyric acid (GABA). In earlier experiments, melatonin was found to enhance the high affinity binding of the GABA agonist, ^{3}H-muscimol by about 20% in rat synaptosomal membranes. The greater consistency of this effect in Tris-HCl as compared with Tris citrate and the ability of KCl to augment melatonin's effect, suggested a chloride ion dependency. In addition, treatment of rat cortical membranes with Triton X-100 decreased or abolished the enhancing effect of melatonin.

We now report that preincubation of synaptic membranes with melatonin at 4°C for 60 minutes, significantly enhances ^{3}H-GABA binding by 30-60%. Saturation studies of high affinity ^{3}H-muscimol (0.3-20 nM) binding indicate that melatonin (10^{-4}M) causes a decrease in binding affinity while enhancing the concentration of binding sites. Moreover, melatonin's enhancing effect on ^{3}H-muscimol binding is blocked by metergoline but not by the benzodiazepine antagonist, Ro 15-1788.

These preliminary findings suggest that melatonin's pharmacologic effects on GABA binding are not mediated via a benzodiazepine site but may involve a serotonergic binding site.
(Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada.)

171.12 RELEASE OF ENDOGENOUS ADENOSINE FROM RAT BRAIN SYNAPTOSOMES. W.F. MacDonald* and T.D. White. Dept. of Pharmacology, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

There is evidence that adenosine is an extracellular inhibitory modulator of neuronal activity in the brain. In order for adenosine to exert effects extracellularly it must first be released, either as the nucleoside or as a nucleotide which is subsequently broken down to adenosine. Radiolabel methods for studying nucleotide/nucleoside release suffer from the serious limitation that the actual amounts of nucleotide/nucleoside cannot be determined since one has no knowledge of the specific activities of the radiolabelled compounds being released. In the present study we investigated extrasynaptosomal accumulation of endogenous adenosine using HPLC with fluorescence detection to monitor the etheno adenosine derivative formed by reaction with chloroacetaldehyde.

When rat brain synaptosomes were incubated for 10 min at 37°C, a single peak was obtained following chromatography of the incubation medium. The peak had a retention time identical to an adenosine standard and was abolished when preincubated with adenosine deaminase, indicating it was adenosine. Basal accumulation of adenosine in the medium was 66 pmol per mg protein per 10 min. Elevated K^+ (24 mM) evoked an additional accumulation of 193 pmol per mg protein per 10 min and 50 μM veratridine evoked 583 pmol adenosine accumulation per mg protein per 10 min. K^+- and veratridine-evoked accumulation of adenosine did not arise from microsomal or mitochondrial contaminants of the synaptosomal preparation since purified microsomes and mitochondria did not exhibit evoked accumulation of adenosine in the medium. K^+-evoked accumulation of extrasynaptosomal adenosine was Ca^{2+}-dependent, whereas veratridine evoked accumulation of adenosine was increased in Ca^{2+}-free medium. In the presence of a combination of α, ß-methylene ADP and GMP, which inhibits ecto-5'-nucleotidase, conversion of added ATP and AMP to adenosine was inhibited by 90% in synaptosomal suspensions. However, inhibition of ecto-5'-nucleotidase only reduced basal extrasynaptosomal accumulation of adenosine by 74%, veratridine-evoked accumulation of adenosine by 46% and K^+-evoked accumulation by 33%. Most of the basal accumulation of extrasynaptosomal adenosine appears to be derived from released nucleotide, probably ATP, but about half of the veratridine-evoked accumulation of adenosine and most of the K^+-evoked accumulation may arise from adenosine released in its own right, rather than from a released nucleotide. (Supported by the MRC of Canada).

171.13 BUFOTENINE ANTAGONIZES SEROTONIN'S EFFECTS ON HERMISSENDA TYPE B PHOTORECEPTORS. R. Wu and J. Farley, Program in Neuroscience, Princeton University, Princeton, N.J. 08540

10^{-4} to 10^{-6} M concentrations of 5-HT enhance the peak and steady-state light-induced depolarizing generator potentials of B cells, through a reduction of two K^+ currents (I_A and I_{K-Ca}) and enhancement of a voltage-dependent calcium current (I_{Ca}) [Wu & Farley, Soc. Neurosci. Abstr., 1984]. Because these effects are similar to those produced by associative training of the animal, and 5-HT is an endogeneous neurotransmitter released during training (Auerbach et al., this volume), we have attempted to identify 5-HT antagonists with the eventual aim of determining how much of the training-produced change in B cells is attributeable to 5-HT's actions.

Pretreatment (8 min.) of Type B cells with 10^{-4} M bufotenine ASW blocked the usual response changes seen when 10^{-4} M 5-HT was subsequently added. Neither the steady-state light response nor membrane resistance were significantly increased under these conditions [$t(5)=1.02$, n.s.; $t(5)=0.0$, n.s.] However, the transient light-induced peak response decreased significantly [$t(6)=2.31$, $p < .05$].

10^{-4} M bufotenine alone (dissolved in methanol/ASW) had no significant effects on input resistance, membrane potential, peak, and steady-state light response of B cells, [$t(6)=0.0$, n.s.; $t(6)=-1.46$, n.s.; $t(8)=-.50$; $t(8)=-.55$] indicating that its effects upon B cells were relatively specific to its antagonism of 5-HT's actions.

Experiments to determine which of the 5-HT-induced ionic conductance changes are affected by bufotenine are in progress.

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171.14 ACTIVATION OF ADENOSINE A_1 RECEPTORS ENHANCES Ca^{2+}-MEDIATED POTASSIUM CONDUCTANCE IN HIPPOCAMPAL CA_1 NEURONS. P.H. Wu,* N. Gurevich and P.L. Carlen, Neurology and Clinical Pharmacology Programs, Addiction Research Foundation; Playfair Neuroscience Unit, Toronto Western Hospital; and Departments of Medicine and Physiology, University of Toronto, Toronto, Canada.

Adenosine (AD) is known to be released both in the peripheral and central nervous system and AD receptors in the central nervous system have been identified. There are three extracellular AD receptors; A_1 (high affinity), A_2 (high affinity), A_2 (low affinity) (Daly, J., et al: Cell. Mol. Neurobiol. 3: 69-80, 1983) and intracellular AD receptors. Although pharmacologic characterization of A_1 receptors has been very successful, the electrophysiological function of A_1 receptors has not been demonstrated conclusively. In the present study, hippocampal slices from male Wistar rats (150-200 g) were prepared and perfused with standard artifical CSF or a Ca^{2+}-free medium containing 200 μ M $CdCl_2$. In some experiments, tetrodotoxin (TTX) 1 μ M and TEA 5 and 10 mM were added to the perfusate. 3 M K-acetate or KCl microelectrodes (50 to 180 Meg ohms) were used for intracellular recordings. AD, L-phenylisopropyl-adenosine (L-PIA), N-ethylcarboxamide adenosine (NECA), 8-para-sulfophenyl theophylline (8-PSPT) and caffeine were dissolved in the medium and subperfused onto the slice or focally applied by pressure ejection. AD (0.5 to 100 μ M), L-PIA (10 μ M) and NECA (1 μ M) applied onto stratum oriens (SO) consistently produced a hyperpolarizing response, especially when the cell was firing either spontaneously or from passive depolarization. The hyperpolarization induced by 5 μ M AD was completely blocked with 10 μ M 8-PSPT application which acts extracellularly. Similarly, adenosine deaminase (2 units/ml) also blocked AD hyperpolarizing responses, suggesting that the AD receptors associated with CA_1 cells and are extracellularly located. The ionic mechanism of the AD-mediated hyperpolarization was examined. The long-lasting afterhyperpolarization (AHP) following a train of spikes was elicited by a depolarizing current pulse. The early part of the AHP, which is a non-calcium-mediated potassium conductance, was not affected by AD or caffeine, whereas the later part of the AHP, which is mainly a calcium-mediated potassium conductance (Ca-gK), was enhanced by AD, and was blocked by 8-PSPT and caffeine (60 μ M). TTX and TEA block Na^+-dependent spikes and voltage-dependent K^+ conductance, bringing out higher threshold Ca^{2+} spikes. AD delayed the onset of Ca^{2+} spikes. We conclude that AD enhances Ca-gK by activating an extracellular, post-synaptic A_1 adenosine receptor. The parallel localization of AD receptors and the distribution of AD containing neurons in the hippocampus (Braas, K.M., et al: Neurosci. Abs. 165: 14, 1984), suggested that AD plays a significant role in the modulation of hippocampal neuronal excitability.

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171.15 HETEROGENEITY OF RESPONSE TO SEROTONIN IN MECHANOAFFERENTS OF THE CEREBRAL GANGLION OF APLYSIA. S.C. Rosen*, A.J. Susswein, K.R. Weiss, and I. Kupfermann (SPON: H. Chiel). Center for Neurobiology & Behavior, Columbia Univ., New York, NY 10032; New York State Psychiatric Institute, N.Y., and Dept. Life Sci., Bar-Ilan Univ., Ramat-Gan, Israel 52100.

Each hemi-cerebral ganglion contains two adjacent clusters of small neurons, many or all of which are primary mechanoafferents with receptive fields on the skin of the head and/or muscles of the buccal mass (Rosen et al., J. Neurophysiol., 1979, 1982). In most respects, these neurons are very similar to other clusters of mechanoafferents that have been described in Aplysia. For instance, the EPSPs produced by cerebral mechanoafferents exhibit profound low frequency depression. A striking difference between at least some of these neurons and other mechanoafferents that are known is that the increase of synaptic effectiveness the cerebral mechanoafferents exhibit following strong nerve stimulation is due to a post-synaptic mechanism, rather than to presynaptic facilitation. Heterosynaptic facilitation of mechanoafferents in the abdominal and pleural ganglia is due to the presence of adenylate cyclase-linked receptors that are stimulated by serotonin (5-HT) which produces inactivation of K^+ channels, resulting in spike broadening. As a first step to examine whether cerebral mechanoafferents possess similar cellular properties, spike width in the presence of 50 mM tetraethylammonium was determined in the presence and absence of 5-HT and other neuromodulators. In contrast to other similar neurons, many of the cerebral neurons in the mechanoafferent cluster, including previously identified interganglionic mechanoafferents, exhibited marked spike-narrowing in the presence of 5-HT (threshold approximately 10^{-6}M). Another population of cells in the mechanoafferent cluster showed spike-broadening, and exhibited enhancement of the EPSP they evoked in follower cells located in the cerebral B-cluster. These results suggest the hypothesis that the mechanosensory cluster of the cerebral ganglion contains at least three types of neurons that may be differentially modulated in order to perform different functions associated with feeding behavior.

171.16 EFFECT OF HISTAMINE ON IPSPs IN RAT HIPPOCAMPAL CA1 PYRAMIDAL NEURONS, S. A. Springfield and H. M. Geller, Dept. of Biology, City College of CUNY, New York, NY 10031 and Dept. of Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854.

Investigations using extracellular recording techniques have indicated that one prominent action of histamine (HA) may be through a reduction in the potency of central GABAergic systems (Springfield et al., Neurosci. Abst., 9: 1154, 1983). To understand the mechanism of this action of HA we have studied its effect on the inhibitory postsynaptic potential (IPSP) and the accompanying conductance changes on hippocampal CA1 pyramidal neurons.

Hippocampal slices (350 μm) prepared from 125-200 g male rats were constantly superfused with Yamamotos' medium equilibrated with 95% O_2/5% CO_2 and maintained at 32-34°C. A bipolar stimulating electrode was placed in the alveus to antidromically activate the pyramidal neurons. Low intensity (0.1-10 V) stimuli evoked an IPSP. Intracellular recordings were made with glass micropipettes filled with 3 M KCl. Hyperpolarizing current pulses (4 nA, 10 msec) were passed at the peak of the IPSP to assess membrane resistance/conductance changes. Histamine (10^{-6} M) was added to the perfusate and changes in membrane resistance and IPSP noted. Histamine was then eliminated to assess the return to control conditions.

The pyramidal neurons had resting membrane potentials of -65.7 to -79.9 mV and input resistances of 35.2-52.8 Mohm. The IPSPs had amplitudes of 18-30 mV and durations of 70-120 msec. Histamine application resulted in a reduction in the potency of the IPSPs, made evident by a reduction in the conductance increase recorded near the peak of the IPSP as well as a reduction in the amplitude of the IPSP. In every instance the IPSP conductance increase was diminished by HA, the mean reduction being 52.6%. The amplitude of the evoked IPSP was reduced by 5 to 53% when HA was applied. All changes were reversed by elimination of HA from the perfusate.

By demonstrating a depression of the IPSP conductance increase, these intracellular experiments support the conclusions already drawn from the extracellular observations; that HA may be causing either a reduction in the release of GABA from local interneurons impinging on the pyramidal cell, or that HA may be causing a reduction in the sensitivity of the pyramidal cell membrane to GABA. In either case, the resultant effect at the postsynaptic membrane of the pyramidal cell, a reduction in the IPSP amplitude and the associated conductance change, can be interpreted as further evidence for modulatory actions by HA. Additional experiments are in progress to distinguish between these possibilities.

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171.17 EFFECT OF REM SLEEP DEPRIVATION ON ADENOSINE A1 AND A2 RECEPTORS IN RAT BRAIN REGIONS. G. YANIK, N.M. PORTER, M. RADULOVACKI., DEPARTMENT OF PHARMACOLOGY, COLLEGE OF MEDICINE, UNIVERISITY OF ILLINOIS, CHICAGO,IL 60612

It has become increasingly clear that endogenous adenosine (Ado) may play a role in the regulation of the sleep-wake cycle. Studies in dogs, (Haulica et al. J. Neurochem. 21: 1019-20, 1973) together with studies from this laboratory in rats have shown that ado and synthetic ado analogs can increase deep slow wave sleep and REM sleep (Radulovacki et al., JPET 228: 268, 1984; Virus et al., Neuropharm. 22: 1401,1983). In addition, Ado A1 receptors have been shown to increase in specific rat brain regions following 48 h. of REM sleep deprivation (RD) by the "flower pot method" (Yanik et al., Soc. Neurosci. Abstr., Vol. 10 Part 2 p. 959, 1984). In order to differentiate between the effects of immobilization stress and RD we subjected rats in the present study to 96h of RD and added a large platform "stress control" group. In addition to the analysis of A1 receptors we also analyzed A2 receptors (assay adapted from Yeung and Green, Nuanyn-Schmeideberg's Arch Pharmacol. 325: 218-25, 1984) since stimulation of A2 receptors is thought to contribute to waking (Radulovacki et.al., 1984, op. cit.). Male Sprague Dawley rats (300-350 g) were assigned to one of three groups: cage controls (CC), large platform controls (LP) , or small platform REM sleep deprived group (SP). LP and SP groups were exposed to the REM deprivation set-up for 96 h while CC were housed in their home cages for the duration of the experiment. Following 96 h of RD, animals were killed by decapitation and their brains were rapidly removed and dissected. All brain samples (cortex, striatum, hippocampus, thalamus and cerebellum) were kept frozen at -70 C until assayed. A1 receptor binding was assayed using H3 PIA, and A2 receptor binding in corpus striatum was estimated using H3 PIA and H3 NECA. A statistically significant increase in Bmax was found only in the cortices ($p<.05$ as measured by Kruskal-Wallis nonparametric ANOVA) of RD animals while LP and CC groups were not significantly different. This finding confirms the previously reported A1 receptor changes seen after 48 hr RD. However, estimations of A1/A2 receptor populations in corpus striatum failed to elucidate any clear-cut role for A2 receptors in REM sleep deprivation. This study indicates that REM sleep deprivation produces increases in A1 receptors in rat brain, and suggests a role for these receptors in REM sleep. (Supported by ONR contract N000 14-79-C-0420)

171.18 EFFECTS OF CHRONIC INTRACEREBROVENTRICULAR INFUSION OF ADENOSINE AGONISTS AND DEOXYCOFORMYCIN ON BRAIN ADENOSINE RECEPTORS & SLEEP IN THE RAT. N.M. Porter, F.M. Clark*, R.D. Green, M. Radulovacki, Dept. Pharmacology, Univ. of Illinois at Chicago.

Two types of adenosine (Ado) receptors have been identified in the CNS: A1 receptors mediate the inhibition while A2 receptors the stimulation of adenylate cyclase (AC). Previous studies have demonstrated that chronic administration of Ado antagonists, such as caffeine and theophylline, up-regulate Ado A1 receptors in the CNS. The present studies were undertaken to determine the effects of chronic administration of Ado agonists on Ado receptors in the brain.

Male Sragues-Dawley rats (300-400 gm) were implanted with cannula into the lateral cerebral ventricle. ALZET osmotic pumps, implanted subcutaneously, were connected to the cannula and one of the following drugs administered: L-phenylisopropyladenosine (L-PIA, 0.50 nmoles/hr/2 wk), 5'-N-ethylcarboxamide adenosine (NECA, 0.04 nmoles/hr/2 wk) and deoxycoformycin (dCF, 5 nmoles/hr/1 wk). Each group had its own vehicle control. Rats were sacrificed 24 hr after the end of the infusions and ligand binding assays performed in cortex and striatum (ST). Ado A1 receptor binding was measured with ^{3}H-PIA, while A2 receptor binding was estimated by the NECA minus PIA binding assay performed in the presence of saturating concentrations of ^{3}H-PIA and ^{3}H-NECA (Yeung and Green, Naunyn-Schmiedeberg's Arch Pharmacol 325: 218, 1984). Chronic administration of L-PIA, an Ado A1 agonist, produced no change in A1 or A2 receptor binding. However, treatment with NECA, a mixed A1/A2 agonist, or with dCF, a potent inhibitor of endogenous adenosine metabolism, while producing no effect on A1 receptor binding, produced significant decreases in A2 receptor binding in ST (40% and 60%, respectively). Studies examining the effects of these treatments on AC activity are currently in progress. Preliminary results in rats treated with NECA suggest desensitization of the AC to the A2 stimulatory response.

In the second study, the effects of chronic administration of NECA on sleep were determined. Rats implanted with EEG and EMG electrodes were polygraphically recorded during the first 48 hr of drug withdrawal. During the first 24 hr, NECA withdrawn animals showed a significant decrease in wakefulness and an increase in deep slow-wave (SWS2) and REM sleep. The increase in SWS2 persisted during the second 24 hr of recording.

These data show that treatment with NECA and dCF results in a down-regulation of Ado A2 but not Ado A1 receptors. Furthermore, the results suggest that these Ado A2 binding sites may represent functional receptors since biochemical and behavioral changes are associated with the loss of these sites. (Supported by ONR contract N00014-79-C-0470)

171.19 THE EFFECTS OF SYSTEMIC AND LOCAL ADMINISTRATION OF ADENOSINE ANALOGS ON HIPPOCAMPAL EVOKED RESPONSES. T.V. Dunwiddie and M.S. Brodie, Dept. Pharmacol., Univ. Colorado Health Sci. Ctr. and Veterans Administration Medical Res. Service, Denver, CO 80262.

Adenosine and its analogs have potent depressant effects on the behavior and physiology of intact animals. Intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) injections of adenosine analogs such as R-phenyl-isopropyladenosine (PIA) and N-ethylcarboxamidoadenosine (NECA) produce profound reductions in body temperature, heart rate, blood pressure and spontaneous locomotor activity. However, there has been no demonstration of a direct effect of systemically administered adenosine analogs on electrophysiological activity in the central nervous system. We examined the effect of i.p. and i.c.v. administration of adenosine analogs on the hippocampal evoked field EPSP (fEPSP), a response that is sensitive to the actions of adenosine in vitro.

Sprague-Dawley rats were anesthetized with urethane (1.2 g/kg) and surgically prepared for stereotaxic implantation of injection cannulae, recording and stimulating electrodes. The anterior hippocampal commissure was stimulated contralaterally to the recording electrode, which was located in the CA1 region of the hippocampus. Microinjection cannulae were implanted in either the anterior or posterior region of the lateral ventricle ipsilateral to the recording electrode. Adenosine analogs (PIA, NECA) were given i.p. in doses from 0.1 to 5.0 mg/kg, and NECA was injected i.c.v. in doses from 0.4 to 135 ug. Adenosine and PIA were also applied from multibarrel recording pipettes by micropressure ejection. The adenosine receptor antagonist theophylline was injected i.p. in doses from 50 to 150 mg/kg or applied directly via micropressure application.

Local ejection of adenosine (5 mM in the pipette) profoundly depressed the amplitude of the hippocampal fEPSP in a theophylline reversible fashion. The fEPSP amplitude was also reduced by local ejection of PIA; systemic or local injection of theophylline partially reversed this response. Similarly, i.c.v. injection of NECA into the posterior (but not anterior) aspect of the ipsilateral ventricle reduced the amplitude of the fEPSP. However, systemic injection of adenosine analogs had no significant effect on the fEPSP amplitude, even at doses up to 100X higher than those required to elicit behavioral responses.

These results demonstrate that although the hippocampus in situ is sensitive to the actions of locally applied purinergic agonists, systemic administration of these agents has no effect. This suggests that these drugs do not reach these sites following i.p. administration in physiologically significant amounts, and would indicate that the hippocampus is unlikely to mediate the behavioral actions of these drugs. (Supported by DA 02702 and the Veteran's Administration Medical Research Service).

171.20 DOPAMINE NEUROMODULATION WITHIN THE RAT CAUDATE NUCLEUS L.A. CHIODO AND T.W. BERGER. Lab. Neurophysiology, Center for Cell Biology, Sinai Hospital of Detroit, 6767 W. Outer Dr., Detroit, MI 48235 and Depts. Psychol. and Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260.

Numerous studies have demonstrated that DA is the neurotransmitter released by nigral efferents within the caudate nucleus. Debate still exists, however, over the precise physiological role of DA at postsynaptic sites. A number of laboratories have reported excitatory effects of microiontophoretically applied DA and substantia nigra stimulation on caudate neuronal activity while other groups have demonstrated that DA inhibits the discharge of these cells. Recently, it has been suggested that DA may not act as a simple excitatory or inhibitory neurotransmitter but instead may exert a neuromodulator role on postsynaptic membranes. That is, as a neuromodulatory the primary physiological role of DA may be to alter the efficacy of other neurotransmitters impinging upon the same postsynaptic membrane. Given these observations, we examined the ability of microiontophoretically applied DA to modulate the co-iontophoretic application of either the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) or the excitatory amino acid glutamate (GLU). All spontaneously active rat caudate neurons sampled were of the type 1 previously described (Skirboll and Bunney, Life Sci., 1979). When GABA was iontophoresed onto these neurons (5-25 nA), there was a rapid decrease in the spontaneous activity (% inhibition = 44.6±1.9 [mean ± SEM]; n=20) while local application of GLU (0-5 nA) increased neuronal discharge (% excitation = 64.6±2.6; n=20). The co-iontophoresis of DA, at ejection currents which inhibited spontaneous activity (10-35 nA; % inhibition = 50.9±3.6; n=10), differentially altered the response of caudate neurons to these two neurotransmitters. While significantly increasing the effectiveness of iontophoretically applied GABA by 112% ($p < .001$; n=10), it decreased the response of caudate neurons to GLU application by 44% ($p < .001$; n=10). In contrast, when DA was microiontophoresed at currents which had no effect on spontaneous activity, there was an augmentation of the response of caudate neurons to both neurotransmitters. That is, the effectiveness of both GABA and GLU iontophoresis was increased by 96% and 186% respectively ($p < .001$; n=10 for both). These data agree with the previous findings which demonstrate that DA exerts a neuromodulatory role at postsynaptic sites within the mammalian CNS.

172.1 A DORSOLATERAL SPINOTHALAMIC PATHWAY IN SQUIRREL MONKEY. M.W. Jones*, A.V. Apkarian, R.T. Stevens and C.J. Hodge, Jr., Dept. of Neurosurgery, Upstate Medical Center, Syracuse, New York 13210.

Recent investigations in our laboratory have demonstrated a crossed dorsolateral spinothalamic pathway in cat which originates primarily from neurons within lamina I, and constitutes the primary lamina I projection to the thalamus. This study was designed to determine if a similar pathway exists in primates.

In 4 squirrel monkeys HRP-WGA (2%; 1µl total) was injected stereotactically into medial and lateral thalamic nuclei known to receive spinothalamic terminations. Just prior to the thalamic injection one of a series of lesions designed to block HRP-WGA transport through the dorsal columns, the ventral quadrant or the dorsolateral funiculus was made in the ipsilateral thoracic spinal cord. After a 5 day survival period tissue was processed for horseradish peroxidase histochemistry. Spinal lesions and thalamic injection sites were examined histologically. The HRP-WGA injection site did not include the midbrain. The locations of HRP-labeled spinothalamic neurons were plotted from transverse sections of the lumbar enlargement (L6-S1).

In an animal that received no spinal lesion and served as a control, labeled neurons were found in laminae I-VIII, X (7.8 total cells/section). The main populations of labeled neurons were located contralaterally in lamina I (14%), the reticular and compacta zones of lamina V (38%) and in laminae VII, VIII (32%). In an animal that received a combined ventral quadrant-dorsal column lesion (1.6 total cells/section), the main HRP-labeled spinothalamic populations were found contralaterally in lamina I (37%) and in the pars compacta of lamina V (24%). In an animal with a lesion of the dorsolateral funiculus (3.6 total cells/section) there were very few labeled neurons in lamina I (3%) and the main HRP-labeled population was found contralaterally in the reticular zone of lamina V (34%) with smaller populations in the pars compacta of lamina V (17%) and in laminae VII, VIII (25%).

These findings demonstrate distinct ventral and dorsolateral spinothalamic projections in the primate which cross segmentally within the spinal cord. This is similar to the cat which also has distinct dorsolateral and ventral spinothalamic pathways. In the squirrel monkey the dorsolateral projection originates predominantly from neurons within lamina I and the pars compacta of lamina V and the ventral projection originates primarily from neurons within the pars reticulata and compacta of lamina V and laminae VII, VIII.

172.2 MEDIAL AND LATERAL THALAMIC TERMINATIONS OF THE DORSOLATERAL AND VENTRAL SPINOTHALAMIC PATHWAYS. R.T. Stevens, A.V. Apkarian, M.W. Jones* and C.J. Hodge, Jr., Dept. of Neurosurgery, Upstate Medical Center, Syracuse, New York 13210.

We have recently demonstrated in cat the presence of two distinct pathways contributing to the spinothalamic tract (STT): a dorsolateral spinothalamic pathway (DSTT) originating primarily within lamina I and a ventral spinothalamic pathway (VSTT) originating mainly within laminae IV, V, VII and VIII (Jones et al., accepted in Brain Res.). Both pathways cross segmentally and terminate within the thalamus. The purpose of this study was to determine the components of each pathway which terminate within the medial thalamus or the lateral thalamus and those which terminate in both the medial and lateral thalamus.

Cats were injected with two retrogradely transported fluorescent dyes. Evans Blue (10% W/V) was injected into the lateral thalamus to include the ventrobasal complex and the ventrolateral thalamus. Granular blue (2% W/V) was injected into the medial thalamus to include the centrolateral and centromedian nuclei and nucleus submedius. After a 10 day survival period, cats were perfused with 10% formalin, tissue was sectioned and viewed on a Zeiss epifluorescence microscope system.

Within the lumbar spinal cord, contralateral lamina I neurons, the primary component of the DSTT, terminated primarily within the lateral thalamus. The percentage of contralateral STT lamina I neurons which terminated with the lateral thalamus ranged from 54% to 78% while those projecting to the medial thalamus comprised 16% to 28%. The percentage of the contralateral STT, lamina I neurons which projected to both the medial and lateral thalamus ranged from 6% to 18%.

Cells of origin of the VSTT (contralateral laminae IV-VII, X) demonstrated approximately equal numbers of cells which projected exclusively to the medial thalamus or the lateral thalamus. Only within lamina VIII were cells identified which projected to both the medial and lateral thalamus (less than 5% of total lamina VIII).

These results show that cells of origin of both the DSTT and VSTT terminate within the medial and the lateral thalamus. Some cells within each pathway bifurcate and terminate within both the medial and lateral thalamus.

172.3 THALAMIC TERMINATIONS OF LAMINA I DORSAL HORN NEURONS IN THE CAT OBSERVED WITH THE PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L) ANTEROGRADE TRANSPORT METHOD. A. D. Craig. Physiologisches Institut der Universität, Röntgenring 9, D-8700 Würzburg, F.R.G.

Spinothalamic(STT) and trigeminothalamic(TrT) lamina I neurons include nociceptive-specific and thermoreceptive-specific cells, as well as multireceptive cells responsive to both modalities; in the cat's STT, these neurons project in a functionally specific manner to medial and/or lateral thalamus (Craig & Kniffki, J. Physiol. (London), in press). The need for an anterograde identification of the multiple lamina I termination sites in thalamus is emphasized by the widespread distribution of STT and TrT projections demonstrated previously. The resolution and sensitivity required for this analysis, which exceed those provided by current tracing methods, may be attainable with the recently introduced anterograde transport method based on the immunohistochemical localization of the lectin PHA-L (Gerfen & Sawchenko, Brain Res. 290:219, 1984).

In the present study, iontophoretic injections of PHA-L are made at sites of physiologically characterized lamina I neurons in the medullary or spinal dorsal horn in anesthetized cats. After a survival time of 1-3 wks, animals are perfused with 4% paraformaldehyde and 0.2% picric acid at low and high pH. Serial 40 µm frozen sections are processed with the indirect immunofluorescence method or with a biotin/avidin/Texas Red® marking procedure. Major incubation parameters are (23°C): 1st antibody 1 µg/ml, 40 hrs; 2nd antibody 5 µg/ml, 4 hrs; labeled avidin 5 µg/ml, 4 hrs.

Several injections have been restricted to lamina I, while some include laminae I and II. Although labeling of severed fibers has occurred, no evidence of retrograde or transneuronal labeling has been observed. In cases with injections at the site of nociceptive trigeminal lamina I neurons responsive to input from intra- and/or peri-oral structures (inc. teeth), bouton-bearing axonal branches or terminal arbors have been observed in several contralateral loci, including ZI, the caudal portion of Po_m, the ventral margin of VPM and VM_b, the region of the oral representation in VPM, and Sm_d. An injection at the site of a cold-responsive lamina I neuron resulted in additional terminations at the dorsomedial border of VPM. Morphologically distinct termination patterns have consistently been observed in several sites.

These results demonstrate that discrete analyses of ascending projections can be made with the PHA-L method in the cat. The direct identification of lamina I termination sites in the diencephalon provides a substantive basis for study of the functional organization of nociceptive and thermoreceptive input to thalamus.

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172.4 ANATOMICAL BASIS FOR AN INTERACTION BETWEEN THE SPINOTHALAMIC AND LEMNISCAL SYSTEMS IN THE VENTROBASAL COMPLEX OF THE RAT THALAMUS. AN ELECTRON MICROSCOPIC STUDY. W. Ma*, M. Peschanski and H.J. Ralston III, Dept. of Anatomy, University of California, San Francisco, CA 94143 and INSERM, U 161, 2 rue d'Alesia, 75014 Paris, France.

In a recent light microscopic study (Neurosc. Abst., 10, 1984, 143-10), we have demonstrated the existence of a somatotopically organized overlap of the projections originating from the spinal cord and the dorsal column nuclei (DCN) in the ventrobasal complex of the rat thalamus (VB). Such an overlap, visualized at the light microscopic level, suggested that some interactioin between the two systems might occur at the level of single VB neurons receiving afferents from the two sources. The present experiment was designed to test this hypothesis by determining the existence of differentially labeled terminals impinging upon the same neuron in a study using a double-labeling anterograde strategy at the ultrastructural level.

Anesthetized Male Sprague-Dawley albino rats received an injection of a 10% solution of wheat-germ agglutinin conjugated to HRP (WGA-HRP) in the left side of the lumbar or cervical enlargements of the spinal cord 48 hours before sacrifice. In the same time, or 24 hours later, the left DCN were lesioned. One to two days later, the animals were perfused transcardially with heparinized saline followed by a phosphate buffered solution (0.1 M, pH 7.4) of 3% glutaraldehyde, 1% paraformaldehyde and 4% sucrose. Vibratome sections of the right VB were cut (50 µm-thick) and reacted using benzidine dihydrochloride as a chromogen then osmicated, dehydrated and flat-embedded. VB areas containing HRP labeled terminals originating from the spinal cord were trimmed out and cut on an ultramicrotome for further analysis with the electron microscope. As expected from previous studies, labeled spinothalamic terminals were large (2 to 7 µm in diameter), contained numerous round vesicles and formed several asymmetrical (Gray type I) synapses with large dendrites and, more rarely, somas of VB neurons. In the same fields, electron-dense, electron-lucent and neurofilamentous degenerating large terminals were visible. The degenerating terminals (0.5 to 3 µm in diameter) were often seen to contain round vesicles and to form asymmetrical Type I synapses with large dendrites and somas of VB neurons. Terminals from the cord or the DCN were sometimes close from each other, less than 1 µm apart, and in a few cases they were observed contacting the same post-synaptic element.

This study confirms at the ultrastructural level that terminals from the spinothalamic tract and the lemniscal system are present in the same areas of the VB. The existence of some convergence has been demonstrated which provides an anatomical basis for a direct effect of the two systems on single thalamic neurons. These results are in agreement with electrophysiological studies demonstrating the presence of noxious and non noxious responsive neurons in the VB of the rat. (Supported by NS 11614 to HJR, and by INSERM, France).

172.5 THE SPINO-RETICULO-DIENCEPHALIC PATHWAY IN THE CAT. A. Blomqvist* and K.J. Berkley. Dept. of Psychology, Florida St. Univ., Tallahassee, FL 32306.

A pathway from the spinal cord to the diencephalon via the reticular formation is generally believed to be of importance for the emotional and motivational aspects of pain sensation. However, little is known about the organization of this spino-reticulo-diencephalic pathway. The purpose of the present experiment was to examine this pathway in the cat by comparing the termination pattern of the spinal projection to the reticular formation with the distribution of the reticular neurons projecting to the diencephalon, using a variety of single and double orthograde and retrograde tracing techniques. Our preliminary results show that whereas several brainstem regions, not generally associated with the reticular formation, receive a dense spinal input, the spinal projection to the reticular formation is sparse, and overlaps only to a limited extent with the location of the reticulo-thalamic neurons. Furthermore, the reticular neurons projecting to the intralaminar nuclei and the zona incerta, two main rostral targets of the reticular formation, seem at least in part to be differently distributed. The findings thus suggest that 1) few reticulo-thalamic neurons receive a direct spinal input and 2) that the information transmitted from the reticular formation to the intralaminar nuclei and the zona incerta, respectively, may be different in character.

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172.6 **Interstitial Nucleus of the Spinal Trigeminal Tract: A Specific Nociceptive Relay for Oral and Perioral Structures?** **R.C. Shults, A.R. Light, and S. Donaghy* Univ. of North Carolina Sch. of Medicine, Chapel Hill, NC 27514**

As part of an investigation of intra-oral and peri-oral sensory/nociceptive pathways in the trigeminal system of adult cats, peroxidase-anti-peroxidase immunohistochemistry and diaminobenzedine were used to visualize antibodies specific for substance P (S-P), leu enkephalin (L-ENK), 5-hydroxytryptamine (5-HT), somatostatin (SRIF) and vasoactive intestinal protein (VIP). As previously reported for nucleus caudalis (medullary dorsal horn) and spinal dorsal horn, S-P was located primarily in layers I and II-outer (IIo), L-ENK in layers I, IIo, II-inner (IIi), and III, and SRIF in layers IIo, IIi and III. S-P and L-ENK reactivity were also observed superficial to the spinal trigeminal tract (SVT) in the paratrigeminal nuclei. In more rostral sections, where nucleus caudalis is continuous with nucleus interpolaris, layers I and II are penetrated and dispersed by deep axon bundles. In the overlying SVT, islands of neuropil become evident as the interstitial nucleus of SVT (Falls and Phelan, Soc. Neurosci. Abs. 10: 482, 1984). These islands stain darkly for S-P, L-ENK and 5-HT. It is important to stress that these islands appear as layers I and II disappear from the underlying nucleus as if layers I and II had migrated into the tract overlying nucleus interpolaris and nucleus oralis. Islands of neuropil were observed with increasing frequency from the nucleus caudalis-interpolaris border, rostrally, to mid levels of nucleus interpolaris. More rostral sections contain progressively fewer islands in the tract over nucleus oralis.

While the interstitial nucleus of SVT (IN-SVT) may represent a novel structure with an unknown function, we would like to propose that it is homologous to layers I and II (marginal zone and substantia gelatinosa) in nucleus caudalis and the spinal dorsal horn and that it may represent a specific nociceptive relay for pulpal, intra- and peri-oral inputs. The following facts support this hypothesis: 1) patterns of localization of S-P, L-ENK and 5-HT are similar for IN-SVT, and layers I and II of nucleus caudalis and the spinal dorsal horn; 2) nociceptive primary afferent fibers have been shown to terminate in IN-SVT (Jacquin, M. personal communication) in a pattern similar to nociceptive afferent terminations in layers I and II of nucleus caudalis and the spinal dorsal horn; 3) close proximity of IN-SVT to major pulpal projection pathways (Westrum et al., Soc. Neurosci. Abs. 10: 993, 1984); and 4) projections to the thalamus from IN-SVT (Falls and Phelan, Soc. Neurosci. Abs. 10: 482, 1984). This projection pathway may explain sparing of oral and peri-oral pain and recurrence of pain following trigeminal tractotomies caudal to obex. Supported by NINCDS grants NS-16433 and NS-00534.

172.7 ULTRASTRUCTURAL ANALYSIS OF SUBSTANCE P AND OTHER SYNAPTIC PROFILES INNERVATING AN IDENTIFIED PRIMATE SPINOTHALAMIC TRACT NEURON. S.M.Carlton, C.C.LaMotte, C.N.Honda, D.J.Surmeier, N.C. deLanerolle and W.D.Willis. Mar. Biomed. Inst., Univ. Texas Med. Branch, Galveston, TX 77550 and Neurosurg. Sec., Yale Univ. School Med., New Haven, CT 06510.

In an effort to define the terminations of primary afferent and modulating systems onto spinothalamic tract (STT) neurons, we have combined intracellular staining of identified STT neurons with immunohistochemistry. STT cells in the lumbosacral cord of anesthetized monkeys (M. fascicularis) were antidromically activated from the contralateral thalamus, and after physiological characterization were intracellularly marked with HRP. Following perfusion with 2.5% paraformaldehyde, serial vibratome sections (50μm) were reacted for HRP and then immunohistochemically labeled for substance P using the PAP technique. Sections were flat embedded for light microscopic reconstructions. For ultrastructural analysis, serial EM sections were collected on formvar coated slot grids.

The one cell analyzed to date was a WDR neuron responsive to activation of low and high threshold cutaneous afferents innervating the foot. The soma was located at the lateral border of lamina V; the dendritic tree extended 700μm dorsoventrally, 1500 μm mediolaterally, and 1300μm in the rostrocaudal plane. The cell was characterized by numerous somatic and dendritic spines. Several types of terminals contacted the STT neuron, including: R terminals with small, clear, round, and occasionally dense core vesicles; DCV terminals with several dense core and clear, round vesicles; LGV terminals with numerous larger granular vesicles; P terminals with pleomorphic vesicles; and F terminals with flattened vesicles. Although examples of each type of terminal were found to contact the STT soma, dendrites, and somatic and dendritic spines, the frequency of each varied. The R terminal was by far the most common contact onto each STT structure, comprising 30-40% of the sample. The DCV and P types each accounted for about 20-35% of the terminals.

Most SP synaptic profiles were R terminals (with or without 1 or 2 dense core vesicles); they were found in contact with the STT somata, somatic spines and dendrites. Other SP profiles were LGV terminals usually observed in long varicose chains along the lateral V border; they contacted STT dendrites. A few SP profiles were DCV terminals, contacting STT dendrites and spines.

The results indicate that the entire soma of this WDR neuron, as well as its dendritic tree, is subject to a large variety of synaptic inputs, including different types of SP terminals which probably arise from several sources. (Supported by NIH grants NS13335, NS11255, NS09743, NS07185, NS07062, NS07216 and NS07574.)

172.8 ULTRASTRUCTURE OF PEPTIDERGIC AND SEROTONERGIC SYNAPTIC TERMINALS CONTACTING SPINOTHALAMIC AND OTHER LAMINA V NEURONS OF THE MONKEY CERVICAL CORD. C.C. LaMotte, Section of Neurosurgery, Yale Univ. School of Medicine, New Haven, CT. 06510.

The lateral reticulated border of lamina V contains both spinothalamic neurons and a high concentration of several peptides as well as serotonin. This study describes terminals in this region that are immunoreactive to a variety of peptides or 5-HT, and their synaptic contacts with spinothalamic neurons.

Spinothalamic neurons and their processes were retrogradely labelled following sterotaxic injections of HRP into the VPL region of the thalamus. Immunoreactive terminals were identified with specific antibodies to the neurochemicals, using the avidin biotin complex (ABC) method.

Substance P terminals are of three types. The most common are R terminals, containing small round clear vesicles and forming synaptic contacts onto the soma and dendrites of dorsal horn neurons. Some SP-R terminals are simultaneously presynaptic to another R terminal as well as a dendrite. A few SP terminals contain several dense core vesicles (DCV) as well as round vesicles. Other SP terminals contain many large granular vesicles (LGV) and are part of long varicose chains located along the lateral edge, in a region continuous dorsally with the marginal zone.

Somatostatin (SS) terminals are R terminals and contact somata, large and small dendrites, and spines. Some SS dorsal horn terminals are large and contain many mitochondria. Some SS terminals are postsynaptic to unlabelled R terminals.

Met-enkephalin terminals contain pleomorphic clear vesicles and several dense core vesicles; they form asymmetrical junctions on somata, large dendrites, and, more frequently, synapse on small dendrites and spines. Some ENK terminals are presynaptic to R or pleomorphic terminals.

Serotonin terminals are R type. However, 5-HT terminals contact somata and proximal dendrites with symmetrical junctions, but contact small dendrites with asymmetrical junctions. A few 5-HT terminals are large glomerular C terminals with round clear vesicles and scalloped edges. 5-HT terminals of either type rarely contain dense core vesicles.

Many of the above types of SP, SS, ENK and 5-HT terminals are found in synaptic contact with the soma and dendrites of retrogradely labelled spinothalamic tract neurons. Some of these STT neurons have somatic and dendritic spines that are also postsynaptic to some of these terminals. Although lateral lamina V contains many of the same neurochemicals identified in the superficial dorsal horn, its synaptic organization is quite different from that which we have previously described for laminae I and II. Furthermore, each of these neurochemical systems directly impinges onto STT cells. (Supported by NINCDS NS13335).

172.9 LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS OF ENKEPHALIN-IMMUNOREACTIVE PROFILES IN THE NUCLEUS SUBMEDIUS OF THE CAT THALAMUS. J.A. Coffield* and V. Miletic. Dept. of Struct. & Funct. Sci., School of Vet. Med., Univ. of Wisconsin, Madison, WI 53706.

The nucleus submedius (Sm) of the cat thalamus reportedly receives direct projections from neurons located in lamina I of the medullary and spinal dorsal horns (Craig and Burton, J. Neurophysiol., 45:443-466, 1981). Since lamina I neurons respond principally or exclusively to noxious cutaneous stimulation, the nucleus submedius might be a thalamic relay center for the processing of nociceptive information. In the present study we have employed the peroxidase-antiperoxidase (PAP) immunohistochemical technique to study the presence and distribution of enkephalin (ENK) in the Sm.

Adult cats were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde in 0.1M phosphate buffer. Fifty μm coronal and parasagittal Vibratome sections were processed for ENK with the PAP technique. For light microscopy (LM), 0.75% Triton X100 was added to the 1^{o}, 2^{o} and PAP incubations to improve antibody penetration. No detergents were added to the material used for electron microscopy (EM).

The ENK immunoreactive fibers appear confined to a small area (100-200μm in diameter) in the dorsal and caudal part of the Sm, and are located from 40 to 100μm from the dorsal edge of the Sm. This area appears to overlap at least in part with the Sm area shown by Craig and Burton to contain projections from the lamina I neurons. At the LM level at least two different types of immunoreactive axons could be seen based on the size of their varicosities. At the EM level preliminary results show that the ENK immunoreactive endings predominantly contact cell bodies and dendrites.

These data indicate a limited and concentrated presence of ENK immunoreactive fibers in the cat Sm. It remains to be established whether any of these fibers originate from lamina I, and what their possible function in sensory information processing might be.

172.10 IMMUNOCYTOCHEMICAL INVESTIGATION OF MONOAMINERGIC INTERACTION IN BRAIN STEM RAPHE NUCLEI OF THE CAT. S. Pretel and M.A. Ruda. Neurobiology & Anesthesiology Br., NIDR, NIH, Bethesda, MD 20205.

The serotonergic (5-HT) neurons of the raphe nuclei are involved in the modulation of sensory, motor and autonomic functions at the spinal level. The neurochemical identity of the afferents which innervate these raphe neurons is not known, however, behavorial data suggest an interaction between noradrenaline afferents and 5-HT neurons in the raphe nuclei. We examined the possible interaction between these two monoamines in n. raphe magnus (NRM) obscurus (NRo) and pallidus (NRp) using a double-label immunocytochemical method.

Cats were perfused with 4% paraformaldehyde. Following cryoprotection, 18 μm transverse or sagittal sections of the brain stem were cut with a cryostat. Monoamines were identified using antisera to 5-HT, dopamine-ß-hydroxylase (DBH, the synthesizing enzyme for noradrenaline which is present in noradrenaline and adrenaline neurons) and tyrosine hydroxylase (TH, an enzyme present in dopamine, noradrenaline and adrenaline neurons). Since there is little evidence for the presence of a catecholamine other than noradrenaline in axons of the caudal raphe nuclei, it is likely that most of our DBH and TH immunoreactive axons contained noradrenaline. The tissue was processed sequentially for each antigen, using first the PAP immunocytochemical method and second the indirect immunofluorescence method with FITC as the fluorochrome. Both labels could be viewed simultaneously using epi-fluorescence and a blue fluorescence filter combination.

Numerous 5-HT cell bodies with labeled proximal dendrites were seen in the NRM, NRo and NRp as well as DBH or TH immunoreactive axons. Some 5-HT labeled axons were also visible. When viewed under high magnification with differential focusing, some of the DBH-immunoreactive varicosities appeared to make contact with 5-HT neurons and proximal dendrites. These contacts were found more often on the cell bodies than proximal dendrites. In addition, we observed 5-HT axons which appeared to contact 5-HT neurons in the NRM, NRo and NRp, but these were found less frequently than DBH varicosities contacting the 5-HT neurons.

These observations suggest that 5-HT neurons in the NRM, NRo and NRp receive input from NA and 5-HT afferents and that these two monoaminergic systems interact within the raphe nuclei. Since afferents from NRM likely distribute to the dorsal horn while the more caudal NRo and NRp afferents terminate mainly in the ventral horn, noradrenergic modulation of 5-HT neurons in the brain stem presumably effects the activity of sensory as well as motor systems.

172.11 IMMUNOHISTOCHEMICAL LOCALIZATION OF GLUTAMATE, GLUTAMINASE AND ASPARTYL AMINOTRANSFERASE NEURONS IN THE SPINAL TRIGEMINAL NUCLEUS OF THE RAT. K.R. Magnusson*, A.J. Beitz, A.A. Larson, J.E. Madl* and R. Altschuler. (SPON: G.W. King). University of Minnesota, St. Paul, MN 55108 and NINCDS, Bethesda, MD 20205.

In an attempt to identify glutamatergic neurons in the spinal trigeminal nucleus (STN), three different glutamate markers were compared using immunohistochemical techniques. Polyclonal antibodies against glutaminase (GLNase) and aspartate aminotransferase (AATase) and a monoclonal antibody to carbodiimide-fixed glutamate (Glu) were used. STN glutamatergic neurons projecting to the thalamus were identified by a combined retrograde transport-immunohistochemical technique. Three Sprague-Dawley male rats were perfused intracardially with paraformaldehyde (4%) and glutaraldehyde (0.1-0.2%) and sections were subsequently reacted with either the GLNase or AATase antibody. Three additional rats were perfused with a solution of 5% carbodiimide and 0.5% glutaraldehyde in phosphate buffer followed by 5% glutaraldehyde. The sections were post-fixed in 5% glutaraldehyde, washed and then reacted with the monoclonal glutamate antibody. Sections incubated with either the polyclonal antisera or the monoclonal antibody were subsequently processed utilizing the avidin-biotinylated peroxidase technique and reacted with diaminobenzidine to visualize the immunoreactive cells. Horseradish peroxidase (HRP) was injected into the ventroposterior nucleus of the thalamus and the combined retrograde transport-immunohistochemical procedure was performed as previously described (Beitz, A.J., Neurosci, 7:2753, 1982). Immunohistochemically reactive neuronal profiles were found in all three subnuclei of STN with each of the three markers. The caudal nucleus of STN (Sp5C) had the highest number of immunoreactive profiles, followed by the interpositus nucleus of STN (Sp5I) and the oral nucleus of STN (Sp5O). The AATase antiserum was found to label more neuronal profiles than GLNase and Glu. This may suggest that the AATase antiserum is staining another population of cells in addition to glutamate. Following large HRP injections into the thalamus, HRP labeled cells were found in all three of the trigeminal subnuclei. Retrogradely labeled neurons were most prominent in the Sp5I and Sp5O. Neurons containing both retrogradely transported HRP and glutamate or glutaminase immunoreactivity were evident predominantly in Sp5I and rostral Sp5O. Approximately 20-35% of HRP labeled neurons were double-labeled. These results suggest that glutamatergic neurons are present throughout the STN and that some of these neurons project to the thalamus. Supported by NIH grants DE06682 and NS19208 to A.J.B. and NIH grant NS17407 to A.A.L.

172.12 DISTRIBUTION OF PONTOSPINAL NORADRENERGIC AXONS IN RAT LOWER CERVICAL SPINAL CORD. R.M. Fay and D.J. Mayer. Dept. of Physiology, Med. Coll. of Virginia/Va. Commonwealth Univ., Richmond VA 23298.

A powerful bulbospinal antinociceptive system is known to descend via the dorsolateral funiculus (DLF), but its neurochemical nature is a matter of controversy. Noradrenaline (NA) is a neurotransmitter candidate for this system because pontine noradrenergic (NAc) cell groups are known to project to the spinal cord, and NA has been shown to play a role in descending inhibitory systems. In order to determine the spinal funicular distribution of descending NAc fibers, we have developed a sensitive procedure which involves combined retrograde and immunohistochemical labelling. Some features of this procedure are: (1) implanting 30% horseradish-peroxidase (HRP) slow release gel in a selected cord target area (appropriate lesions and/or a specialized delivery apparatus is used to minimize undesired labelling when implanting areas deep within the cord); (2) cutting and staining cords for HRP to verify actual labelling; (3) use of a diaminobenzidene-stabilized tetramethylbenzidene (TMB) reaction to optimize retrograde-HRP staining in combined-labelling sections (Rye, J. Histochem. Cytochem. 32:1145); (4) use of the peroxidase-anti-peroxidase technique for immunohistochemical staining; and (5) use of the ultra-sensitive TMB stain for retrograde HRP on alternating sections in order to evaluate the efficacy, and extrapolate the maximum expected amount, of retrograde staining in the combined-labelling sections. All areas of spinal-cord white matter have been selectively implanted, with results as follows. Implantation of the dorsal columns, the lateral funiculus (LF) adjacent to lamina V, and the outer margin (approx. one-third of their depth) of the LF and ventrolateral funiculus (VLF) resulted in practically no combined labelling. Implantation of the ventral funiculus (VF) and the internal two-thirds of the VLF and LF, however, resulted in consistent, strong labelling of the ventral locus coeruleus (primarily ipsilateral), the subcoeruleus nucleus (ipsilateral only), A5 (ipsilateral only), and A7 (bilateral) cell groups. DLF implants, on the other hand, produced a consistent but light combined label in a smaller percentage of NA cells in the same general locations as LF, VLF, and VF implants. Although it is clear that the majority of NAc fibers descends in a crescent-shaped bundle within the LF and VLF (the distribution pattern in the VF is yet to be determined), these findings alone are insufficient to distinguish whether the NAc DLF fibers arise from disparate pontospinal NAc cells or arise as collaterals from NAc axons in the more-ventral funiculi. Resolution of the cause of this difference may provide information on the role of the DLF in antinociception.

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172.13 **THE EFFECTS OF SEROTONIN AND SUBSTANCE P ON SPINAL PAIN REFLEXES.** R.M. Murphy* and F.P. Zemlan (SPON: R.J. Hitzemann). Dept. Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

Several lines of evidence indicate that the descending bulbospinal serotonin system suppresses incoming noxious input to the spinal cord and mediates narcotic analgesia. Recent immunohistochemical studies have shown that 5-HT and substance P (SP) coexist in the same bulbospinal neurons, and are colocalized in the same spinal cord dense core vesicles. The present behavioral studies examined the relationship(s) of 5-HT and SP in the control of three spinal pain reflexes.

Two days after spinal transection, 5-HT_1 receptors were stimulated by ip administration of the 5-HT agonist 5-MeODMT (1.5 mg/kg) which resulted in the expansion of the receptive field (RF) area for all three spinal pain reflexes (ventro-, 16% increase from baseline; dorsi-, 51% increase; lateral, 219% increase). Intrathecal SP administraion (0,0.25,1.25, 7.5 ng) in rats pretreated with 5-MeODMT, produced a significant reduction of pain reflex RF area for all three spinal reflexes at all three doses of SP. The largest reduction occurred with the 0.25 ng dose of SP (ventro-, 54% decrease; dorsi-, 55% decrease; lateral, 65% decrease). However, intrathecal SP administration in rats not pretreated with 5-MeODMT produced a dose-response related expansion of pain reflex RF area for all three reflexes (eg. ventro-, 80% increase; dorsi-, 110% increase; lateral-, 275% increase after the 7.5 ng SP dose).

The present data, consistent with other studies in intact animals, indicate that administration of SP alone facilitates spinal cord pain transmission probably by activation of SP primary afferent receptors. Similarly, 5-HT agonist administration facilitated pain transmission in the present study consistent with earlier reports. However, SP inhibited the effect of 5-MeODMT on spinal reflexes when administered after the 5-HT agonists. Thus, SP demonstrated the opposite effect following 5-MeODMT treatment suggesting a second mechanism of action. This second mechanism may be related to the descending 5-HT/SP system. At this meeting, we report electrophysiological data suggesting a functional interaction of 5-HT and SP released from bulbospinal 5-HT/SP neurons. Also, we report an apparent coupling of a subpopulation of spinal 5-HT and SP receptors suggested by receptor binding studies. In the present study, the inhibition by SP of the 5-MeODMT induced facilitation of spinal pain transmission may be related to an interaction between 5-HT and SP associated with the bulbospinal 5-HT/SP system.

(Supported by NS 18326).

OPIOID RECEPTORS II

173.1 DERIVATIVES OF 7α-AMINOMETHYL-6,14-endo-ETHENOTETRAHYDROTHEBAINE AS POTENTIAL PHOTOAFFINITY LABELS OF OPIOID RECEPTORS. J.C.Schaeffer, J.J. Kopcho* and M.A. Byer*. Department of Chemistry, California State University, Northridge, California 91330.

Considerable pharmacological and biochemical evidence indicates the presence of multiple opioid receptors in the brain. In order to establish whether these apparently different receptors are either simply different conformations of the same molecule stabilized by different membrane environments, different binding sites on a single stable molecular conformation, or totally different molecular species, specific irreversible labels would be most useful. Toward this end, a series of potential photoaffinity labels derived from 7α-aminomethyl-6,14-endo-ethenotetrahydrothebaine (I) containing an 4-azido-2-nitroanilino moiety that can be photoactivated at wavelengths greater than 350nm has been synthesized. The relative binding potencies of these compounds were ascertained by a binding assay using a crude rat brain membrane preparation and 1nM naloxone ± 1μM levallorphan in the presence or absence of sodium.

Compound I was synthesized from thebaine in three steps. It was converted to 7α-N-(4-azido-2-nitrophenyl)aminomethyl-6,14-endo-ethenotetrahydrothebaine (II) by reaction with 4-azido-2-nitrofluorobenzene. Compound II had an I_{50} of 1000nM. Monodemethylation of II at the 6 or 3 (III) position gave analogs with I_{50}s (100mM Na) of 320nM and 5.6nM, respectively. Demethylation at both the 3 and 6 positions gave an analog (IV) with an I_{50} (100mM Na) of 1.7nM, while replacing the N-methyl group in III with a cyclopropylmethyl group gave analog V that had an I_{50} (100mM Na) of 3.7nM. The 3-demethylation of I afforded the corresponding oripavine derivative (VI). The reaction of VI with N-hydroxysuccinimidyl N-(4-azido-2-nitrophenyl)-3-aminopropionate gave the corresponding 7α-amide derivative (VII) that had an I_{50} (100mM Na) of 0.32nM. In the absence of sodium the I_{50}s of III, IV, V, and VII were 0.6nM, 0.4nM, 2.1nM, and 0.38nM, respectively. The sodium ratios suggest that III is probably a pure agonist, IV and V are mixed agonists/antagonists, and VII is a pure antagonist.

Compounds III,IV,V, and VII are potent inhibitors either in the absence or presence of sodium of tritiated naloxone binding, and thus are excellent candidates for further evaluation as photoaffinity labels.

173.2 HIGH RESOLUTION RADIOAUTOGRAPHIC VISUALIZATION OF DELTA OPIOID RECEPTORS IN RAT BRAIN USING IODO-AZIDO DTLET, A HIGHLY SELECTIVE PHOTOAFFINITY PROBE. F Pasquini*. A Beaudet. P. Bochet*, C. Garbay Jaureguiberry* J. Rossier and B.P Roques* Montreal Neurol Inst., 3801 University St. Montreal Canada H3A 2B4; Lab. de Physiol Nerv , CNRS, 91190 Gif-sur Yvette, France; U E.R. Sc. Pharmac. et Biol.. Univ. René Descartes, 4, ave. de l'Observatoire, 75270 Paris, France.

^{125}I-azido DTLET is a pure monoiodinated derivative of the δ opioid selective photoaffinity ligand azido DTLET (Tyr-DThr-Gly-pN_3Phe-Leu-Thr), which binds specifically and with high affinity to rat brain membrane preparations (Kd=14 nM) and shows a displacement profile by DAGO (Ki=370 nM) and DTLET (Ki=2 nM) similar to that of (^{3}H)DTLET (Bochet et al., INRC, 1985). The possibility of applying this selective photoaffinity probe to the high resolution radioautographic detection of brain δ opioid receptors was investigated using 20 μm-thick cryostat sections from rat neostriatum. Incubation of unfixed tissue sections for 45 min at room t°, with 0.2-0.7 nM ^{125}I-azido DTLET yielded remarkably high specific/total binding ratios (> 90%). Pre-fixation of the brain with fixative solutions containing 0.75-2% paraformaldehyde or 0.1% acroleine almost totally abolished this specific binding. However, in sections pre-fixed with weak concentrations of glutaraldehyde (0.3-0.5%) associated or not with 0.2% paraformaldehyde and 1% tannic acid up to 65% of the original specific binding was still detectable. The radioautographic distribution of the bound radioactivity was characterized in both fresh and pre-fixed sections by a dense and homogeneous labeling of the caudate-putamen, similar to that observed using other δ selective ligands. Irradiation of the labeled sections (pre-fixed or not) with a single 254 nm U.V. flash allowed retention of 20-30% of the specifically bound radioactivity during subsequent post-fixation and dehydration steps, irrespective of the nature (glutaraldehyde, paraformaldehyde or a mixture of both) and concentration (1-4%) of the post-fixative used. In contrast, in sections that were not exposed to U.V. light, the radioactivity was totally washed out from the sections in the course of fixation and dehydration. Similar patterns of radioautographic labeling were observed before and after U.V. flashing followed by histological processing, suggesting that the radiolabeled molecules had been cross-linked onto or in the vicinity of their specific binding sites during irradiation Finally, the topographic distribution of covalently bound ^{125}I-azido DTLET observed in radioautographs prepared by classical dipping techniques was similar to that revealed by film radioautography. These results indicate that ^{125}I-azido DTLET is an ideal tool for the selective light microscopic radioautographic localization of brain δ opioid receptors and should be applicable to the visualization of these sites at the electron microscopic level. Supported by the C.N.R.S. (France) and the MRC (Canada).

173.3 CYCLOFOXY, A FLUORINATED OPIATE RECEPTOR LIGAND: HIGHLY SELECTIVE LABELING OF BRAIN OPIATE RECEPTORS, IN VIVO. N.L. Ostrowski, T. Burke*, K.C. Rice*, A. Pert and C.B. Pert+. Section on Behavioral Pharmacology, Biological Psychiatry Branch, NIMH, *Section on Medicinal Chemistry, Laboratory of Chemistry, NIADDK, +Section on Brain Biochemistry, Clinical Neuroscience Branch, NIMH, 9000 Rockville Pike, Bethesda, MD 20205 U.S.A.

Cyclofoxy, a fluoro-analogue of naltrexone, has been designed specifically to permit labeling of opiate receptors in living animals and humans (Burke, Rice & Pert, Heterocycles, Vol. 23, p. 99, 1985). Recently, using positron emission tomography (PET) 3-[^{18}F]-acetylcyclofoxy was shown to accumulate in opiate receptor-rich brain regions of a live baboon and to be stereospecifically displaced by injections of (-)- but not (+)-naloxone (Pert, et al., FEBS Letters., Vol. 177, p. 281, 1984). We now present autoradiographic evidence that ^{3}H-cyclofoxy labels a population of opiate receptors in brain that is virtually identical to that labeled by ^{3}H-naloxone. Furthermore, the binding patterns of ^{3}H-cyclofoxy in brain are similar after injections into live rats and under in vitro binding conditions. Intraveous injections of ^{3}H-cyclofoxy into rats yielded optimal brain to cerebellar binding ratios in homogenates, supernatants and in 24-micron thick brain sections 60 minutes after 30μCi per animal (s.a. = 16.4 Ci/mmol). Autoradiographs revealed the typical opiate-antagonist binding profile with marked retention of ^{3}H-cyclofoxy in striatal patches, the subcallosal streak, thalamus and medial habenula and little retention of label in the cerebellum. ^{3}H-cyclofoxy binding was reversible; that is, radioactivity could be removed from brain slices by washing slide-mounted sections for 30 minutes. The same brain sections, after washing, again bound ^{3}H-cyclofoxy or ^{3}H-naloxone in the same pattern, in vitro. However, when pre-washed brain sections were incubated in the presence of cold naloxone, ^{3}H-cyclofoxy binding was reduced to background levels. These data show that ^{3}H-cyclofoxy labels the well-described naloxone-sensitive opiate receptor in vivo and in vitro. The present results combined with evidence that cyclofoxy demonstrates low levels of toxicity in animals suggest that cyclofoxy is an excellent tool with which to study the physiological role of opiate receptors in living animals and humans using in vivo autoradiography and PET.

173.4 BINDING PROPERTIES OF [^{3}H] DIPRENORPHINE AND [^{3}H] ETHYLKETOCYCLAZOCINE IN GUINEA PIG BRAIN. Hurlbut, D.E.* and Leslie, F.M. Department of Pharmacology, University of California, Irvine, CA 92717, U.S.A.

The pharmacological characteristics of [^{3}H] diprenorphine (^{3}HDIP) and [^{3}H] ethylketocyclazocine (^{3}HEKC) binding to the putative kappa opioid receptor in guinea pig brain have been examined. Well washed membrane preparations were incubated at 22°C for 90 min in 50 mM Tris buffer (pH 7.4) containing enzymatic inhibitors (bacitracin, 1 mM and bestatin, 10 µM) and appropriate labelled and unlabelled drugs. Specific binding was defined in the absence and presence of levallorphan (1µM). The affinity of the two radioligands was determined by Scatchard analysis. Dose response curves for inhibition of radioligand binding by a number of unlabelled drugs were then generated and compared. Two methods were used to analyze the pharmacological properties of radioligand binding sites in anatomically defined brain regions: regional dissection and autoradiographic analysis. For autoradiography, brain sections were cryostat cut and incubated as described above. Radioligand binding was then visualized by exposure to tritium-sensitive film. Grain density was quantified by computerized image analysis (Altar et al, Science 228:597, 1985). In guinea pig cerebellum, ^{3}HDIP and ^{3}HEKC exhibited monophasic Scatchard plots with similar Bmax values. Selective µ and δ blockers did not displace radioligand binding, confirming previous reports that this tissue contains only "kappa" opioid binding sites (Robson, L.E. et al, Neuroscience 12:621, 1984). A number of other inhibitors, including dynorphin A, exhibited biphasic inhibition curves, with Hill coefficients significantly less than 1. Similar biphasic inhibition curves were obtained in guinea pig whole brain homogenate in the presence of selective µ and δ blockers (D-Pro4-morphiceptin, 300 nM, and DSLET, 100 nM). Curves could be fitted to a two site receptor model using computerized non-linear least squares analysis. There were two lines of evidence to suggest that these high and low affinity sites were independent, rather than interconverting forms of the same receptor: 1) various opioid drugs exhibited different rank orders of potency for inhibition of the two binding sites and 2) proportions of the two binding sites were found to vary in different regions of the brain. These data suggest possible heterogeneity of "kappa" opioid binding sites in guinea pig brain.

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173.5 N-ETHYLMALEIMIDE TREATMENT PREVENTS FORMATION OF A HIGH AFFINITY δ OPIOID AGONIST RECEPTOR COMPLEX WITHOUT ATTENUATING GUANINE NUCLEOTIDE INHIBITION. J.W. Spain* and C.J. Coscia, Dept. Biochem., St. Louis Univ. Sch. Med., St. Louis, MO 63104, U.S.A.

Previously, we demonstrated multi-step association of both µ and δ opioid agonists to partially purified bovine hippocampal synaptic membranes (SPM's) (Scheibe et al., J. Biol. Chem. 259, 13298-13303, 1984). Subsequent experiments suggested the involvement of a GTP binding protein (N_i) in the formation of a high affinity complex for the δ selective agonist, D-ala^2-D-leu^5-enkephalin (DADL). In the present study, NEM was used to alkylate bovine hippocampal SPM's in an attempt to gain additional insight into the mechanism of GTP action. Dissociation of ^{3}H-DADL (in the presence of 20 nM D-ala^2-mephe^4-gly-ol^5 enkephalin) from SPM's pretreated with 0.2 mM NEM for 20 min (quenched with 2 mM DTT) was compared to controls. Computerized analysis of kinetic data indicated that NEM treatment prevents the association-time dependent formation of the slowly dissociating state which has been attributed to a putative ternary complex of receptor-ligand-N_i. This interpretation is consistent with the known ability of NEM to block receptor mediated inhibition of adenylate cyclase. Computer modeling of the multi-step association, with or without NEM uncoupling, simulated binding patterns for dissociation and association that were comparable to those observed experimentally.

The effect of the GTP analog, Gpp(NH)p, on steady state DADL binding to the NEM treated SPM's differed from that previously reported for less specific enkephalins in tissue containing more µ than δ sites. The observed enhancement of Gpp(NH)p inhibition at δ sites following NEM treatment resulted in 15-20% decreased binding at 1-10 µM while in controls the same effect required 50-100 µM Gpp(NH)p. In parallel experiments the expected decrease in potency of Gpp(NH)p with µ-specific ligands was observed. If Gpp(NH)p was added to NEM-treated SPM's at the onset of dissociation, the off-rate was significantly increased and proved to be association time-independent. In control membranes, the effect of Gpp(NH)p upon dissociation was most prominent following prolonged association time periods and was negligible after a brief association. Within the framework of the ternary complex theory, these results suggest the existence of a discrete GTP binding site in addition to the GTPase present on N_i. This site, when occupied by guanine nucleotide, causes the dissociation of δ agonist. As evidenced by independent studies in our labs, this binding attenuation is reversible by divalent cations. Supported in part by NSF BNS 81-14947 and NIH HL 07050 grants.

173.6 INTERACTION OF RELATIVELY SELECTIVE DELTA OPIOID LIGANDS WITH HIGH AFFINITY MU$_1$ OPIOID BINDING SITES. Y. Itzhak and G. W. Pasternak, The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021 U.S.A.

Previous pharmacological and biochemical studies indicated that both opiate alkaloids and enkephalins which cross react with mu and delta sites display similar high affinity to mu$_1$ opioid binding sites. This study was undertaken in order to elucidate whether relatively selective delta-ligands: DSTL, [D-Pen^2, L-Pen^5] - and [D-Pen^2, L-cys^5]enkephalins display affinity for mu$_1$ sites in addition to their interaction with delta sites.

Control and naloxonazine (NAZ) treated rat brain membranes were washed extensively and binding of ^{3}H-DSTL and ^{3}H-DAGO (mu) was tested. Saturation analysis of ^{3}H-DSTL binding to control membranes revealed a biphasic Scatchard plot K_D = 0.2 nM; Bmax = 0.7 fmole/mg tissue and K_D = 3.9 nM; Bmax = 7.4 fmole/mg tissue. In contrast a linear Scatchard plot was obtained in NAZ-treated membranes (K_D = 4.4 nM, Bmax = 7.5 fmole/mg tissue). Inhibition of ^{3}H-DAGO binding by delta-ligands or ^{3}H-DSTL mu-ligands (DAGO and morphine) was biphasic, i.e. low contractions (0.5-10 nM) inhibit 5-25% of the corresponding ^{3}H-ligand binding while considerably higher concentrations were required to inhibit the rest of that binding. However, treating NAZ eliminated the inhibition of binding observed with low concentrations leaving a monophasic inhibition of ^{3}H-ligand binding.

The results presented in this study indicate that selective delta-ligands still display high affinity for mu$_1$ sites.

173.7 SYNTHESIS AND BIOLOGICAL ACTIVITY OF POTENT ANALOGS OF DYNORPHIN-(1-13) WITH HIGH AFFINITY FOR THE κ OPIOID RECEPTOR. M. Dumont, L. Lachance* and S. Lemaire. Département de Pharmacologie, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.

Various analogs of Dynorphin-(1-13) (Dyn-(1-13)) substituted in position 8 or 10 were synthesized by the solid-phase method and their specific activity was measured by their binding potency to κ opioid receptors on guinea pig cerebellum and their ability to inhibit the electrically-induced contractions of the guinea pig ileum and the mouse vas deferens. The synthetic peptides were cleaved from the resin with liquid HF and purified by successive chromatographies on Sephadex G-10 and Nucleosil C-18. Their purity was assessed by thin layer chromatography on silica gel, analytical high pressure liquid chromatography on μ-Bondapak C-18 and amino acid analysis. Binding assays were performed at 22°C for 30 min with 2 ml aliquots of the membrane preparation (Ca 1 mg wet weight) and 2 nM [^{3}H]-ethylketocyclazocine ([^{3}H]-EKC). Bacitracin (2.5 x 10^{-5} M) was added to the incubation medium to protect the peptides from enzymatic degradation. The bound ligand was separated from free by filtration though GF/B Whatman filters. Introduction of Ala in position 8 produced a marked increase in the activity of the peptide on the κ sites (Ki:0.05 nM as compared with 0.11 nM for Dyn-(1-13)) and in the mouse vas deferens (IC_{50}:11 nM as compared with 21 nM for Dyn-(1-13)). Its activity on the guinea pig ileum was slightly decreased as compared with that of the parent peptide. Other substitutions in position 8 such as the introduction of D-Ala, Trp or D-Trp caused either no change or slight decreases in the biological activity of the peptide. On the other hand, the introduction of D-Pro in position 10 of Dyn-(1-13) induced no change in the potency of the peptide for the κ receptor while its activity on the guinea pig ileum was lesser than that of the parent peptide. Finally, replacement of position 10 by Trp or D-Trp caused important decreases in the activity of the peptide in all assays. It is concluded that more potent analogs of Dyn-(1-13) with high affinity for the κ opioid receptor can be obtained by modification of the hydrophobicity and steric bulkiness of the residue in position 8.

(Supported by the Medical Research Council of Canada. M.D. is a recipient of F.R.S.Q. studentship).

173.8 BINDING SITE SPECIFICITY OF ANTI-IDIOTYPIC ANTI-OPIATE RECEPTOR ANTIBODIES TO NEUROBLASTOMA x GLIOMA OPIATE RECEPTORS J.A. Glasel and W.E. Myers, University of Connecticut Health Center, Farmington, CT 06032

We have recently reported the production and purification of rabbit polyclonal anti-idiotypic (anti-Id) anti-opiate receptor antibodies (Glasel & Myers, Life Sci., *in press*). Following affinity purification, these antibodies competitively, and reversibly inhibit binding of ^{3}H-morphine to rat neural membranes. In addition, the anti-Ids displace ^{3}H-naloxone, and ^{3}H-DADLE in a binding site-specific manner (unpublished results).

As an approach to investigating similarities between rat brain opiate receptors, and opiate receptors expressed by neuroblastoma x glioma hybrid cells, we have examined the effects of our antibodies on opiate binding to the hybrid cell receptors.

Mouse neuroblastoma x rat glioma (NG-108-15) hybrid cells were grown to confluency in culture flasks. ^{3}H-morphine, ^{3}H-naloxone and ^{3}H-DADLE binding to whole cells was determined using the standard filter assay. The effect of our antibodies on opiate binding was established by the addition of 20ug of the affinity purified antibodies to the incubation mixtures following pre-incubation with radioligand.

The anti-Id antibodies completely blocked ^{3}H-morphine binding, reduced ^{3}H-naloxone binding by greater than 50%, but had no effect on binding of ^{3}H-DADLE. This is in contrast to what we have observed in rat neural membrane assays, suggesting receptor subclass differences between the two sources.

173.9 OPIATE RECEPTORS IN RAT PITUITARY ARE CONFINED TO THE NEURAL LOBE AND ARE EXCLUSIVELY KAPPA. M. Herkenham, K. C. Rice*, A. E. Jacobson*, V. Bykov* and R. B. Rothman. Lab. Neurophysiol., NIMH (MH), Lab. Chemistry, NIADDK (KR and AJ), Bethesda, MD 20205 and Lab. Preclinical Pharmacol., St. Elizabeths Hospital, NIMH, Washington, DC 20032 (VB and RR).

The distribution, density, and subtype specificity of opiate receptors in the rat pituitary were examined by quantitative autoradiography after in vitro binding optimized to label mu, delta or kappa opiate receptors. After pre-incubation, sections for mu and delta binding were respectively incubated at room temperature for 90 min in 2 nM [^{3}H]D-ala^2-MePhe^4-Gly-ol^5-enkephalin or 5 nM [^{3}H]D-ala^2-D-leu^5-enkephalin plus 30 nM oxymorphone. Sections for kappa binding were pretreated with etonitazene and fentanyl derivatives, BIT and FIT, to irreversibly alkylate the mu and delta sites, washed, and incubated at 0°C for 3 hrs in 2 nM [^{3}H]bremazocine plus 0.4 M NaCl. Binding to BIT and FIT treated brain membranes in the 0°C/NaCl condition gave an apparent single class of receptors with a Kd of 0.45 nM, a Bmax of 378 fmole/mg prot, and a kappa profile of ligand displacement by opiate analogs. To replicate earlier studies, some sections were incubated at 0°C in 2.5 nM [^{3}H]naloxone plus 100 mM NaCl. All sections were washed, dried and apposed to LKB film for two months.

Film densitometry showed that mu and delta binding was undetectable in the pituitary but showed characteristic subtype patterns in co-processed rat brain sections. On the other hand, [^{3}H]bremazocine bound moderately densely (about the same density as in the substantia nigra and periaqueductal gray) to the neural lobe. A thin outer rim of binding had about 3-fold higher density than the central portion of the neural lobe. Binding in the anterior and intermediate lobes was not significantly higher than the blank (generated by adding 1 μM etorphine). [^{3}H]naloxone binding showed the same pattern as did [^{3}H]bremazocine, but with only about one-tenth the density.

These results indicate that opiate receptors are confined to the neural (posterior) lobe and are exclusively kappa. Specific [^{3}H]naloxone binding can be attributed to its affinity for the kappa receptor. Morphine and enkephalin bind weakly in neural lobe homogenates compared to brain (Simantov and Snyder, Brain Res., 1977, 124: 178), supporting the selective presence of kappa receptors in the pituitary. The kappa-preferring endogenous ligand, dynorphin, is densely localized to nerve terminals in the neural lobe. Future studies may show the functional relationship of kappa receptors to the endogenous peptides. In the meantime the rat pituitary, because it contains only kappa receptors, is a useful tissue for biochemical characterization of opiate receptor subtypes.

173.10 THE LABELING IN VIVO OF SIGMA OPIOID RECEPTORS IN MOUSE BRAIN WITH (+)-[^{3}H]-SKF10047. R.M. Ferris, A. Russell, F.L.M. Tang and R.A. Maxwell. Dept. of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The sigma opioid receptor, so named because of the distinct pharmacological profile produced by its prototypic agonist SKF 10047 (N-allynormetazocine), is believed to mediate mania and other psychotomimetic effects in man. Recently, a site that binds (+)-[^{3}H]-SKF10047 has been identified in guinea pig and rat brains in vitro. While this sigma receptor has received extensive biochemical and pharmacological characterization in vitro, little information is available on the nature of the sigma site in vivo. In the present study, we describe the in vivo labeling of sigma opioid receptors in mouse brain using (+)-[^{3}H]-SKF10047 as a ligand, and have attempted to compare the relative potencies of various drugs on sigma sites in vivo and in vitro. Mice were injected with 5 μCi of (+)-[^{3}H]-SKF10047 into the tail vein. After various time intervals, the mice were decapitated, their brains were rapidly removed, weighed and homogenized in 50 mM Tris-HCl buffer, pH 7.7 and total and particulate (specific and nonspecific) bound radioactivity were determined (detailed methodology will be presented). Specifically bound (+)-[^{3}H]-SKF10047 in the particulate fraction was defined as the difference in total radioactivity in the particulate fraction obtained from vehicle injected mice minus the radioactivity in the particulate fraction from haldol (2 mg/kg i.p.) injected mice. Specifically bound (+)-[^{3}H]-SKF10047 in the particulate fraction reached peak levels 30 min. after i.v. injection declined rapidly over the next 120 min., and constituted 87% of the total particulate radioactivity. Labeling of the sigma opioid sites could be blocked in vivo by injecting mice i.p. with the drug 30 min. before the i.v. injection of the ^{3}H ligand. Under these conditions, haldol was found to be the most potent compound in reducing specific (+)-[^{3}H]-SKF10047 binding with an ID_{50} of 0.75 ± 0.024 mg/kg. Other neuroleptics such as thioridazine and chlorpromazine had good potency with ID_{50} values of 8.9 ± 2.02 mg/kg i.p. and 19.2 ± 1.4 mg/kg i.p. Clozapine and spiperone were very weak inhibitors in vivo. Specific (+)-[^{3}H]-SKF10047 binding was also reduced in vivo by imipramine, dl-propranolol, bupropion, BW 234U, and (+)-EKC; but was not reduced by apomorphine, clozapine, sulpiride, naloxone and SCH23390 at doses of 50 mg/kg i.p. Phencyclidine at doses as high as 10 mg/kg i.p. only produced 36% inhibition of (+)-[^{3}H]-SKF10047 binding. The relative potencies of these agents obtained in vivo correspond well with their relative affinities obtained in vitro. One exception was noted, (+)-butaclamol was found to be approximately 300X weaker than its (-)-isomer in vitro but 8X more potent than its (-)-isomer in vivo. Thus, this in vivo binding assay should be a useful new technique for studying the effects of drugs on sigma sites in the intact animal.

173.11 DIFFERENCES IN THE MU$_1$ BINDING OF ^{3}H-DADL-ENKEPHALIN IN THE CORTEX AND THALAMUS. B. Adler, R. R. Goodman and G. W. Pasternak. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Considerable work has been directed towards the biochemical and pharmacological characterization of mu$_1$ receptors. One difficulty in these studies has been the relatively low percentage of total binding corresponding to mu$_1$ sites. Autoradiographical studies have indicated that a large portion of the binding of radiolabeled enkephalins to the thalamus is easily displaced by low concentrations of morphine sulfate, suggesting that morphine-sensitive mu$_1$ binding represents a high percentage of total ^{3}H-enkephalin binding in this region. In contrast, radiolabeled enkephalin labels predominently delta sites in cortex. We therefore compared the binding of radiolabeled enkephalins to homogenates of the rat thalamus and cortex. Competition studies indicate that 40-60% of ^{3}H-DADL-enkephalin binding in the thalamus is inhibited by low concentrations of morphine (2-5 nM). In contrast, morphine lowered ^{3}H-DADL-enkephalin binding by only approximately 5-15% in the cortex. IC_{50} determinations of morphine against ^{3}H-DADL-enkephalin from 5 independent experiments show values of 3.4 nM in the thalamus and 60 nM in the cortex. These results support the previous observations noted in autoradiography studies and illustrate the differing ratios of mu$_1$ to delta binding in these two brain regions. These differing ratios may prove useful in the development of mu$_1$ binding assays and emphasize the advantages of using tissue regions in the characterization of opiate receptor subtypes.

173.12 EFFECTS OF SIGMA AGONISTS ON LOCAL CEREBRAL GLUCOSE UTILIZATION. M. R. Kozlowski and M. J. Higgins*. Department of Medicinal Sciences, Pfizer Inc., Groton, CT 06340

The 14C-2-deoxyglucose technique has been used by several investigators to determine the effects of drug action on local cerebral glucose utilization (LCGU). In the present report, this technique is used to examine the effects of sigma receptor agonist compounds on brain activity in the rat. In order to insure that the effects observed were due to sigma receptor stimulation alone, 3 structurally different sigma agonist compounds were used: the phenylcylohexylamine, phencyclidine (PCP); the benzomorphan, SKF-10,047 (SKF) and the dioxolanylpiperidine, dexoxadrol (DEX).

PCP, SKF and DEX (3.2 umol/kg, s.c.) produced qualitatively similar patterns of altered LCGU. The largest increases in LCGU occurred in the frontal cortex, nucleus accumbens, anterior cingulate cortex, anterior caudate and the ventromedial thalamic nucleus. Smaller, but still statistically significant increases in LCGU were found in the motor and sensory cortices, sibiculum, and hippocampus. LCGU levels were also elevated in the majority of other structures examined, however these increases did not reach statistical significance. In addition to the common pattern of altered brain activity produced by all sigma agonists, increases in LCGU not common to all 3 compounds were seen in several structures including the subcallosal cortex (PCP only), posterior caudate (PCP only), posterior cingulate cortex (PCP and DEX), entopeduncular nucleus (SKF only) and inferior colliculus (SKF only).

We propose that the common pattern of altered LCGU seen in this study is the result of sigma receptor stimulation, the biochemical action shared by all of these compounds, whereas the effects not common to all 3 compounds are due to other mechanisms.

173.13 ISOLATION, PURIFICATION, AND CHARACTERIZATION OF RAT BRAIN OPIATE RECEPTORS. (SPON: N. Buckholtz). C.C. Smith, J.B. O'Neill, B. Zipser*, S.M. Shreeve, C.M. Fraser, J.C. Venter, R.J. Weber and C.B. Pert*. Neuroimmunology Unit, Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Building 10, Room 3N256, 9000 Rockville Pike, Bethesda, Maryland 20205-1000.

We have used (3-[^{125}I]iodotryosyl^{27}) β-endorphin (^{125}I-βE) as a high specific activity ligand probe to study rat brain opiate receptors. Binding of ^{125}I-βE is reversible (approximately 90%), stereospecific, and saturable. Competition experiments with [D-Ala^2, D-Leu^5]enkephalin, dynorphin$^{1-13}$, morphine, and etorphine demonstrate that ^{125}I-βE binds to opiate receptors with mu, delta, and kappa type characteristics. ^{125}I-βE was bound to rat brain membranes which where then solubilized in 1% digitonin, resulting in a relatively stable (t/½ for dissociation approximately 40 hours) ^{125}I-βE opiate receptor complex, which could be assayed on Whatman GF/C filters coated with polyethyleneimine. We then used a combination of lectin affinity chromatography, preparative isoelectric focusing and high-performance size exclusion chromatography to characterize the ^{125}I-βE opiate receptor complex and isolate it in increasingly pure form. These procedures demonstrate that the digitonin solubilized opiate receptor ^{125}I-βE complex adheres to wheat germ agglutinin:sepharose and can be eluted off with 0.2 M N-acetylglucosamine, has an isoelectric point of 4.55 ± 0.1, and a Stoke's radius of approximately 72 Angstroms. SDS gel electrophoresis of the radioiodinated isoelectrically focused, wheat germ agglutinized fraction, for example, revealed several minor bands at 14 and 19 Kd, major bands at 34 and 40 Kd and at 58 Kd, which corresponds to the molecular weight of the opiate receptor in agreement with previous reports (Klee, W.A., et al., FEBS Lett. 150: 125-128, 1982; Newman, E.L. and Barnard, E.A. Biochemistry 23: 5385-5389, 1984) and Zipser, et al., this meeting.

173.14 COMPARISON OF THE BINDING AND REGIONAL DISTRIBUTION OF SIGMA OPIOID AND PHENCYCLIDINE RECEPTORS. Patricia C. Contreras, Remi Quirion and Thomas L. O'Donohue, Exper. Ther. Branch, NINCDS, NIH, Bethesda, MD 20205 and Douglas Hosp. Res. Ctr., Verdun, Quebec, Canada H4H1R3.

The similarities between the psychotomimetic effects of sigma opioids, such as SKF10,047 and cyclazocine, and phencyclidine (PCP) support the suggestion that PCP receptors and sigma opioid receptors represent the same binding site. However, recent studies by Tam (Proc.Natl.Acad.Sci. USA 80:6703,1983) and Martin et al.(J. Pharmacol. Exp. Ther. 231:539,1984) have shown differences in the binding of PCP and SKF10,047. It has also been suggested that haloperidol, a dopamine antagonist, and N-n-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP), a dopamine agonist, exert some of their effects by binding to sigma opioid receptors. The purpose of our investigations was to examine whether PCP and sigma opioid receptors represent one class of receptors and whether haloperidol and 3-PPP bind to sigma/PCP receptors by comparing the binding and regional distribution of ^{3}H-TCP (1-(1-(2-thienyl)cyclohexyl) piperidine), ^{3}H-PCP, ^{3}H-dexoxadrol, ^{3}H-(+)SKF10,047, ^{3}H-haloperidol and ^{3}H-3-PPP. ^{3}H-TCP was found to label the same binding sites as ^{3}H-PCP because the order of potency of PCP analogs and sigma opioids for inhibition of binding of ^{3}H-PCP and ^{3}H-TCP were the same. Also, a high density of binding sites were labeled by both ^{3}H-PCP and ^{3}H-TCP in the cortex, hippocampus. and dentate gyrus. Moderate densities were found in the medial geniculate nucleus, nucleus accumbens, caudate nucleus, interpeduncular nucleus, superior colliculus, periaqueductal grey and cerebellum. Very little binding of ^{3}H-TCP and ^{3}H-PCP were found in the spinal cord, most of branstem and hypothalamus. Dexoxadrol produced PCP-like stereotyped behavior and ataxia, but the binding of ^{3}H-dexoxadrol was different from that of ^{3}H-PCP as there were different orders of potency for inhibition of binding of each labeled ligand. Also, unlike ^{3}H-TCP, ^{3}H-dexoxadrol labeled binding sites in the hypothalams. The binding sites labeled by ^{3}H-dexoxadrol in the hypothalamus and cortex were different as measured by the potency of drugs to displace ^{3}H-dexoxadrol. Also, haloperidol only displaced the binding of ^{3}H-dexoxadrol in the hypothalamus and not in the cortex. The binding of ^{3}H-(+)SKF10,047 was determined because only the (+) isomer produces stereotyped behavior. ^{3}H-(+)SKF 10,047 did not label υ,κ or δ receptors as naloxone, ethylketocyclazocine and dynorphin did not inhibit the binding of ^{3}H-(+)SKF10,047. The order of potency of drugs for displcing ^{3}H-(+)SKF10,047 was different from that found with ^{3}H-PCP or ^{3}H-dexoxadrol. The binding and regional distribution of ^{3}H-haloperidol and ^{3}H-3-PPP was also determined. These results suggest that PCP and sigma opioids bind to a heterogeneous population of receptors and that PCP and sigma opioid receptors are different.

173.15 QUANTITATIVE LIGHT MICROSCOPIC VISUALIZATION OF SIGMA OPIOID/PCP RECEPTORS WITH [^{3}H]TCP, [^{3}H](+)SKF10,047 AND [^{3}H]PCP. S.R. Zukin and R. Sircar. Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Phencyclidine (PCP), its active analogs including N-(1-[2-thienyl]cyclohexyl)piperidine (TCP) and sigma opioids such as N-allyl-normetazocine (SKF10,047) exert unique behavioral effects in animals and psychotomimetic effects in humans. Drugs of these classes have been shown by ourselves and others to bind saturably, reversibly and with high affinity to the same specific brain receptors. These receptors exhibit a characteristic heterogeneous regional distribution pattern distinct from that of any other receptor type. In binding as well as behavioral experiments, the PCP analog TCP (N-(1-[2-thienyl]cyclohexyl)piperidine) has been demonstrated to be significantly more potent than SKF10,047 or PCP. In the present study quantitative autoradiographic patterns of [^{3}H]TCP, [^{3}H]PCP and [^{3}H](+)SKF10,047 binding were determined in rat brain in order to assess the relative specificities of these ligands for sigma opioid/PCP receptors. 20 micron thick sections of fresh-frozen rat brains were incubated with 5 nM [^{3}H]TCP (58.2 Ci/mM), 10 nM [^{3}H](+)SKF10,047 (25.5 Ci/mM) or 10 nM [^{3}H]PCP (49.9 Ci/mM) respectively in 5 mM Tris-HCl, pH 7.4 for 45 min to one hr. Adjacent sections were incubated under the same conditions but in the presence of excess nonradiolabeled ligand. Slides were washed, dried and tightly juxtaposed against tritium-sensitive film for 2-6 weeks. 10 micron sections were cut from a tritium standard block ([^{3}H]Microscale, Amersham) and dry-fixed to slides; one such slide was placed against each film. Densitometric readings of each brain region as well as each standard of the microscale were recorded. Receptor densities were expressed as fmol/ mg tissue. Distribution patterns of [^{3}H]TCP, [^{3}H](+)SKF10,047 and [^{3}H]PCP binding were very similar. The highest binding densities of all three tritiated ligands were found in CA_1, CA_2 and dentate gyrus of the hippocampus. Certain brain regions rich in mu opioid receptors, including central gray, substantia nigra and interpeduncular nucleus displayed lower levels of [^{3}H]TCP than of [^{3}H]PCP or [^{3}H](+)SKF10,047 binding, suggesting higher specificity of [^{3}H]TCP for sigma opioid/PCP receptors and little cross-reactivity at other receptor classes. On the basis of its greater specificity as well as potency, [^{3}H]TCP appears superior to [^{3}H]PCP or [^{3}H](+)SKF10,047 as a molecular probe of brain sigma opioid/PCP receptors.

173.16 DOPAMINE RECEPTOR-REGULATED MU- AND DELTA-PROFILE OF AN OPIOID RECEPTOR COMPLEX COUPLED TO ADENYLATE CYCLASE. A.N.M. Schoffelmeer*, J.C. Stoof* and A.H. Mulder* (SPON: H.W.M. Steinbusch). Dept. of Pharmacol. and Neurol. (JCS), Med.Fac., Free University, van der Boechorststraat 7 1081 BT Amsterdam, The Netherlands.

Adenylate cyclase activity (determined as the efflux of cAMP) in rat neostriatal slices is regulated by stimulatory D-1 dopamine receptors and inhibitory D-2 receptors (Stoof, J.C. and Kebabian, J. W., Nature 294, 366-368 (1981)). Recent studies, examining ligand binding to rat brain homogenates (Rothman, R.B. and Westfall, T.C., J. Neurobiol. 14, 341-351 (1983)) as well as smooth muscle bioassays (Lee, N.M. et al., Biochem. Psychopharmacol. 33, 75-89 (1982)) have suggested that mu- and delta-opioid receptors might not always be independent entities, but different recognition sites of an opioid receptor complex. Here we report on the inhibitory effects of mu- and delta-opioid receptor activation on dopamine receptor regulated cAMP efflux from rat neostriatal slices. Both morphine and D-ala^2-D-leu^5-enkephalin (DADLE) inhibited D-1 receptor induced cAMP efflux (30/uM dopamine + 10/uM (-)sulpiride) with an EC_{50} of 800 and 3 nM, respectively. The mu-opioid receptor antagonist naloxone was more potent in reversing the inhibitory effect of morphine, whereas the delta-opioid receptor antagonist ICI 174864 was a much more potent antagonist for DADLE, suggesting the involvement of mu- and delta-opioid receptors. Upon simultaneous activation of D-1 and D-2 receptors (by 30/uM dopamine in the absence of (-)sulpiride) the EC_{50} of morphine remained unchanged, while that of DADLE was strongly enhanced to 100 nM. In this case, naloxone was an equipotent antagonist for both opioid receptor agonists, suggesting the involvement of mu-opioid receptors only. Similar results were obtained upon stimulation of D-1 receptors with the D-1 agonist SKF 38393 and stimulation of D-2 receptors with the D-2 agonist LY 141865. Moreover mu-opioid receptor efficacy appeared to be strongly diminished upon activation of delta-opioid receptors. Therefore these mu- and delta-opioid receptors cannot be regarded as independent entities, but appear to be functionally coupled. Our data provide functional evidence for the existence in the neostriatum of an opioid receptor complex with closely associated mu- and delta-recognition sites. The pharmacological profile of this receptor complex appears to be regulated by dopamine receptors.

PROCESS OUTGROWTH: GUIDANCE MECHANISMS, GROWTH CONES

174.1 REARRANGEMENT OF AXON FASCICLES IN THE DECUSSATION OF THE PYRAMIDAL TRACT. K. Kalil and C. Norris*. Dept. of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

Axons of the hamster pyramidal tract are grouped into well-defined fascicles along their entire trajectory from the sensorimotor cortex to the spinal cord, an arrangement particularly striking in young animals. We were interested in whether axons maintain the same spatial relationships along this pathway such that selective fasciculation could play a role in target innervation.

In one series of experiments, HRP was injected into the sensorimotor cortex of animals 1-4 days of age. In some cases small lesions were made in the center of a large injection site. Labeled axons within the internal capsule are arranged in fascicles that reflect the topography of their cortical neurons, as shown by the fact that within the internal capsule the lesions create empty unlabeled spaces normally occupied by several contiguous fascicles. Small injections of HRP in the opposite hemisphere (corresponding to the location of the ipsilateral lesion) resulted in the labeling of a small contiguous group of fascicles similar in position to the unlabeled regions of the other side. This topographic order is maintained until the pyramidal tract enters the decussation in the caudal medulla at 40-44 hours after birth. As fibers cross, the overall pattern of axon fascicles is rearranged, reversing the order of fascicles from right to left as they enter the dorsal column.

In further experiments, HRP-coated glass pipettes applied to the medullary pyramid filled axons locally in the region of the decussation in animals 2 days to 2 weeks of age. At the decussation individual fibers do not maintain their original positions but join new fascicles. Some axons pass over many fascicles before finding the appropriate partners. When axons are followed into the rostral spinal cord, it is apparent that those that were contiguous in the medullary pyramidal tract are now widely separated in the dorsal column. Thus, the spatial order of axon fascicles in the spinal cord is not determined by merely passive channeling of axons to their targets in the same topographic order in which they leave the cortex. Further, temporal order does not seem to be a factor in determining nearest neighbor relationships in the decussation, since lead fibers are often widely separated from later-arriving axons. The results suggest that fibers actively seek out specific fascicles at the decussation, a process which may play a role in their ability to find appropriate targets in the spinal cord.

Supported by NSF Grant BNS-8311517 and NIH Grant NS-14428.

174.2 PHYSICAL AND MOLECULAR FACTORS THAT INFLUENCE AXONAL GUIDANCE AT THE DEVELOPING OPTIC CHIASM. M. Poston*, U. Rutishauser and J. Silver (SPON: I.R. Kaiserman-Abramof). Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

We are interested in the physical and molecular characteristics of boundaries that may play a role in axonal guidance along the optic pathway in the developing vertebrate embryo. We have studied the development of the presumptive optic chiasm in 2-5 day chicken embryos. When pioneering optic fibers approach the chiasm they never grow rostrally into the olfactory region of the telencephalon, even though a potential avenue of tissue joins these two areas. Conversely, olfactory tract axons grow as far caudally as, but never cross, the forward boundary of the optic chiasm.

A region of specialized neuroepithelium immediately rostral to the optic chiasm, first described by Silver (J.C.N. 223, 1984) in the mouse embryo and called the "knot", exhibits unusual cellular and molecular properties which may influence axonal guidance at this major intersection. The "knot" consists of a very dense cluster of cells which lack long radial processes and whose cell bodies are situated immediately along the basal lamina of the neuroepithelium, thus eliminating the marginal zone where axons tend to grow. We saw no indication that the neural cell adhesion molecule (NCAM), an adhesion promoting membrane glycoprotein that is expressed elsewhere along neuroepithelial endfeet within the optic pathway (Silver and Rutishauser, Dev. Bio. 106, 1984), was expressed by the cells in the knot region. NCAM is produced by cells of the presumptive chiasm (both before and after the arrival of optic axons on day 4) and by the ventrocaudal telencephalon (presumptive olfactory tract) rostral to the knot. The absence of NCAM staining at the knot was consistent across the diencephalic/telencephalic junction. A tremendous amount of cell death was also observed pre-axonally and precisely in the pre-knot region.

We propose that the knot, through its elimination of the marginal zone endfeet and its lack of NCAM, functions as an axon-refractory, physiochemical barrier which effectively separates the optic and olfactory systems. If correct, this proposal would offer a possible explanation of a fundamental observation concerning forebrain sensory projections, that retinal ganglion cell axons do not normally directly innervate the telencephalon and that olfactory axons do not travel caudally into the diencephalon to form direct connections with the thalamus. Supported by NSF(BNS8218700)

174.3 AGE AND SECTORAL ORIGIN OF GANGLION CELL AXONS ARE MAPPED ON TWO ORTHOGONAL AXES IN THE OPTIC PATHWAY OF GOLDFISH. R. Bernhardt and S.S. Easter, Jr., Univ. of Michigan, Ann Arbor, MI 48109.

The polar coordinates of the parent ganglion cell somata in the goldfish retina are reflected by the locations of their axons in the cross-sectioned optic nerve near the nerve head. The distance from the optic disc, r, is mapped on an axis extending from base to base of the roughly trapezoidal cross section. Sectoral origin (θ) is mapped on a second, orthogonal axis. Ventral retinal axons occupy the slanting sides of the trapezoid, axons from dorsal retina occupy the center, and temporal and nasal axons, intermediate positions (Easter et. al., J. Neurosci., 8:739, 1981), similar to the pattern in cichlid fish (Scholes, Nature, 278:620, 1979).

To study whether a similar map also exists more centrally in the optic pathway, we have labeled two groups of axons in the same nerve by punctate application of HRP to 1) fascicles in the retinal fiber layer, labeling axons from a common sector (similar θ), and 2) fascicles in the tectal stratum opticum, labeling fibers of similar age, originating from a common annulus in the retina (similar r). The labeled axons were traced in serial transverse sections from the retina through the tectum.

Axons of similar age remain clustered throughout the optic pathway, whereas axons of common sectoral origin spread over a larger cross-sectional area in the proximal nerve and contract again as they approach the tectum. The precise map observed near the optic nerve head is obscured in the nerve by: (1) this dispersal of fibers of common θ, (2) some ribbon-like folding, and (3) the general increase in diameter of the optic nerve. However, the two labeled populations remain roughly orthogonal to each other throughout the nerve and tract.

Mapping along the two orthogonal axes is obvious in cross-sectioned ventral and dorsal brachia, which carry fibers of dorsal and ventral hemiretinal origin, respectively. The youngest axons (large r) run close to the pial surface, the oldest axons (small r) are located deep, on the opposite side of the brachium. θ is mapped orthogonal to this age axis. Axons of temporal retinal origin run on the central side of the brachium, close to the nucleus rotundus, axons of nasal retinal origin are farthest from it, axons from intermediate retinal locations occupy intermediate positions. This fiber arrangement anticipates the order of exit of the axons from the brachium into the tectum, i.e. the temporal ones exit rostrally, the nasal ones, caudally. Fibers rearrange between the retina and the tectum, but the principle of orthogonally mapping sectoral and radial origin is retained, suggesting that common mechanisms might govern axon topography throughout the optic pathway. (Supported by EY-00168 to SSE.)

174.4 PROJECTION PATTERNS OF HIPPOCAMPAL TRANSPLANTS IN ADULT HOST HIPPOCAMPUS VISUALIZED BY THY-1.1 IMMUNOHISTOCHEMISTRY. C-F. Zhou*, R.J. Morris*, R.M. Lindsay*, P.J. Seeley*and G. Raisman., Lab. of Neurobiology and Development, National Institute for Medical Research, Mill Hill, London, NW7 1AA, United Kingdom.

Transplantation of neural tissue can be used to shed light on the adult brain's capacity for change and the rules that govern such change. In our transplant experiments, grafts of embryonic mouse hippocampus were placed in the hippocampal formation of host adult mice. Donor tissue, either as primordia or dissociated cell fractions transplanted in a plasma clot, was from the mouse strain A/Thy-1.1 and therefore carried the 1.1 allelic form of the Thy-1 antigen. Host animals were congenic strain A mice expressing the alternative Thy-1.2 allele. Fibres projecting from the transplant could therefore be visualized by Thy-1.1 immunohistochemistry as positive stain against a negative (Thy-1.2) host background.

Two main types of projection were observed: mossy-fibre-like and fimbria-like. Mossy-fibre-like projections generated by transplanted granule cells were visualized as strong staining along the host suprapyramidal layer and weaker infrapyramidal staining along the normal mossy fibre pathway. These projections occurred where transplants interrupted host CA3 and their form depended on transplant position along CA3 and on the location of damage caused by the transplantation procedure. It appeared that deafferentation of neuropil supplied by host mossy fibres was necessary for the transplant projections. Fimbria-like projections from transplanted pyramidal cells occurred either along the commissural/associational band of the dentate molecular layer for transplants positioned at the mouth of the dentate gyrus or along host stratum oriens and stratum radiatum for transplants intersecting host CA3 at its juncture with the fimbria.

Thus projections from transplants appear to invade regions of denervated neuropil and are organized according to rules that are similar to those pertaining to normal fibre projections during development.

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174.5 TRANSPLANTATION OF FETAL LATERAL GENICULATE NUCLEUS TO THE OCCIPITAL CORTEX: CONNECTIVITY WITH HOST'S AREA 17. M. A. MATTHEWS. Dept. Anat., L.S.U. Medical Center, New Orleans, LA. 70119.

The developing lateral geniculate complex was excised from fetal albino rats at 18 days of gestation and implanted into the occipital cortex of host animals at 5 days of postnatal age. Groups of host animals were sacrificed at 10, 20 and 30 days following this procedure. The transplant tissue of selected animals was stereotaxically lesioned two days prior to scheduled sacrifice and their brains subjected to either Fink-Heimer or electron microscopic analysis of the distribution and density of degenerating efferents from the transplant. The remaining animals were analysed by means of Bodian, Golgi-Cox or electron microscopic techniques.

Transplanted neurons displayed typical dendritic branching patterns of geniculate relay neurons by 20 days following implantation. Intrinsic neurons, characterized by a small ovoid soma and two main stem dendrites, only became evident in transplant tissue by 30 days and were much reduced in number. Synapses developed by 10 days and rapidly increased in number by 20 and 30 days. Most complexes were simple axo-dendritic, asymmetric junctions. Multiple serial and reciprocal complexes, as well as the characteristic glomerular complex, failed to appear.

Analysis of Bodian stained material revealed a dense network of fibers coursing about the transplant. Distinct bundles of these fibers were observed extending from the medial edge of the transplant into area 17 by 20 days following implantation. A Fink-Heimer analysis of animals whose transplants were stereotaxically lesioned revealed degeneration in Layers II-VI of the primary visual cortex but the majority of these fibers terminated within the lateral two-thirds of Layer IV. Few degenerated fibers could be found in the underlying white matter indicating that efferents from the transplant found their way to their "correct" target zone by growing through a complex neuropil which provided minimal physical substrates to guide such growth. Most of the contacts formed by these fibers were simple junctions along the shafts of dendrites with a wide range in diameter.

It is concluded that the nearby host visual neurons, which are the correct target cells for the efferents arising in the transplant, induced a directed growth of these fibers.

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174.6 TRANSPLANTATION OF LABELED BRAIN CELL SUSPENSIONS BETWEEN SPECIES. J. Wells, B.P. Vietje, D.G. Wells, M. Boucher and R.P. Bodony. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Cross-species cell suspension transplants to the hippocampal formation have proven to be successful in restoring function after fornix lesions. In such transplants the cells do not remain aggregated and have been difficult to locate in the host. In order to describe the migration and differentiation of the transplanted cells, the cell suspensions were labeled prior to injection into the denervated host hippocampus. The transplanted cells were labeled with 1% and 5% HRP, 1% HRP conjugated to wheatgerm agglutinin (WGA) and by an antibody to WGA. A variety of fluorescently labeled compounds (WGA/FITC, Lucifer yellow and PHA-L) were also used, but, because there was autofluorescence and phagocytosis of fluorescent particles in the host, the separation of host cells from donor cells could not be made unequivocally. Cells to be labeled and injected were from E15-E17 mouse embryos and were transplanted into adult rat hosts. The hosts were sacrificed 7-11 days after transplantation. Control injections into the dentate gyrus using the same procedures but injecting only 1% HRP with no cells showed only lightly labeled contiguous cell bodies in the dentate granule cell layer.

Labeled neurons and glia survived in the host hippocampal formation. Many cells had migrated away from the injection site and it was rare to see neurons and glia within the injection site. The labeled cells had differentiated and grown to exhibit extensive processes. Some of the labeled processes had structures which appeared to be growth cones and filopodia. The position of the transplanted cells was dependent on the injection site in the hippocampal formation. When the injection was deep, there was a propensity for the cells to be oriented along the dentate granule cells. In those labeled cells which were found in the dentate granule cell layer, the differentiating processes were most frequently oriented toward the molecular layer rather than the hilus. None of the labeled neurons looked like dentate granule cells. If the injection was between hippocampus and dentate, most cells were oriented along the obliterated hippocampal fissure with processes that bridged the fissure. In more dorsal injections, cells were seen oriented along the pyramidal cell layer. Since the HRP reaction product is electron dense, the transplanted cells can be observed ultrastructurally. Distinct synapses on labeled processes were difficult to discern at these survival times, but vesicle-containing profiles were observed in close apposition to labeled membranes. This method will be an important adjunct to the techniques for studying the migration, growth and differentiation of neurons and glia.

174.7 **LIGHT MICROSCOPIC ANALYSIS OF MIGRATION OF SYMPATHETIC PREGANGLIONIC NEURONS IN CHICK EMBRYO.** Anita Prasad* and Margaret Hollyday. Dept. Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

We have been looking at the normal development of the Terni Column (TC) in the chick embryo. The TC is comprised of visceral motoneurons in the spinal cord which project to sympathetic ganglia via the ventral roots. From their origin in the ventricular zone these neurons, along with the somatic motoneurons, migrate ventro-laterally. As originally described by Levi-Montalcini (1950) the TC cells subsequently undergo a secondary migration traveling medially and dorsally to their adult position adjacent to the central canal.

As a preliminary study for experiments addressing factors controlling this migration, we have reexamined the migration of these cells using retrograde transport of HRP. HRP conjugated to WGA was injected into the ventral root. The HRP reaction product was visualized by processing the tissue with DAB following a cobalt intensification step. This technique produces Golgi-like staining of the labeled cells which enabled us to study the morphology of the TC cells during migration. We identify Terni cells as those cells positioned medial to the commissural (arcuate) fibers which separate the motor column from the ventricular epithelium and labeled by virtue of having an axon in the ventral root.

Before stage 26, the HRP labeled cells formed a compact, seemingly homogeneous, cluster of cells in the ventrolateral portion of the spinal cord. Some of these cells showed signs of early dendrite formation but the majority were spherical and none of these cells had labeled processes extending medial to the commissural fibers. We interpret this cluster as consisting of both somatic motoneurons and Terni cells prior to their secondary migration. Presumptive migrating Terni cells were first seen at stage 26. These cells had slender processes extending medially, which typically interdigitated with cells of the ventricular epithelium. While some cells were bipolar in shape, the majority of the labeled cells had two or more processes extending medially and/or dorsally to the neuron soma. No growth cone-like specializations were visible at the ends of these processes. The number and complexity of the processes extending from the cell bodies increased in older embryos. Neurons appeared to reach their permanent location and to initiate dendrite formation as early as stage 30. In contrast to the processes extending from labeled cells along the migratory pathway, these incipient dendritic processes were thicker and more irregularly shaped, and commonly ended in enlarged club-shaped specializations. Some of these dendrites extended across the dorsal midline.

Labeled axons were seen in the sympathetic ganglion in stage 26 embryos, the stage when the retrograde migration begins. We are pursuing experiments to determine whether normal migration of the Terni cells depends on the presence of sympathetic ganglion cells.

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174.8 WHOLEMOUNT VIEWS OF AXONAL GROWTH CONES IN THE DEVELOPING SPINAL CORD OF XENOPUS. R.H.Nordlander. Dept. Oral Biology, Case Western Reserve University, Cleveland, OH 44106.

Growth cones at the tips of developing longitudinal spinal axons of Xenopus embryos and larvae were filled anterogradely with horseradish peroxidase (HRP) from a number of sites in the brainstem, the spinal cord, and the periphery. After a suitable interval, usually one hour or less, animals were fixed in a solution of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.3. Spinal cords were dissected free of surrounding tissues, reacted with the reagents of Hanker et al (Histochem.J., 9:789, 1977), and viewed whole in glycerin.

The configurations of the growth cones observed here varied quite widely. Those of primary sensory (Rohon-Beard) neurons were only slightly enlarged and displayed almost no filapodia either at their ascending or descending tips. Those of dorsal root afferents were also generally simple though some filapodia were occasionally observed. Growth cones at the lead of their finer collatral branches, however, were delicate and frilly.

Growth cones of descending axons in the ventral and lateral fasciculi were labeled from sites in the brainstem or spinal cord and belonged to a number of different cell types (Nordlander,R.H., J.Comp.Neurol., 228:117, 1984). These were best seen in lateral views of the cord and were the most variable in shape. Most of these growth cones were bulbus in outline with a few filapodia usually at their apices. Others seemed to be flattened in a plane parallel to the surface of the cord with several webbed filapodia. Still others spread widely over the lateral fasciculus with parallel filapodia reaching into multiple parallel longitudinal tracts.

Axons trailing behind most of these growth cones were straight and constant in dorsoventral position over their entire lengths. In a few cases, however, the trailing axon "hopped" from one parallel course to another and back again as if it had been pulled caudally in the wrong fascicle for a short time and then had corrected its course. Variocosities along the lengths of these developing axons were most common at younger stages.

Growth cone morphology in this system is probably influenced by the same factors that control growth cone shape in vitro (Letourneau,P.C., Devel.Biol. 44:77 and 92, 1975). These include the configuration of the substratum and the adhesiveness of the substratum.

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174.9 FUNCTIONAL INTERACTIONS BETWEEN IDENTIFIED GROWTH CONES AND MUSCLES IN EMBRYONIC ZEBRAFISH. Judith S. Eisen, Paul Z. Myers*, and Monte Westerfield. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Embryonic zebrafish first exhibit spontaneous muscle contractions at about 18 hrs post-fertilization (17 somites at 28.5°C). These contractions begin as localized twitches in individual segments with no apparent coordination between segments or between left and right halves of the same segment. Twitches can first appear in any trunk segment (1-15), and later develop in other segments in no apparent sequence. We have correlated the first appearance of primary motoneuron growth cones with the onset of twitching, and suggest that these early muscle contractions are neurogenic in origin.

Primary motoneurons were identifiable prior to axogenesis by their size and position in the spinal cord. The first primary motoneuron to initiate axogenesis in each segment invariably was located on the ventrolateral aspect of the spinal cord and in the middle of the segment. Within a few minutes after the growth cone of this identified cell left the spinal cord, localized twitches were observed in that segment. These twitches consisted of single contractions that repeated at irregular intervals of 2 sec to 2 min and were restricted to 2-4 muscle fibers at the region of the horizontal septum separating the ventral and dorsal regions of the muscle. These fibers are the first to express mature properties including cross striations and acetylcholine esterase activity. The growth cone was within 20 µm of these fibers when contractions were first observed.

These early contractions appear before primary motor growth cones make obvious contacts with the muscle fibers and certainly before organized neuromuscular junctions are present; this may indicate that subsequent coordinate development of the muscle and motoneurons depends on neuromuscular activity.

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174.10 REGENERATING OPTIC NERVES PRODUCE STEADY CURRENTS AT THEIR TIPS. John A. Freeman. Dept. of Anatomy, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

Previous studies have shown that growth cones of retinal ganglion cells in culture generate steady inward calcium currents across their tips as they grow (Freeman et al, J Neurosci. Res., 1985). The present study was undertaken to determine whether such currents are also produced during regeneration of the optic nerve in vivo, which would imply that they may play a critical role in the process of nerve growth. Small (1.0-1.5 mm) incisions were made in the medial branch of the optic tract of adult toads (Bufo marinus) on the dorsal surface of the optic tectum. Field potentials evoked by stimulation of the distal portion of the optic tract were recorded to verify which portion of the tract had been severed, and the site of the injury was recorded with a video camera/ frame buffer and stored in a PDP 11/34 computer. Five days later, when the majority of cut optic nerve fibers have developed growth cones and have begun to regenerate, the animals were re-anethetized, and the regions of the optic tract containing the regenerating fibers were mapped with a 2-dimensional vibrating microprobe (Freeman et al, op cit). A consistent pattern of current densities was found at each active site. This consisted of a distribution of positive currents flowing inward at the sites of regeneration, and oriented parallel to the axis of the optic nerve fibers. The current densities ranged in amplitude from 5×10^{-6} A/cm^2 to 7×10^{-5} A/cm^2 at a distance of approximately 100-200 um from the site of the regenerating fiber tips. The video system was used to superimpose the measured current density vectors on the image of the region from which they were recorded, for subsequent microscopic correlation. Application of a Ringers solution containing 0.5mM Ca^{2+} and 100 um La^{2+}, which is known to block calcium currents through calcium channels, abolished the measured currents within 20-30 minutes, suggesting that the regeneration currents are carried principally by calcium ions. Control lesions of the optic tract mapped at the time of injury generated small transient currents, which in contrast to the regeneration currents, decayed rapidly over 1 hour, and were not blocked by La^{2+}. These results complement those of Borgens et al (PNAS, 1980) who found that persistent currents entered the transected lamprey spinal cord. I conclude that regenerating nerve fibers generate substantial steady currents across their tips, which are likely to play a significant role in nerve growth, possibly related to the transport and fusion of membranous vesicles, the electrophoretic transport and alignment of membrane macromolecules, and the release of neurotransmitter, as previously suggested (Freeman et al, op cit).

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174.11 SPINAL CORD EXPLANTS CULTURED ON CARBON FILAMENTS AND STIMULATED WITH DIRECT CURRENT: AN EM STUDY. T. Khan, G. Gaik, J. Luberda*, Rehab R&D Center, VA Hospital, Hines, IL

In previous studies, we observed that carbon filaments provided a supporting framework or scaffolding for growing processes in cultures of spinal cord explants. In addition, morphometric techniques demonstrated that direct electric currents applied to carbon filaments enhanced growth of neurites within the cultures. This study focuses on the electron microscopic appearance of the growing explants on non-stimulated and electrically stimulated carbon filaments.

The culture assemblies consisted of approximately 3000 parallelly aligned carbon filaments (8-10 mm diameter) that were attached to the bottoms of 35 mm plastic petri dishes. In electrically stimulated chambers, the ends of the filaments extended through and beyond the dishes and were attached to a direct current source. Two millimeter thick lumbar spinal cord segments from 17 day old rat embryos were prepared under sterile conditions. All explants were grown for three weeks in modified Dulbecco Eagle medium supplemented with 20% fetal calf serum, 0.6% glucose, and 10% penicillin-streptomycin at 37 degrees C in a humidified 95% air and 5% CO2 atmosphere. Specimens for EM observation were fixed at room temperature for 1 hr in 2% glutaraldehyde and 2% formaldehyde in 0.1M phosphate buffer PH 7.4. After rinsing tissues were postfixed in 1% osmium textroxide for 1 hr, dehydrated and embedded in araldite.

Light microscopic observation of living cultures of both non-stimulated and stimulated explants showed that the explants grew between and parallel to the long axis of the carbon filaments. It was noted in the 1 micron plastic sections that some of the cells had migrated along the carbon filaments beyond the boundries of the original explant.

With the electron microscope, the non-stimulated cultures appeared to consist of immature neurons with few neurites and well developed glial cells. The cytoplasm of the neurons contained swollen-appearing golgi and smooth endoplasmic reticular cisternae, scattered fragments of rough edoplasmic reticulum, and some mitochondria. Neurites were few in number, and no synapses were observed. The ultrastructural morphology of the stimulated explants differed in that the neurons were mature in their appearance. The cytoplasm of these cells consisted of a well defined golgi apparatus, nissl material, and mitochondria. Many large dendrites and axons containing well defined microtubules were noted. Axodendritic and axosomatic synapses were frequently observed. The glial cells were well developed.

In conclusion, our electron microscopic data confirmed our previous morphometric observation that growth of spinal cord explants cultured on carbon filaments was enhanced by stimulation with direct current. (supported by VA and PVA Vaughan Chapter)

174.12 NEUROPEPTIDES OR THEIR PRECURSORS ARE PRESENT IN GROWTH CONES OF APLYSIA NEURONS IN VITRO. M. S. Flaster, R. T. Ambron, and S. Schacher. Ctr. for Neurobiol. & Behav., Depts. Anat. & Cell Biol., Columbia Univ., College of P&S, and NYS Psychiat. Instit., New York, NY 10032

Recent in vitro studies indicate presence and release of conventional neurotransmitter at growth cones. Are these findings generalizable to peptide neurotransmitters? Here we report evidence that neuropeptide transmitters or their precursors are present in neuronal growth cones in vitro.

Identified neurons were removed from the abdominal ganglion of the marine mollusc Aplysia californica and placed into cell culture as previously described (Schacher, S., & Proshansky, E., J. Neurosci., 3:2403, 1983). Reagents used for indirect immunofluorescence included anti phe-met-arg-phe-amide (FMRFa) antiserum (Peninsula Laboratories) and 3 anti-R3-R14 peptide antisera of differing sequence specificity (gifts of Dr. R. Scheller).

Anti-FMRFa antisera on cells R2, L2-L6, L12 and L13 yields positively staining cell bodies and intensely staining varicosities and growth cones. All these cells have previously been shown to contain FMRFa-like immunoreactivity in vivo (Brown, R.O. et al., Soc. Neurosci. Abstr., 10:691, 1984). Other cells including L11 and the right upper quadrant (RUQ) cells are non-immunoreactive in vivo and also fail to stain in vitro.

RUQ cells fluoresce intensely and specifically with all 3 anti-R3-R14 antisera as has been previously reported for these cells in vivo (Kreiner, T. et al., J. Neurosci., 4:2581, 1984). Cell bodies stain positively and growth cones and varicosities stain intensely. In all growth cones, fluorescence was more intense in the proximal portion and far less intense in distal regions displaying filopodia. In well spread growth cones, the staining was punctate. RUQ growth cones can be isolated for biochemical analysis (Flaster, M. et al., Soc. Neurosci. Abstr., 10:923, 1984). Following a 24 hr exposure to ^{35}S-methionine, growth cone fractions were collected and compared to RUQ cell bodies, primary RUQ neurites free of growth cones, and the neurites and cell bodies of other identified neurons using SDS-PAGE and fluorography. A prominent band migrating with M_r of 11 kD was found to be uniquely present in all RUQ cell fractions including growth cones. A species of similar M_r has been previously reported to be the R3-R14 propeptide (Kaldany, R-R. et al., Ann. Rev. Neurosci., 8:431, 1985). The presence of the propeptide in the growth cone is consistent with our immunocytochemical results, although smaller, more fully processed peptides may also contribute to the immunocytochemical signal. We also find a prominent peptide species with M_r of 14.5 kD uniquely present in RUQ cell bodies, but absent from other cells and the neurites or growth cones of RUQ cells. The identity of this species remains to be established but it may be related to the R3-R14 pre-propeptide which is similar in size (Nambu, J.R. et al., Cell, 35:47, 1983).

While the presence of neuropeptides in growth cones in vivo remains to be established, the presence of neuropeptide or precursor in the growth cones of neurons in vitro suggests a possible role for these peptides in the earliest interactions between synaptic pairs.

174.13 ULTRASTRUCTURAL LOCALIZATION OF IONS IN NEURONS BY ELECTRON PROBE MICROANALYSIS. D.A. Spero*, K.J. Doane*, I.C. Piscopo* and F.J. Roisen (SPON: G. Krauthamer). Dept. Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854 and Philips Electronic Instruments, Mahwah, NJ 07430.

We employed a combination of established morphological techniques in a novel manner to determine the regional distribution of ions in Neuro-2a murine neuroblastoma and primary cultures of dissociated 9-day embryonic chick dorsal root ganglia (DRG). These techniques involved electron probe analysis of quick-frozen, freeze-substituted whole-cell mounts at the ultrastructural level. Neuro-2a and DRG neurons were prepared and grown as described previously (Roisen et al., J. Neurobiol., 4:347-368, 1972; Spero and Roisen, Dev. Brain Res., 13:37-48, 1984). Cells were plated on Formvar-carbon-coated gold grids treated with poly-L-lysine. Cultures were grown for 24-48 h in an atmosphere of 5% CO_2 in air at 35°C. Cultures were drained free of residual medium and quick-frozen by immersing into Freon 22 cooled to its melting point with liquid nitrogen. Samples were freeze-substituted in a mixture containing 1% glutaraldehyde and 0.02 M cacodylate buffer (pH 7.2) in absolute ethanol at -80° C for 2 days. Cells were critical-point-dried in CO_2, coated lightly with carbon and examined with a Philips EM420T equipped with an EDAX 9100 X-ray microanalysis system. Whole-cell transmission electron micrographs were taken of representative cells at 80 kV. Multiple electron probes of 1 um diameter were made in representative intracellular regions including the soma, neurite shaft and growth cone to determine quantitatively the presence of ions in these regions. Background counts obtained from noncellular areas were stored in a microprocessor and substracted from those obtained in cellular regions. The Na, Mg, P, Cl, K and Ca concentrations of the various regions of neurons were not significantly different. However, the concentration of zinc in the neurite and growth cone were increased when compared to the soma in both Neuro-2a and DRG neurons. A comparison of the ion concentration in neurons with non-neuronal cells in DRG cultures revealed higher Ca levels in the non-neuronal cells. These results indicate that the quick-freeze, freeze-substitution method yields high quality ultrastructural preservation, while retaining elements for microprobe analysis. Studies are in progress to determine the developmental changes in the distribution of zinc and its association with a subcellular compartment during neuritogenesis.
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174.14 POLARIZED COMPARTMENTALIZATION OF ORGANELLES IN GROWTH CONES FROM DEVELOPING OPTIC TECTUM. T.P.O. Cheng* and T.S. Reese, Lab. of Neurobiology, NINCDS, NIH, at the Marine Biological Lab., Woods Hole, MA 02543.

Computer-assisted reconstructions of continuous serial sections have been used to study the cytoplasmic organization of growth cones in vivo. Optic tecta from 6¼-6½ day old chicken embryos were quick-frozen and then freeze-substituted in acetone-osmium tetroxide (5%) or, for comparison, prepared by conventional fixation (2% glutaraldehyde, 2% formaldehyde and 0.5% acrolein in 0.2M cacodylate buffer, pH 7.2). Ten growth cones were reconstructed from aligned serial electron micrographs. In the freeze-substituted growth cones, numerous lumenless membrane-bound sacs and arrays of multilamellated stacks replaced the abundant smooth endoplasmic reticulum (SER) found after chemical fixation. Microtubule fascicles were well organized in the neurites, but progressively diverged from their typical fascicular organization in the initial segment (transition zone) of the growth cone, and were essentially absent in its varicosity and more distal segment. In contrast, mitochondria were concentrated in the proximal segment of the varicosity while the multilamellated stacks and the endosomal vacuoles were concentrated in the distal segment. Coated pits and vesicles were concentrated near the terminal filopodium in the most distal and organelle-poor domain of the growth cone. Five structural domains are thus delineated along the longitudinal axis of the growth cones on the basis of the spatial distributions of the intracellular organelles: terminal neurite; transition zone; proximal and distal segments of the varicosity; and terminal filopodium.

Measurements were made of the distribution density of coated pits, microtubules, mitochondria, coated vesicles, endosome-like vacuoles, and multilamellated stacks of lumenless membrane-bound sacs (membrane discs) in the structural domains along the growing axis of the growth cone from their serial reconstructions. The polarization of the cytoplasmic compartments, as defined by the distribution of intracellular organelles in the growth cones, was evident when these measurements were pooled, supporting the observed differences in the distribution of various organelles listed above.

In conclusion, the morphological observations suggest that dilation of and fusion among the lumenless membrane-bound sacs, resulting from chemical fixation, may have given rise to the SER network, while the three-dimensional reconstructions suggest that cytoplasmic components of growth cones are polarized along the longitudinal axis of growth, which may reflect their role in membrane recycling.

174.15 RAPID QUANTITATION OF NEURITIC OUTGROWTH WITH A MICROCOMPUTER-BASED IMAGE PROCESSOR. T.A. Dahlberg*, T.S. Ford-Holevinski* and B.W. Agranoff. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

The quantitation of neurite outgrowth in explant culture is typically based on visual estimates of outgrowth density, average length, grid crossings or area of outgrowth which may then be incorporated into a formula determining total outgrowth. These methods tend to be tedious and subject to operator bias. Taking advantage of current microprocessor technology, we have constructed an inexpensive device of commercially available components which will accurately determine the degree of neuritic outgrowth in our explant cultures in 30 sec entirely independent of user input. The components of this system are: an Apple II microcomputer, a black and white video camera, a circuit board to digitize the video signal (Dithertizer II; Computer Stations, Inc., St. Louis, MO) and a 68000 coprocessor board to store and analyze the digital images (Dtack Grande; Digital Acoustics, Santa Ana, CA). Software written in 6502 and 68000 assembly languages allowed this device to be used as an image processor. Interfaced with a Leitz Diavert microscope and utilizing pseudo-darkfield microscopy, the image processor was used to acquire and store images of goldfish retinal explants in culture. The final digital image was composed of a matrix of 280 X 192 picture elements (pixels) each of which was represented by one byte (256 gray levels). At the magnification used, the pixels measured 9 X 10 micrometers in the horizontal and vertical dimensions, respectively. The images were mathematically manipulated by algorithms designed to maximize visualization of the neurites growing out from the explants and to minimize the presence of the explant body itself. Finally, a pattern recognition scheme was used to remove non-neuritic debris as well as to restore the continuity of the digitized neurites. The number of pixels that were "lit" in the final binary image were then counted and used as the measure of growth for the explant at each time point examined. The coefficient of variation for repetitive scans of a single explant is 2.2% while the correlation coefficient is 0.94 when results obtained with this device are compared to those obtained by a visual estimation technique. (Supported by NEI Grant EY 05947. T.F.-H. was a trainee on NIH Training Grant MH 15794.)

174.16 LAMINAR DISTRIBUTION OF MICROTUBULE-ASSOCIATED PROTEIN 2 (MAP2) IN EMBRYONIC MOUSE CORTEX. J.E. Crandall, M. Jacobson* and K.S. Kosik*. Southard Lab., E.K. Shriver Ctr., Waltham and Dept. Neurol., Harvard Medical School, Boston MA

Dendrites of neocortical pyramidal neurons grow and differentiate in the following order: apical tufts, basilar branches and apical collaterals. This growth sequence is correlated roughly with the order in which proliferating axons invade the molecular and subplate layers prior to the cortical plate. Using a monoclonal antibody, 5F9, (Kosik et al., P.N.A.S., 81:7941, 1984) to microtubule-associated protein 2 (MAP2) that selectively stains dendrites and neuronal somata, we have investigated the time and location of appearance of MAP2 immunoreactivity in the embryonic neocortex of the mouse from embryonic day 13 (E13), prior to cortical plate formation, until birth (E19). Embryos were removed from timed-pregnant females and perfused transcardially with or immersed in a 2% paraformaldehyde-lysine-periodate fixative (McLean and Nakane, J. Histochem. Cytochem., 22:1077, 1974). Coronal and tangential 50-70 um vibratome sections were processed for immunocytochemical analysis at both light and electron microscopic levels. The secondary antibody was conjugated to peroxidase and the reaction product was visualized with diaminobenzidine.

Using this staining protocol there was no detectable MAP2 immunoreactivity above background control levels at E13. From E15 to birth the staining pattern of MAP2 immunoreactivity was laminar specific and similar in all neocortical regions surveyed. The molecular layer and subplate of the developing cortex contained the most dense staining of both cell bodies and processes. In these two laminae reaction product was seen in dendrites and cell somata at the ultrastructural level at E17. The cortical plate exhibited weak to moderate staining of single or grouped radial process and cell somata. The ventricular, subventricular and intermediate zones did not stain above background levels in control sections incubated with secondary antibody only or with MAP2- preadsorbed antibody. The laminar pattern of staining may be due to the higher density of differentiating dendrites present in the molecular and subplate layers or to compartmentalization of MAP2 within an individual differentiating neuron. Supported by NIH grants NS12005 and NS20213.

REGENERATION: PATTERNS AND RESPONSES

175.1 SIMULTANEOUS RECORDING OF INHIBITORY INTERACTIONS BETWEEN TWO TASTE NERVES IN ONE PERIPHERAL FIELD. D. R. Riddle*, C. R. Belczynski*, S. E. Hughes*, L. L. Morton* and B. Oakley. Div. of Biol. Sci. Univ. of Mich., Ann Arbor, MI 48109.

The right chorda-lingual nerve will regenerate into the distal stump of the left lingual nerve of gerbils. Subsequent electrophysiological recording from both chorda tympani nerves and histological analysis of the tongue indicated that the left half of the tongue had been supplied with two chorda tympani nerves and the contralateral lingual nerve. In spite of the presence of additional taste axons from the contralateral chorda tympani, no additional taste buds were formed. We conclude that the character of the epithelium, and not the number of taste axons, sets an upper limit to the number of taste buds which can be formed. In these animals it was possible to record impulse discharges from both chorda tympani nerves simultaneously while the tongue was stimulated with 0.3 M NaCl, 0.5 M sucrose, 0.3 M KCl, 0.3 M NH_4Cl, 0.01 M HCl, 0.01 M quinine hydrochloride, 0.3 M $CaCl_2$ and a concentration series of sucrose and of NaCl. The two nerves responded to all chemicals and had similar apparent dissociation constants for sucrose, but the latter values were 2-4 times higher than normal. Responses of the foreign chorda tympani were slower both to rise and to fall from maximal levels. Taste stimulation often inhibited spontaneous activity in multi-unit recording, especially in the foreign chorda tympani nerve. Periods of vigorous activity in the native chorda tympani sometimes coincided with depressed activity in the foreign chorda tympani. Moreover, electrical stimulation of the native chorda tympani frequently suppressed taste responses from the foreign chorda tympani, sometimes for several minutes. We suggest in these hyperinnervated tongues that the sluggish responses to stimulus onset and offset, the inhibition of spontaneous activity, and the inhibitory effects of electrical stimulation reflect inhibitory interactions between taste cells or taste axons that may be similar to the processing of sensory information in normally innervated tongues. Supported in part by NIH Javits Award No. NS07072.

175.2 THE EFFECT ON THE RAT HYPOGLOSSAL AND DORSAL MOTOR NUCLEUS OF THE VAGUS NERVE OF VINBLASTIN APPLIED TO THE INTACT HYPOGLOSSAL AND VAGUS NERVES. H. Aldskogius and M. Svensson (SPON: G. Grant). Dept. of Anatomy, Karolinska Institutet, Stockholm, Sweden.

It has been proposed that axotomy-induced retrograde changes in neurons and their environment develop because the transport of trophic substances from the periphery to the nerve cell bodies is interrupted. Electrophysiological and ultrastructural studies on sympathetic ganglia following application of colchicin to postganglionic axons support this notion (Purves, D., J. Physiol. 259:159, 1976). However, in a recent study using the 2-deoxyglucose technique to evaluate retrograde responses in the hypoglossal nucleus after injection of colchicin in the hypoglossal nerve, the characteristic axotomy-induced increase in 2-deoxyglucose uptake was not observed (Singer, P.A. et al., J. Neurosci. 2:1299, 1982).

In the present study the possible effects of blocking retrograde transport in peripheral nerves with vinblastin have been examined histologically in the hypoglossal nucleus and dorsal motor nucleus of the vagus nerve.

Experiments were performed on anesthetized (chloral hydrate 35 mg/100 g b.w. i.p.) adult, female Sprague-Dawley rats (200-250 g b.w.). Cuffs were prepared by soaking a piece of artificial hemostatic material in a solution of vinblastin (0.05, 0.025, 0.01 or 0.005%) in saline. In control experiments cuffs containing saline alone were used. The cuffs were wrapped around the right hypoglossal and left vagus nerves. A piece of Parafilm was placed under the cuff. After 30 min., the cuff and Parafilm were removed and the area flooded with saline. In one series of experiments, the effects on the retrograde transport of wheat germ agglutinin conjugated horseradish peroxidase from the tongue and pyloric region were examined. Tracer injections were made two days after the cuff application, and the animals were aldehyde-perfused after another two days. Serial, frozen, tetramethyl benzidine processed sections from the brain stem and nodose ganglia were examined. In a second series of experiments, the animals were aldehyde-perfused seven or 12 days after the cuff applications. The brain stem was embedded in paraffin, serial 6 µm sections cut and stained with cresyl violet, and examined qualitatively for the presence of chromatolytic neurons and quantitatively with regard to the number of perineuronal glial cells.

A dose of 0.01% or more blocked retrograde transport of HRP or HRP conjugate almost completely in the hypoglossal nerve and a dose of 0.005% or more in the vagus nerve. At these doses, the majority of neurons ipsilateral to vinblastin treatment were chromatolytic and a perineuronal glial cell reaction was present. These results are compatible with the proposal that interruption of axonal transport of trophic substances from the periphery is a "signal" for the induction of retrograde neuronal and associated non-neuronal changes.

175.3 PATTERNS OF AXONAL REGENERATION AFTER NEONATAL SCIATIC NERVE CRUSH. C.-B. Jeng and R.E. Coggeshall. Marine Biomedical Institute and Depts. of Anatomy and of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

It has been reported that neonatal peripheral nerve damage results in death of primary sensory and/or motor neurons. The present study is an extension of this work in that we present axonal numbers in the distal stump and various tributaries of the damaged nerve. To do this, we crush the sciatic nerve in neonatal rats (< 24 hrs. old). Eight weeks later, all axons in the sciatic nerve distal to the crush (sciatic), the sural nerve (SN), the digital nerve to the 2nd toe (DN), the nerve to the medial gastrocnemius muscle (NMG), the nerve to the lateral gastrocnemius muscle (NLG) and the nerve to the flexor digitorum longus muscle (NFDL) are counted by electron microscopy.

The axon counts are:

	Sciatic		Sural		DN		NLG		NMG		NFDL	
	MY	UN	MY	UN	MY	UN	MY	UN	MY	UN	MY	UN
Operated	5700	8000	460	850	30	160	680	600	480	340	340	330
Normal	8000	16000	1000	3500	142	380	400	660	280	430	400	430

Note that in the regenerated sciatic nerve, there are 25% fewer myelinated and 50% fewer unmyelinated axons than in the normal. This pattern is not repeated in the tributary nerves. For the cutaneous nerves (SN & DN), there is a considerably greater loss of axons, whereas in the muscle nerves (NLG, NMG, NFDL) there is only a slight loss of unmyelinated axons and myelinated axon numbers are increased in NLG and NMG. Further work on mechanisms that lead to these different patterns and to relate these patterns to the sensory and motor cell loss are underway. This work emphasizes the differences in patterns of axon regeneration when a nerve is damaged in a neonate as compared to an adult.

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175.4 AXONAL GROWTH AND REGROWTH AFTER SPINAL CORD LESIONS IN NEWBORN RATS. B. S. Bregman, M. McAtee*, and S. Sobhani*, Dept. Anatomy, University of Maryland School of Medicine, Baltimore, Maryland 21201.

After spinal cord lesions sustained neonatally, even late-developing, uninjured axons are unable to grow through the site of a lesion, but can take an aberrant route through adjacent undamaged spinal cord tissue. The current experiments were designed to determine whether transplants of fetal spinal cord tissue could provide a terrain for axonal elongation that is more favorable than that encountered at the glial scar at the lesion site, and serve as a bridge for both injured and later developing pathways to reach spinal cord segments caudal to the lesion. In the current experiments, newborn rats (< 72 hours) received spinal cord transections at a mid-thoracic level. Transplants of fetal spinal cord tissue from fetuses 13-14 days in gestation were placed into the lesion site. Normal littermates and littermates with spinal cord lesion but not receiving a transplant served as controls. We have used peroxidase-antiperoxidase immunocytochemical techniques to examine the descending serotonergic projection from the medullary raphe nuclei to the spinal cord in order to determine: 1) Do the transplants receive serotonergic innervation from the host CNS? and 2) Do the serotonergic axons grow across the transplant and back into the host spinal cord caudal to the lesion?

At the time of transplantation, the fetal spinal cord (E13-14) does not contain serotonergic (5-HT) innervation. The newborn spinal cord contains 5-HT immunoreactive fibers throughout its length. At 1 mo. to 1 yr. survival, there is a moderate to dense 5-HT innervation throughout the transplant and fibers can be observed crossing between host and transplant. No 5-HT immunoreactive cell bodies were identified within the transplants. Neonatal transection abolished the 5-HT innervation of the spinal cord caudal to the lesion by 7 days post-transection. In animals with transplants, however, 5-HT axons were identified both within the transplant and within the host spinal cord caudal to the transection. In the host spinal cord caudal to the transplant, fascicles of 5-HT fibers were often located in an ectopic position (e.g. dorsal column). Despite their abnormal position within the white matter, the 5-HT fibers innervated normal targets within the host spinal cord, e.g. the intermediolateral cell column. Growth was not limited to spinal cord segments immediately adjacent to the lesion and transplant; 5-HT fibers were identified throughout the host cord as far as the lumbar enlargement. The density of 5-HT innervation was far less than that encountered normally. Thus, late-developing or regrowing serotonergic axons were able to cross the site of a neonatal spinal cord transection by growing through the transplant and were able to innervate the host spinal cord caudal to the lesion.

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175.5 **Behavioral Analysis of the Recovery of Command System Function Following Spinal Transection in Larval and Adult Sea Lampreys.** D. Kaufman*, L. Margolin* and J. Ayers. (Spon: R. Schatz). Dept of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908.

We have used a computer algorithm for the analysis of undulatory behaviors (Science 221: 1312-1314) to compare the movement parameters of freely behaving recovered spinally transected larval and adult sea lampreys to the behavioral output evoked in these same specimens by electrical microstimulation of locomotor command tracts located in the rhombencephalon. Experimental animals were fully transected at the ninth myotome caudal to the last gill arch and allowed to recover for 90-120 days until they achieved the criterion for complete recovery of swimming (Brain Res. 279: 238-240). Specimens were prepared for focal extracellular stimulation by exposing the brain and rostral spinal cord. The anterior quarter of the body was pinned to a Sylgard™ lined dish, allowing the caudal regions of the body to undulate freely. Double barreled microelectrodes were used to deliver biphasic stimuli (ammocoete, 10-30 hz., 20-60 μa; transformers, 10-15 hz., 30-60 μa) to the bilateral command tracts .

Stimulation of the command system in recovered ammocoetes elicited swimming which was always shorter in period and increased in intersegmental phase lag when compared to the recovered behavior. As in normal specimens, command tract evoked swimming was bilaterally assymmetrical in curvature (>58% of occurrences). Increasing stimulus current had no direct effect on period and intersegmental delay, but produced a slight decrease in curvature. Increasing stimulus frequency, elicited variable effects on period and intersegmental delay; whereas curvature was slightly decreased. The recovered behavior of transformers, in contrast, was within the range of normal animals except for an increase in curvature In 40% of recovered transformers, microstimulation evoked slight undulations of the body trunk just caudal to the lesion. In contrast to the robust behavioral recovery, total recovery command system evoked swimming has yet to be observed. In 60% of recovered specimens, stimulation elicited tail flicks and flexions of the caudal half of the dorsal fin. In neither case, was the nature of the command system effect related to the quality of the recovered behavior.

Supported by NSF Grant BNS-8406880.

175.6 **Behavioral Recovery Results from Spinal Cord Regeneration In Larval Sea Lamprey.** G. Eaholtz. Marine Science Laboratory, Northeastern University, Nahant, MA 01908.

Sea lampreys can recover behaviorally from spinal cord lesions as severe as complete transection. To determine whether the recovery of swimming following spinal cord transection depends upon regeneration of fibers within the spinal cord, I tested specimens for coordinated motor discharge anterior and posterior to a complete transection by electromyographic recording after removing all sources of extero-spinal conduction. Larval lampreys were transected at the level of the 9th myotomal segment and allowed to recover. Specimens which had achieved criterion for recovery of swimming (Brain Res. 279: 238-240), were prepared for electromyographic recording anterior and posterior to the transection site (Soc. Neurosci .Abstr. 10: 631). I eliminated all extero-spinal tissue at the transection site in 4 recovered specimens and found that spontaneous coordinated behavior of the head and the tail persist.

In the first preparation a segment of the body musculature and notocord was removed at the site of the previous transection, but the gut and spinal cord were left intact. Specimens prepared in this fashion can swim spontaneously and exhibit such adaptations as changes in speed and turning. Electromyograms recorded rostrally and caudally to the transection site show coordinated rhythmic bursting activity identical to that observed in intact swimming animals

In additional experiments, specimens prepared by removal of six myotomal segments along with all extero-spinal tissues bridging the transection site were pinned to a Sylgard™ slab. Nose pinch stimulation resulted in undulatory contractions which were coordinated across the site and could propagate rostrally or caudally. Whole mounts of the spinal cords were stained with toluidine blue to confirm the transection site. These findings indicate that the recovery of coordinated swimming in the lamprey results from regeneration across the spinal lesion and not from passive mechanical conduction or plasticity of extero-spinal pathways..

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175.7 EPENDYMA-MESENCHYME INTERACTIONS DURING SPINAL CORD REGENERATION: REGENERATION OF LONG DESCENDING AXONS FROM THE BRAIN. S. Pinol-Roma*, K. Pollack*, and S.B. Simpson Jr. Dept. of Biochemistry, Molecular Biology and Cell Biology, and the Graduate Neuroscience Program, Northwestern Univ., Evanston, IL. 60201.

During tail regeneration in the lizard Anolis, ependyma cells regenerate as a continuous epithelial tube and guide hundreds of regenerating central nerve fibers. If however, the lizard cord is injured at mid-back levels, no regenerative response occurs. Histologically the end result is identical to that exhibited by the injured mammalian cord. The neuron cells of origin of the regenerating axons in the tail cord are not known. Obviously, for the lizard cord regeneration system to be of maximal experimental value, at least some of the regenerating central axons should be long descending axons from brain stem neurons.

Using the retrograde transport of HRP and a variety of methods for visualizing the transported marker, we have begun to map both the normal and regenerated long spinal axons in Anolis. We have succeeded in visualizing a large number of neurons in the brain stem that project to caudal levels of the normal anole cord. These neurons are located in the red nucleus, reticular nucleus of isthmus; superior reticular nucleus; vestibular nucleus; nucleus raphe magnocellularis; inferior nucleus of the raphe; and the spinal trigeminal. The projections, so far, appear to be identical to those described for Lacerta by Schwab 1979 in Biology of the Reptilia Neurobiology B., Gans, Northcutt and Ulinski eds. Academic Press.

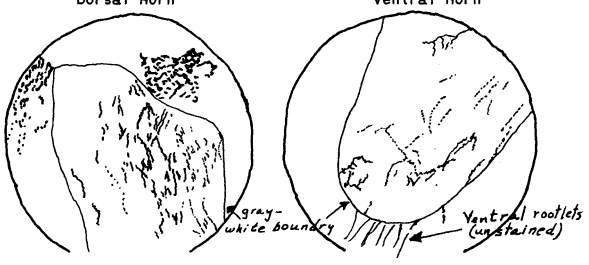
HRP tracing of regenerated spinal axons in the regenerated tail cord, has to date, identified neurons in the reticular nuclei, the vestibular nucleus and the nucleus raphe magnocellularis as regenerating during tail cord regeneration. We are in the process of refining our maps of both normal and regenerated spinal axons. We are also mapping neurons within the spinal cord that also regenerate during tail cord regeneration.
(Supported by NIH grant NS20970)

175.8 PATTERNS OF REINNERVATION IN THE ADULT MAMMALIAN SPINAL CORD BY VENTRAL ROOT AXONS ANASTOMOSED TO PROXIMAL DORSAL ROOTS. R. E. Kingsley. South Bend Center for Medical Education, Indiana University School of Medicine, Notre Dame IN 46556.

The right L7 ventral root was cut and anastomosed to the cut proximal limb of the left L6 dorsal root in five cats. Details of the procedure have been published elsewhere (K. K. Messenger & R. E. Kingsley, 1983; R. E. Kingsley, K. K. Messenger & R. H. Seall, 1984). After various reinnervation intervals (6 - 12 months), the grafted dorsal root was sectioned within 2mm of the spinal cord entry point and HRP crystals applied to the central stumps. After allowing 24 hours for transport, the cats were killed, the spinal cord cut into sections 100u thick, and the HRP visualized with TMB (Mesulam, 1982). The sections were stabilized with amonium molybdate (5%), embedded in Araldite-Epon and cured between sheets of Aclar film.

The TMB procedure has proven to be much more sensitive than the DAB procedures we previously used. I observed five types of reaction product. Two forms were artifacts of the chemical process; long slender crystals of reaction product and a dust-link precipitate on the surface of the sections. Another form of artifact was clearly associated with blood and blood vessels. Since these were all easily recognized as artifact, no effort was made to suppress these reaction products in order that more labeled axons could be seen.

Axons were labeled in two distinctly different ways. Most of the axons were labeled with a small fine granular residue. In the dorsal horn, this residue was dense enough to appear continuous, yet still granular. These axons were distributed along the medial side of the dorsal horn, very few were seen in the lateral half. They were not randomly organized, but were distributed in parallel chains running in the dorsal/ventral direction. The TMB procedure has allowed us, for the first time, to see labeled axons in the ventral horn as well. There they are much fewer in number, but their organization is still principally in parallel chains along the medial half of the horn. Deep in the ventral horn they seem to become a little more disorganized, running more independently and they may even run in the medial/lateral direction. These axons were almost never seen in white matter. The second type of labeled axon , seen only in some animals, was large and very densely labeled. They were almost always in white matter. They are probably not derived from the graft but are axons bruised during the second surgury.



175.9 ADULT DORSAL ROOT GANGLION (DRG) NEURONS REGENERATE THEIR CENTRAL PROCESSES INTO TRANSPLANTS OF FETAL SPINAL CORD. A. Tessler, K. Winkler* and B.T. Himes* Philadelphia VA Medical Center and Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129, and J. Houle and P. Reier, Departments of Neurosurgery and Neuroscience, College of Medicine, University of Florida, Gainesville, FL 32160.

After transection of their central axons, adult DRG neurons elongate beyond the site of injury, but regenerating processes fail to re-enter the spinal cord. This failure may be due to inability to penetrate the PNS-CNS interface or to limited capacity for axonal elongation of adult neurons. Either may be affected by implantation of embryonic tissue. Therefore, we have studied the ability of adult DRG neurons to regenerate central axons into grafts of fetal spinal cord. Adult Sprague-Dawley rats (150-250g) underwent laminectomy at the level of the lumbar enlargement. Dorsal roots on one side were cut and a portion of spinal cord approximately 3mm in length was resected. Spinal cord taken from E14 or E15 Sprague-Dawley donors was implanted into the cavity, and the cut dorsal root stumps which remained attached to the DRGs were placed on top of the graft. 2-7 months later WGA-HRP conjugate was applied to the sciatic nerve in order to test the possibility of transganglionic transport of HRP into the transplants. Reaction product was observed within grafts located in lumbar segments. Reaction product generally consisted of finely granular punctate staining or clumped deposits and less commonly had the appearance of axons. Its characteristics resembled the staining found in normal adult spinal cord labeled by the same method. These results indicate that the cut central processes of adult DRG neurons are able to regenerate into transplants of fetal spinal cord. Either or both of two conclusions therefore follow: (1) the regenerative capacity of adult DRG neurons suffices to permit elongation into CNS terrain which is conducive to growth; or (2) embryonic transplants increase the capacity of these neurons to regenerate after axotomy.

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175.10 RESTITUTION OF DESCENDING SPINAL PATHWAYS AFTER SPINAL TRANSECTION IN XENOPUS OCCURS DURING, BUT NOT AFTER, METAMORPHOSIS. G.L. Lopate*, M.S. Beattie, and J.C. Bresnahan, Dept. of Anatomy and Div. of Neurosurgery and Neurosci. Res. Lab. , Ohio State Univ. College of Med., Columbus, OH 43210.

The regenerative capacities of vertebrate axons vary widely between species, between systems within species, and in some cases may be dependent upon the developmental status of the organism. Forehand and Farel (J. Neurosci. 2:654, 1982) showed anatomical and behavioral recovery after spinal transections in the bullfrog, but only if transections were made in larval stages and the animals were allowed to complete metamorphosis. We have examined the question of long tract restitution during metamorphosis in **Xenopus laevis**, and find that in this species also, the process of metamorphosis is accompanied by behavioral recovery and restitution of some descending spinal tracts. In addition, we found that fibers crossing the lesion site were located preferentially in the lateral funiculi, a finding like that reported by Bunt and Fill-Moebs (Dev. Brain Res., 16:307, 1984) in the teleost spinal cord. No recovery occured in juvenile, post-metamorphic spinal frogs, and no fibers were seen crossing the lesions, which showed frank discontinuity.

Spinal cord transections between the fifth and sixth or sixth and seventh vertebrae were made in larval tadpoles (stages 50-61) or juvenile froglets under MS-222 anesthesia, using a knife contructed from a tungsten microelectrode. Animals were allowed to survive for 4-5 weeks (juveniles), or until tail resorption was complete (tadpoles). Behavioral observations were made throughout the post-operative interval. In order to label descending spinal axons, HRP was either injected into the caudal brainstem, or was placed in a spinal transection rostral to the initial lesion to label fibers descending into the lesion area. In additional animals, HRP was placed in a second transection made just after the first and rostral to it in order to assess the completeness of the lesion. These animals were sacrificed 4-8 hrs later, and their spinal cords processed for visualization of HRP-filled fibers at the lesion site. These controls showed complete disruption of descending fibers in most cases. In a few cases, a few axons were spared in the ventral funiculus, but never in the lateral funiculus. In recovered animals, fibers could be traced into the lumbar enlargement after either brainstem or spinal placements of HRP.

These results suggest either that brainstem spinal systems regenerate during metamorphosis, or that new fibers cross the lesion and support behavioral recovery. The study of cellular and systemic events during metamorphosis may provide insights into mechanisms which promote axonal growth and regeneration.

(Supported by NS-10165 and a Roessler Foundation Scholarship)

175.11 PATHWAY CONSTRAINTS ON REGENERATING MOTOR AXONS IN BULLFROG TADPOLES. M.T. Lee and P.B. Farel. Dept. Physiol., Univ. N. Carolina Sch. of Med., Chapel Hill, NC 27514.

Following ventral root (VR) transection at early stages of development, motoneurons of the bullfrog's lumbar lateral motor column are capable of specifically reinnervating appropriate regions of the hindlimb (Farel & Bemelmans, submitted). This specificity is largely lost if the VRs are transected in more advanced tadpoles or juvenile frogs. In an attempt to understand the factors that influence the selection of targets by these regrowing axons, we have begun to examine the paths they follow between the spinal cord and the muscles of the limb.

The three VRs (8, 9, & 10) that innervate the hindlimb were transected on one side of bullfrog tadpoles (Rana catesbeiana) at various stages. After post-operative periods sufficient to allow motor axon regeneration, horseradish peroxidase (HRP) was applied to one of the previously severed VRs and to the corresponding root on the unoperated side. These orthograde labeling studies were complemented by retrograde labeling of particular muscle nerves in other operated animals. At 3-8 days after labeling, the lumbar spinal nerves and major hindlimb nerves were dissected free of surrounding tissues, intensified with cobalt, reacted with diaminobenzidine, and examined in whole-mount.

At stages XII-XIV, when reinnervation is nonspecific, HRP applied to VR 10 on the unoperated side labels axons that contribute to many of the major nerves of the limb; however, those axons that enter the profundus posterior nerve of the thigh are restricted to 2 of its 4 main branches. On the operated side, motor axons regenerating from VR 10 are also found only in these 2 branches, even though they pass close by the points at which the other branches arise. Conversely, retrograde labeling of one of the other branches shows that none of its axons are supplied by VR 10, on either the operated or the unoperated sides. These results, combined with those of previous studies, imply that axons regenerating from a particular VR to inappropriate targets are nonetheless constrained to enter only those nerves normally supplied by that VR.

These findings are consistent with the concept that regenerating axons are channeled down preformed pathways, which may consist of denervated Schwann tubes of the distal nerve stump (Gutman & Young. J. Anat., 78: 15, 1944). One way to test the strength of this channeling influence would be to misdirect regenerating axons into the wrong Schwann tubes at stages when reinnervation is specific following simple transection. Such misdirection might be accomplished by transecting and then cross-joining the lumbar spinal nerves. These experiments are now in progress.

Supported by NIH grants NS16030 and NS14899.

175.12 ALTERATION OF NERVE FIBERS IN MINOR SPINAL CORD INJURY OF GUINEA PIG. Yutaka Naka*,Kunio Nakai,Toru Itakura*,Kazuo Nakakita*,Ichiro Kamei*,Harumichi Imai* and Norihiko Komai*. Dept. of Neurological Surgery,Wakayama Medical College, Wakayama 640 Japan.

Alteration of various nerve fibers in the spinal cord was studied by morphological methods following minor spinal cord injury. Under gas anesthesia with nitrous oxide and halothane, the spinal cord of guinea pigs weighing 250-300g were pinched by 50% of its original size for ten seconds using a custom-made device(2mm in width). Following this mild spinal cord injury the animals showed a temporary paraplegia and sensory disturbance. After this brief period of spinal shock the animal behaved almost normally for up to six hours. Then they again started to show paraparesis and hypalgesia of bilateral lower extremities that lasted for 2-3 days. About 80% of thus treated animals behaved normally by the fifth postoperative day. After various survival period from the injury, the animals were perfused through ascending aorta with 2% paraformaldehyde and 0.2% picric acid in cold phosphate buffer(PH=7.4). Parasagittal sections of the injured spinal cord were then processed for peroxidase anti-peroxidase immunohistochemistry using antisera against dopamine beta-hydroxylase(DBH),substance P(SP) or vasoactive intestinal polypeptide(VIP) as primary antibodies. Immediately after the lesioning of spinal cord we did not observe any change in immunohistochemistry except the directly damaged area by mechanical compression. The size of this primary mechanical lesion was 0.3-0.5mm in width rostrocaudally. In 6 hours after the lesioning swollen fibers immunoreactive to above three antisera began to appear in the white matter near the primary lesion. At this moment immunopositive nerve fibers in the gray matter around the primary lesion seemed to be unaffected. In 24 hours only SP fibers within the gray matter began to decrease its density in the vicinity of the primary lesion. By 72 hours the number of immunopositive fibers to DBH and VIP began to decrease. Numerous immunopositive swollen fibers to all three antisera had appeared in the white matter of three day-survived animal. Interestingly, those swollen fibers showed significantly elevated cytochrome oxidase activity especially near the primary lesion. In a week after the lesioning, the area without immunoreactive nerve fibers was 1.3-1.5mm in width. In two weeks each nerve fiber already began to reappear within the area whose immunopositive fibers had almost disappeared in one week.

In summary, among three types of neurotransmitter system tested, SP fibers seemed to be more vulnerable to mild spinal cord injury. And the swollen nerve fibers with elevated cytochrome oxidase activity following the injury suggest that swollen fibers within the white matter may have high metabolic activity to promote regenerative process.

175.13 REGENERATION OF SEVERED CNS AXONS IN LARVAL CRAYFISH.

K. R. Seshan* and G. D. Bittner (SPON: José P. Segundo), Department of Zoology, The University of Texas, Austin, Texas.

A single abdominal ganglion of the ventral nerve cord was removed or the ventral nerve cord was severed between two adjacent ganglia in larval crayfish Procambarus clarkii, 4-8 weeks old and measuring 1-5 cm in length. In contrast to results obtained using juvenile or adult crayfish in which very few axons regenerate (Bittner, G.D., Ballinger, M.L.,& Larimer, J.L., J. Exp. Zoology,189:13-36(1974), a substantial number of nerve fibers regenerated and established morphological contacts between the lesioned stumps. The outgrowing nerve fibers were grouped together into several small bundles which in cross section showed ultrastructural characteristics of normal axons with axoplasmic organelles. Glial cells and connective tissue elements occurred in close association with the outgrowing axons. Horseradish peroxidase (HRP) injected into axons 1-5 mm away from the lesioned site was readily taken up by the regenerating axons and transported in both rostral and caudal directions across the lesion site to adjacent ganglia. The appearance of HRP reaction product in axons across the lesion site and in adjacent ganglia suggests that at least some of the severed axons may restore functional connections in larval crayfish. Identifiable severed medial and lateral giant axons grow into the lesion site but do not appear to establish morphological contact with their severed surviving segments within our three month period of experimentation. Severed CNS proximal and distal stumps that do not exhibit any significant axonal regeneration are often covered with glial cells. These axonal stumps end blindly and show accumulation of axoplasmic organelles.

Combined with previous results cited above, our present data show that the CNS of immature crayfish retains its capacity for axonal regeneration but that property gradually decreases with increasing age of the organism.

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175.14 REINNERVATION OF THE RAT URINARY BLADDER WITH SOMATIC NERVES. C. L. Lee, L. Monti-Bloch* and E. R. Perl. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.

We have examined the effects of using somatic nerves (femoral, genitofemoral, and obturator nerve) to reinnervate the denervated rat urinary bladder (UB).

Female Sprague-Dawley rats were denervated by unilateral or bilateral isolation and excision of the pelvic nerve (PN) and pelvic ganglia (PG). In bilaterally denervated animals, one somatic nerve was sutured to the detrusor muscle at a site just above the uretrovesicular junction. In the unilaterally denervated animals, the somatic nerve was sutured to the PG or PN. The animals were allowed to survive from 31 to 448 days (total N = 64 animals).

Intrabladder pressure was measured while stimulating the rerouted somatic nerve. Physiological evidence of reinnervation was found in two groups of animals. One-half of the animals (22 out of 44) that had direct femoral nerve to UB detrusor muscle connections responded to the somatic nerve stimulating with transient bladder contraction. Two-thirds of the animals (6 out of 9) with PG connected by a somatic nerve exhibited a pressure response. Only one out of six animals with the genitofemoral nerve connected to the UB detrusor showed a response. None of the animals that had a genitofemoral nerve connection to the PN (2 animals) or obturator nerve connection to the UB detrusor (3 animals) showed a response. The pressure change due to stimulation of the somatic nerve ranged from 0.1 to 7.6 mm Hg; however, none of the animals showed functional spontaneous voiding.

In most animals, the pressure change evoked by somatic nerve stimulation was potentiated by eserine (0.35 mg) and in every case atropine (0.35 mg) abolished the response.

This evidence suggests that physiologically effective reinnervation of the denervated rat urinary bladder by a somatic nerve is feasible, although the functional effectiveness of such reinnervation is yet to be established. Thus the possibility exists of alleviating bladder paralysis secondary to peripheral nerve or spinal injury by using motor outflow from spinal levels above the injury. (Supported by grant NS 14899 from the NIH of the USPHS.)

175.15 **SELECTIVE NEURONAL INVOLVEMENT IN MOTOR NEURON DISEASE (Wobbler Mouse)** H. Mitsumoto, A. Boggs* and N. Sunohara*, Department of Neurology, The Cleveland Clinic Foundation, Cleveland, OH 44106

The reason(s) why certain groups of motor neurons are selectively affected while others are not in motor neuron disease is unknown. The wobbler mouse offers a unique opportunity to study this issue since the disease affects almost exclusively the forelimbs but not the hindlimbs. Morphologically vacuolar degeneration develops in the anterior horn cells, and the spinal ventral root motor axons undergo secondary axonopathy including active axonal regeneration (Mitsumoto and Bradley, Brain, 1983). The difference was analyzed by histometry comparing the C7-C8 ventral roots and the L4-L5 ventral roots. The most distinct differences between these two anterior horn cell groups occurred from 3 to 6 weeks of age: at the cervical level large myelinated fibers (>6 um) were diminished in number by nearly 50% ($p<0.01$) in this period, but regained their prior number by 3 months of age; In contrast, those in the lumbar ventral roots were increased in number parallel to those in the controls in this period, but then diminished in number by 3 months. The absolute number of small myelinated fibers (<6 um) was increased in lumbar ventral roots as compared to control lumbar ventral roots as well as cervical ventral roots ($p<0.05$). These histometric findings suggest that axonal regeneration was more active in lumbar roots. Therefore, we compared the regenerative capacity after nerve crush of the forelimb nerves (cervical anterior horn cells) and sciatic nerves (lumbar anterior horn cells). Our previous study of the regenerative capacity of cervical anterior horn cells by means of radiolabeled axonal transport technique clearly showed at 7 days a diminished rate of axonal elongation ($p<0.01$) and an absence of the distal peak representing a large cohort of growth endings in the cervical anterior neurons (Mitsumoto: Muscle and Nerve, 1985). The same test for lumbar anterior horn cells showed the presence of a normal distal peak at 7 days, identical to controls. Therefore, lumbar anterior horn cells of wobbler mice have a better regenerative capacity compared to cervical neurons, suggesting that the overall neuronal function is better in "unaffected" lumbar anterior horn cells. The difference in the regenerative capacity is perhaps closely associated with the mechanism(s) of selective neuronal involvement in this motor neuron disease (Grant support by NIH, NS-21742).

175.16 HISTOLOGICAL AND HISTOCHEMICAL CHANGES FOLLOWING AXONOTMESIS IN NON-HIBERNATING AND HIBERNATING RODENTS. G. Chaban*, C. Barrett*, E. Donati* and L. Guth*(SPON: J. Krikorian). Dept. of Anatomy, University of Maryland Medical School, Baltimore, MD 21201.

Axonal regeneration in the mammalian CNS may be influenced by microenvironment at the lesion site. We attempted to develop a method of axotomy that minimizes vascular injury and prevents ischemic necrosis (PIN) of neural tissue. We investigated the effect of graded crushes of the optic nerve in rats and crushed optic nerves in non-hibernating and hibernating ground squirrels; in the latter because of depressed adventitial and glial reaction to injury (Guth et al., J.Comp.Neurol., 203: 297, 1981). Retinae and nerves were studied by light microscopy using histological (HOGE, PTAH, protargol) or histochemical (ACHase, acid phosphatase, NADH dehydrogenase, G6PDH and G3PDH) procedures. We crushed rat optic nerves at different points (0,1,4,7,9 mm retrobulbarly) using three levels of force (weak, intermediate, strong) at each position. Blood vessels bridging the site were massaged to reestablish blood flow. Three days postoperatively (dpo), the extent of lesion increased with the severity of crush. However, significant PIN did not result following weak crushes but it frequently did occur when greater forces were used. Presumptive "watershed" area was found (1 mm retrobulbarly), where even strong crushes did not produce PIN. Chromatolytic retinal ganglion cells were seen in the non-hibernators after 3 dpo. Viable tissue bordering the lesion site showed numerous terminal axonal varicosities (TAVs) which were much more prevalent centrally. In the retinal stump, most TAVs occurred at 10 dpo and were morphologically variable, some with thin, sprout-like appendages. Several had reversed direction. Postoperatively, the neurons and their processes diminished in number, however more than 50% of neurons and 20% of optic nerve fibers remained after 90 dpo. This is a greater survival rate than that following neurotmesis (Richardson et al., J.Neurocyt., 11: 949, 1982). The excess of neurons over nerve fibers indicates that many are amacrines. Gliosis was weak and localized in the retina, but strong and disseminated in the optic nerve. In hibernation, the reactive and degenerative changes slowed down by 4 to 30-fold. Between 15 and 30 dpo, uncommonly large and abundant TAVs were seen at the tip of retinal stump, later becoming smaller and denser. Numerous convoluted axons with TAVs recurved in the direction of retina, often in vicinity of macrophages. The lesion site remained loosely organized but changes evident in the non-hibernators soon followed arousal. Histochemical results indicated that significant enzymatic activity persisted in surviving neurons, however, except for acetylcholinesterase, it increased only in glia. Successful axonal regeneration was not observed. It seems that constructive events at the axon tip were not supported by similar changes in the perikaryon. These results support the potential usefulness of this model in studies on CNS regeneration.

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175.17 A BIOASSAY FOR THE ANALYSIS OF NEURITE-PROMOTING FACTORS IN THE EXTRACELLULAR MATRIX OF RAT PERIPHERAL NERVE. A. W. Sandrock* and W. D. Matthew, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

In mammals, severed neuronal axons residing in peripheral nerves regenerate readily, whereas axons of the central nervous system fail to regrow effectively. Studies performed in the laboratory of Albert Aguayo have demonstrated that the limited regenerative capacity of central axons is the result of the relative inability of the CNS tissue milieu to support effective axonal elongation, and that the PNS environment contains the necessary ingredients for successful axonal regeneration--even for many CNS axons. What are these ingredients? In the wake of peripheral nerve injury, neuronal axons regenerate within the confines of the persisting Schwann cell basal laminal tubes of the distal stump. The possibility exists, then, that the extracellular matrix (ECM) constituents of these tubes play an important role in promoting axonal regeneration.

We have developed an in vitro bioassay that is sensitive to the substrate-bound factors of peripheral nerve which influence the elongation of regenerating axons. In this assay, rat superior cervical ganglion explants are plated onto longitudinal sections of fresh-frozen sciatic nerve. Twenty-four to forty-eight hours later, the regrowing axons are visualized by catecholamine histofluorescence. The axons always regenerate along the long axis of the nerve in a parallel array, remaining within the boundaries of the tissue section. The rate of elongation is sensitive to the inhibitory effects of INO, a monoclonal antibody which recognizes and inhibits the neurite-promoting activity of a heparan sulfate proteoglycan-laminin complex derived from PC12 cell conditioned medium (Matthew and Patterson, 1983). Interestingly, INO appears to stain the Schwann cell basal lamina of peripheral nerve, but not CNS fiber tracts. In contrast, the rate of axonal regrowth over sciatic nerve sections is virtually unperturbed by an antiserum which recognizes purified laminin, inhibits this molecule's neurite-promoting activity, and stains the Schwann cell basal lamina of peripheral nerve. Finally, when ganglia are similarly explanted onto longitudinal sections of adult rat optic nerve, axonal regeneration is minimal.

In summary, we report the development of an in vitro axonal regeneration assay which (1) reflects the differential ability of PNS versus CNS tissue to support effective axonal regeneration as observed in vivo, and (2) is sensitive to antibodies which interfere with the neurite-promoting activities of ECM constituents in peripheral nerve. We plan to use this assay system to further probe for molecules affecting axonal regeneration.

[This work supported by the Sloan and McKnight Foundations.]

175.18 ACUTE EFFECTS OF GM1 GANGLIOSIDE ON CNS INJURY: ASSESSMENT OF DOSING SCHEDULE FOR OPTIMAL FUNCTIONAL RESPONSE. Y.S. Li*, M.M. Rapport, & S.E. Karpiak. Div. Neuroscience, NYS Psychiat. Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, Coll. of Physicians & Surgeons, Columbia U., N.Y., N.Y. 10032.

Since we reported that systemic injections of GM1 ganglioside limit the edema associated with CNS trauma, we are studying the short-term (24-48hrs) effects of ganglioside treatment on CNS injury. We hypothesized that this limitation of damage contributes to facilitated recovery. An experiment was designed to assess when ganglioside treatment produces the greatest functional recovery after injury. Sabel [1] reported that in rats injected with GM1 ganglioside, amphetamine-induced rotation is markedly reduced as early as 48hrs after a unilateral hemitransection of the nigro-striatal pathway (NSP). Using this model, we assessed the effects of GM1 ganglioside (20mg/kg i.p.) administered at various intervals: PRE: daily, for 2 days before surgery; POST: 1 day after surgery, with individual groups receiving their 1st injection at 0,2,4,8 or 12 hrs after hemitransection; PRE&POST: daily, 2 days before surgery, day of surgery [8-12hrs after hemitransection] & day after surgery. Ipsiversive rotation was determined in automated rotometers, 48hrs after hemitransection, for 60min starting 30min after s.c. amphetamine [2mg/kg]. Each experimental group was tested in parallel with a saline control group. The average number of ipsiversive turns for all saline controls was 520 (SEM=49.5;N=34). Rats PRE treated with GM1 showed a 25.7% decrease ($p<0.05$) in rotational behavior. Rats POST treated with GM1 at 0 or 2hrs after surgery showed 38.6% & 49.4% decreases in rotation respectively ($p<0.025$ & $p<0.005$). However, rats POST treated at 4,8 & 12 hrs showed respective reductions in rotation of 21.3%, 27.4% & 27.7%, which were not statistically significant. A significant reduction [47.5%] in rotations was seen in the PRE&POST treated group ($p<0.005$). Our data show that the greatest reduction in rotations was achieved when rats were either treated with GM1 PRE&POST surgery, or treated within 2hrs after surgery. GM1 treatments 4hrs or more after surgery failed to reduce rotation significantly. Limiting dosing to the 0-2hr post-surgical period is equally as effective as a PRE&POST injection protocol, where the 1st post-surgical injection is 8-12hrs after hemitransection. The reduction seen in the PRE&POST group must be due to GM1 dosing prior to injury. Effects of GM1 present at the time of surgery may have been sufficient, so that it was not required to dose animals at 0-2hrs after surgery in order to achieve optimal results. Although all GM1 injected rats showed a trend toward reduced levels of rotation after surgical transection of the NSP, the 0-2hrs post-surgery may be a critical time period when GM1 treatment leads to the greatest reduction in functional impairment.

1. Sabel et al., J. Neurosci. Res. 12:429 (1984).

175.19 EFFECT OF CRUSH LESION ON GANGLIOSIDE AND NEUTRAL GLYCOLIPIDS IN RAT SCIATIC NERVE. M. Guzman-Harty*, J. Warner*, M. Mancini*, D. Pearl* and A. J. Yates. Division of Neuropathology and Statistics Laboratory, The Ohio State University, Columbus, OH 43210.

Exogenously administered gangliosides promote axonal sprouting in crushed rat sciatic nerve. If gangliosides are involved in nerve regeneration, alterations in ganglioside metabolism may occur following nerve trauma. However, little is known about the role of neutral glycolipids in nerve regeneration. Because of the structural and metabolic relationships between gangliosides and neutral glycolipids, we studied the effects of crush injury on the synthesis and accumulation of these compounds in peripheral nerve. Left sciatic nerves (SN) of male Sprague-Dawley rats were crushed and allowed to recover for 0, 1, 2, 4, 7, and 14 days. At each time point, both L-5 dorsal root ganglia (DRG) were injected with 100 μCi of [^{3}H]-glucosamine. Two days later, both DRG, lumbosacral trunk (LST), and SN were removed. Ganglioside and neutral glycolipid of each tissue were purified and radioactivity measured (Ledeen, et.al. Soc. Neurosci., Abstr. 71.PO, 1983). The amounts of ganglioside in crushed LST were consistently higher than the controls with the largest difference occurring within 2 days after crush. At Day 0, there was a significant difference between control and crushed nerve ganglioside in the LST. This difference fell off roughly exponentially with time, with the difference in later days significantly less than that in Day 0. The largest difference between crushed and control SN occurred later than that of LST (2-7 days after crush). The ganglioside in crushed and control SN was nearly the same at Day 0 and Day 1, but became significantly different at later times. There was no consistent difference between the DRG values of control and crushed nerves at any of the times studied. There was a slight but consistently higher radioactivity values in the whole crushed nerve (DRG + LST + SN) than the control tissue at all times studied. The neutral glycolipids followed a similar pattern of synthesis and accumulation as the gangliosides in crushed nerves. Preliminary HPTLC studies of DRG gangliosides showed no observable difference in pattern between the left (crushed) and right (control) tissues. These findings indicate that gangliosides and neutral glycolipids are synthesized at normal or slightly elevated rates after nerve crush but accumulate proximal to the distal end of the crush site. This accumulation may increase ganglioside concentration in the growth cone at this site which may lead to axonal regeneration. Supported by NIH Grant NS 10165 and 5T32 N507091.

175.20 ANATOMICAL AND BIOCHEMICAL CHANGES IN CELL BODIES OF AXOTOMIZED AND CONTRALATERAL INTACT MOTONEURONS. G. Ring*, F. Reichert* and S. Rotshenker. Dept. of Anatomy and Embryology, Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel.

The interruption of motor axons is followed by significant anatomical, and biochemical changes in the cell bodies of the axotomized motoneurons. Some of these changes (chromatolysis, nuclear and nucleolar size, and RNA metabolism) are believed to reflect a modification in gene expression that may be an important event in nerve cells' attempt to regenerate their axons. In the present study, we examined some aspects of cell body morphology (cell, nuclear and nucleolar size) in axotomized motoneurons and intact motor nerve cells situated on the opposite side of the spinal cord. The same cells were also examined by quantitative autoradiography for the incorportion of H^3-Uridine into the rapidly labelled pool of RNA molecules.

Significant increases in cell body, nuclear and nucleolar sizes were observed in axotomized motoneurons cell bodies 4 to 34 days after axotomy. Increase above normal was first observed in nucleoli (2 days), cell body (4 days) and nuclei (7 days), all peaked at 14 days and remained elevated for the duration of the experiment (34 days). A small, but significant, increase in nucleolar size was also observed in contralateral intact motoneurons on days 10 and 26.

The incorporation of H^3-Uridine into cell bodies of axotomized motoneurons was complex: it increased 2 days after the operation, reached two peaks (days 4 and 14) and then declined below normal (day 34). The incorporation of H^3-Uridine into cell bodies of contralateral intact motoneurons increased on days 14 and 16 and declined below normal on days 26 and 34.

In axotomized motoneurons, the time course of changes in H^3-Uridine incorporation differed from the time course of changes in cell body, nuclear and nucleolar size. Especially surprising is the dissociation of the time courses of H^3-Uridine incorporation and nuclear size (the organelle into which most H^3-Uridine was incorporated). In contralateral intact motorneurons, the timing of the increase in nucleolar size (10 days) and the increase in H^3-Uridine incorporation (14 and 21 days) correlates with the appearance of contralateral sprouting (about 14 days) (e.g. S. Rotshenker, J. Neurosci. 2:1359, 1980).

175.21 THE RELATIONSHIP OF ORNITHINE DECARBOXYLASE ACTIVITY TO CHANGES IN CHROMATIN STRUCTURE IN AXONAL REGENERATION. M. R. Wells. Neurochemistry Research Lab., V. A. Hospital and Dept. of Physiology, George Washington Univ., Washington, D.C. 20422.

Autoradiographic techniques were used to qualitatively characterize the possible relationship between structural changes in chromatin and ornithine decarboxylase (ODC) levels in rat dorsal root ganglion neurons after crush lesions of the sciatic nerve. Changes in chromatin structure were examined by [^{3}H] Actinomycin D binding to neuronal nuclei in tissue sections (Wells, M.R., Exp. Neurol.,86:303-312, 1984). Alterations in ODC were examined by an autoradiographic technique using [^{3}H] difluoromethylornithine (Wells, M.R., Anat. Rec.,211:212A, 1985). All measurements were made on the L5 DRG after a crush lesion of the sciatic nerve as it emerged from the sciatic notch. The time periods examined included 0,0.5,1,2,3,4,5,7,8,9,11,14, and 30 days postoperation. Counts of autoradiographic grains were made over neurons and/or nuclei and compared statistically to the contralateral ganglion and normal sections on the same slide. ODC levels incresed significantly 0-3 days after injury compared to the contralateral side or normal with a maximum at 0.5 days. This change occurred in the absence of detectable alterations in actinomycin D binding to nuclei. An early increase of actinomycin D binding occurred at 3-4 days postoperation with a peak at day 4. During this time, ODC levels decreased to normal levels at day 4 and began a significant increase from days 5 to 8. Actinomycin D binding decreased at day 5 to normal, dropped to to below control at day 7, and then increased significantly above control levels at day 8. At day 8, both ODC and actinomycin D binding were significantly above control values. From days 9-30, changes in both measures occured in parallel. The pattern observed suggests that increases of ODC occur just prior to increases in actinomycin D binding to nuclei and associated increases in RNA synthesis (Langford, C.J., J. Neurochem.,34: 531-539, 1980). The relationship of ODC and actinomycin D binding appears different in the early phases of the response to axotomy compared to the second week. This may indicate that different mechanisms for ODC regulation are occurring in the early phase of the reaction, perhaps related to injury, as compared to the later response which may be more closely linked to the effort of the neuron to regenerate its axon.

Supported by the Veterans Administration.

175.22 QUANTITATIVE CYTOCHEMICAL (RNA) AND CYTOLOGICAL OBSERVATIONS ON THE AXOTOMIZED RAT RED NUCLEUS. EFFECT OF GANGLIOSIDE INJECTION. K.D. Barron, M. Banerjee* and M.P. Dentinger*. Neurology Service, VAMC and Dept. of Neurol., Albany Med. Col., Albany, N.Y. 12208.

Unilateral rubrospinal tractotomy was performed at C3 in 250-300 g female Sprague-Dawley rats which were sacrificed by perfusion with 3:1 ethanol:acetic acid 3,7,10,14,28 and 60 days later. Operates were paired except that there were 4 subjects for each of the 14 and 28 days postoperative intervals. Additionally, 8 rats were injected daily intraperitoneally with chromatographically homogeneous GM_1 ganglioside, 30 mg/kg body weight. Four of the ganglioside-treated group were killed 14 and four 28 days after surgery. Tissues were embedded in paraffin and, after verification of adequacy of the cord lesion, 6 um sections were cut serially through the red nucleus (RN) from the caudal pole for 300 um rostrally. Counts of neurons and neuroglia and areal measurements of neuronal somas were compared with control data obtained from 4 unoperated rats matched for age and sex. Cytophotometric assay for RNA was by an azure B technic (Barron, K.D. et al, Brain Res. 130:469, 1977). In untreated, tractotomized animals, loss of cytoplasmic RNA from axotomized neurons approximates 20% at three days, 50% at 7-14 days and 65% at 28-60 days postoperative. Nucleolar RNA falls more slowly and is reduced by 41,49 and 58% 14,28 and 60 days after surgery. Somal areas of axotomized neurons averaged 63,50 and 43% of normal 14,28 and 60 days postoperatively. However, counts of neurons in serial sections did not show a significant cell loss in the axotomized RN. Neuroglial proliferation did not occur, in contrast to observations made after axotomy of rat peripheral neurons. The effects of ganglioside treatment are under statistical assessment. In ganglioside-injected rats, neuronal areas were 73 and 65% of normal 14 and 28 days after operation while somal RNA content was 58 and 54% (53 and 36% in untreated animals) and nucleolar RNA 73 and 63% of normal values (59 and 51% in untreated rats). Nucleolar shrinkage also was retarded in ganglioside-treated animals 14 and 28 days after operation (83 and 74% of normal vs 69 and 61%). Light microscopic study of cord lesions did not yield evidence of axonal regeneration in any animals. However, ganglioside treatment may retard the atrophy and loss of RNA that occurs in rubral nerve cells after axotomy.

175.PO "DE MEDINACELI" VERSUS MICROSUTURE: A CRITICAL APPRAISAL OF NERVE REPAIR. J. K. Terzis. Microsurgical Research Center, Norfolk, VA 23501.

Microsurgical nerve coaptations utilize microsutures to maintain close apposition of the cut nerve ends. However, the return of function is typically less than perfect. A new method aimed at circumventing the shortcomings of current techniques has been introduced by de Medinaceli and claims to provide superior results: we have therefore compared the results of nerve repair using the new technique with those using established microsurgical techniques. Results are compared with those after nerve crush, an injury considered to give near optimal conditions for full recovery.

The sciatic nerves of 99 rats were exposed under Nembutal anesthesia and either crushed between watchmakers forceps, or cut and repaired using the standard suture method or the new de Medinaceli method. All operations were performed in random sequence by one surgeon, except for 10 rats in which the nerves were reparied by Dr. de Medinaceli. The rats were coded and their recovery over 3 months evaluated under double blind conditions using the following four methods:

(1) determination every 3-7 days of the twitch tension developed by the middle toe upon supramaximal sciatic stimulus;

(2) analysis of walking tracks obtained every 3-7 days;

(3) measurement of the compound action potentials in response to proximal sciatic stimulation, recorded above and below the lesion in a terminal acute experiment;

(4) histological examination of transverse sections of the sciatic nerve above and below the lesion, and in longitudinal sections through the site of nerve injury.

Examination using the first two methods were hampered by the strong tendency of the rats to mutilate their anesthetic foot (94% of rats in the suture group; 70% of de Medinaceli; 16% of crush).

Preliminary findings suggest that the results obtained with the de Medinaceli method are superior to those obtained with established micro techniques: both techniques yield a result inferior to that obtained with simple nerve crush. The detailed studies will be presented which will reveal any clear advantages of the new method.

DRUG EFFECTS ON RECEPTORS

176.1 SUBSTANCE P AND ITS ANTAGONISTS SHORTEN THE OPEN LIFETIME OF NICOTINIC RECEPTOR CHANNELS IN BC3H-1 CELLS. D.P. Walton* and R.E. Sheridan. Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

The effects of Substance P and a synthetic antagonist of Substance P were examined at nicotinic receptors in the continuous cell line BC3H-1. BC3H-1 cells were grown for 2-4 days in DMEM with 20% fetal bovine serum. The cells were then transferred to DMEM containing either 2% or 0.5% fetal bovine serum to slow cell growth and initiate acetylcholine receptor synthesis. Patch clamp recordings were made on the cells from 4 to 10 days following the change to low-serum media. Single nicotinic receptor channel currents were recorded from intact and excised, inside-out patches of cell membrane exposed to 0.1 to 0.6 micromolar acetylcholine or 1 to 7 micromolar carbachol contained in the patch electrode. Either Substance P or the Substance P antagonist, D-pro^2, D-trp^{7,9} Substance P, were added in micromolar concentrations to the patch electrode. The experiments were conducted in HEPES-buffered saline at 20°C.

Both Substance P and the D-pro^2, D-trp^{7,9} Substance P analog shortened the mean open lifetime of the nicotinic receptor channel. At -125 mV, 20 micromolar Substance P roughly halved the mean open time for receptors opened by either acetylcholine or carbachol. The Substance P antagonist was actually more potent than Substance P and at 20 micromolar reduced the mean open lifetime of carbachol-induced membrane channels by about 5 fold. This "channel shortening" effect of the peptides increased linearly with concentration and increased exponentially with membrane hyperpolarization. However, there was no change in the open-channel conductance of the nicotinic receptors in the presence of either peptide.

The reduction in the mean open lifetime of nicotinic channels in the presence of both Substance P and a Substance P receptor antagonist suggests that this effect is not mediated by a Substance P selective receptor site on the nicotinic receptor. Both the linear dose-response relationship for channel shortening and the strong, exponential dependence on membrane voltage suggest that Substance P interacts with the nicotinic receptor ion channel in a manner analogous to local anesthetics and other channel-blocking drugs at the nicotinic acetylcholine receptor.

176.2 THE OPTICAL ISOMERS OF THE BENZOMORPHAN DERIVATIVE, SKF 10047, HAVE EQUIVALENT EFFECTS ON THE IONIC CHANNEL OF THE PERIPHERAL NICOTINIC RECEPTOR. N. Kapai*, Y. Aracava*, and E.X. Albuquerque. (SPON: Dr. R.G. Grenell) Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

SKF 10047 (N-allynormetazocine) is considered to be the prototype drug for the putative σ opiate receptor. The dextro stereoisomer (+)- and the racemic mixture (±)-SKF 10047 are reported to possess psychotomimetic properties, and the levo (-)-SKF 10047 to have analgesic effects (Martin et al., J. Pharmacol. Exp. Ther. **197**:517, 1976). Binding studies performed on Torpedo membranes have revealed that (-)-SKF 10047 interacts with a site on the acetylcholine receptor-ionic channel (AChR) complex that is distinct from the agonist site as well as that for phencyclidine (PCP) (Oswald et al., Proc. Natl. Acad. Sci. **82**:940, 1985). In light of these findings we attempted to study the effects of the above two steroisomers of SKF 10047 on the nicotinic AChR of the frog (Rana pipiens) neuromuscular junction. The electrophysiological studies were performed using sciatic nerve-sartorius muscle preparations for endplate current (EPC) and isolated fibers from interosseal muscles for single channel current recordings. Our studies revealed that both the stereoisomers (10-60 μM) produced a similar depression of the peak EPC amplitude with a resultant nonlinear current-voltage relationship at increasing hyperpolarized potentials. In addition, the decay time constant of the EPCs (τ_{EPC}) was shortened with an apparent loss in the voltage dependency (10-40 μM) as observed under control conditions. The blocking effects were therefore pronounced at more hyperpolarized potentials. At low concentrations (< 5 μM), only the (+)-isomer produced a slight prolongation of τ_{EPC} with a significant increase in the peak amplitude of the EPC, suggesting the possibility of an anticholinesterase action similar to that observed for psychotomimetic drugs such as PCP. Single channel recordings performed in the presence of both (+)- and (-)-SKF 10047 revealed short channel openings without bursting activity. The increased channel activation and prolongation of the channel open times seen with low concentrations of (+)-SKF 10047 on EPCs were not observed at the single channel level, most likely due to the absence of cholinesterase in the single fiber preparations. The channel open times reached a saturation level at concentrations higher than 20 μM only with the (+)- and not with (-)-SKF 10047. Based on these studies, both stereoisomers of SKF 10047 appear to interact with the neuromuscular AChRs blocking the ionic channel in its open conformation while the (+)-isomer may have in addition other effects. (Supported by USPHS Grant NS-12063 and U.S. Army Med. Res. and Develop. Command Contract DAMD-17-84-C-4219.)

176.3 AGONIST EFFECTS OF NEOSTIGMINE ON SINGLE NICOTINIC ACETYLCHOLINE CHANNEL CURRENTS RECORDED IN RAT MYOTUBES. J.F. FIEKERS, Dept. Anatomy and Neurobiology, University of Vermont Coll. Med., Burlington, Vermont 05405.

The carbamate anticholinesterase agent, neostigmine, in addition to its inhibitory action on acetylcholinesterase, has direct actions on the acetylcholine (ACh) receptor-channel complex (Fiekers, J. Neurosci.5:502-514, 1985). In the present study the direct actions of neostigmine were studied on single channel currents using the gigaohm patch clamp technique. Rat myotubes were cultured in DMEM and 10% horse serum using standard cell culture conditions. Single channel currents were recorded at 20°C on cell attached and inside-out recording configurations. Neostigmine in concentrations ranging from 0.1 nM to 100 uM activated channels in the absence of agonist. The amplitude, duration and frequency of opening of neostigmine-activated channels were increased with membrane hyperpolarization. These currents reversed at approximately zero mV. The frequency of channel opening also increased with increasing concentrations of neostigmine. Concentrations above 10uM exhibited channel openings with abrupt closures similar to effects observed with channel blocking agents. Following a period of increased channel frequency, concentrations above 50uM produced a steady decrease in the frequency of channel opening with complete cessation of channel activation occurring at a rate inversely related to the neostigmine concentration. With high concentrations of neostigmine bursts of channel activity were followed by prolonged silent periods. The half time for onset of this desensitized state was approximately 3 min. for 100uM neostigmine at -100mV.

The frequency of activation of single channel currents was markedly increased above control levels when both neostigmine and ACh (1nM-1uM) were present in the patch electrode. This was followed by a decrease in channel opening frequency which was more rapid than either agent alone. Single channel currents were frequently interrupted by brief closures, i.e. flickering within the open channel and the noise present during a channel opening was increased. Single channel currents recorded in the presence of neostigmine, ACh or both were blocked by prior addition of d-tubocurarine. Similar results were also obtained with experiments on ACh receptor channel complexes in the end-plate regions of adult neuromuscular junctions of snake twitch fibers following enzyme treatment. These results demonstrate that direct actions of neostigmine include (1) agonist effects on the nicotinic receptor, (2) the production of a desensitized state of the receptor and (3) the establishment of an altered state of the receptor in the presence of agonist. (supported by MDA).

176.4 DIRECT INTERACTIONS OF REVERSIBLE AND IRREVERSIBLE CHOLINESTERASE (ChE) INHIBITORS WITH THE ACETYLCHOLINE RECEPTOR-IONIC CHANNEL COMPLEX (AChR): AGONIST ACTIVITY AND OPEN CHANNEL BLOCKADE. Y. Aracava* and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

New aspects of the molecular pharmacology of the classic ChE inhibitors have recently been revealed. Carbamates and organophosphate anti-ChE agents have been shown to have direct effects on the AChR behaving either as a nicotinic agonists or as noncompetitive blockers interacting with sites located on the ionic channel. The current development of a method for obtaining isolated fibers from interosseal muscles of the adult frogs and their suitability for patch-clamp recordings have allowed studies of the kinetics of drug interactions with the nicotinic AChR at the single channel current level. Thus, this biological model was used to evaluate the effects of the carbamates neostigmine and edrophonium, and of the organophosphate agent VX on the neuromuscular AChR. Single channel currents activated by ACh (0.3-0.4 μM) had conductance of 33 pS and mean channel open times of 10-12 msec at -100 mV membrane potential with very few fast closures during the open state. In contrast, neostigmine (1-100 μM)and edrophonium (1-50 μM) when present together with ACh inside the patch electrode produced bursts of very short open events (5-7 openings per burst) with 33 pS conductance as well as of low-conductance channel openings. The individual openings within a burst had a mean duration of 2.8 msec and 0.5 msec in the presence of edrophonium 5 and 50 μM, respectively. Similar effects were observed with neostigmine (50 μM) which decreased the mean lifetime of these conductive intervals to 1.0 msec. The duration of the short closures within a burst (the reciprocal of the backward rate constant of the blocking reaction) were also analysed. In the presence of these carbamates, the mean of these short closed times showed a strong voltage dependence and a prolongation at more hyperpolarized potentials (1.8 msec and 0.52 msec at -100 and 200 mV, respectively). VX (1-50 μM) produced bursts composed of more prolonged open events (3.6 msec at 20 μM). The analysis of the short closed times revealed a slow component (15 msec at 100 mV membrane potential) which suggested a more stable blocked state. In addition, neostigmine and edrophonium showed an agonistic effect, activating bursts of channel openings similar to those observed in the presence of these agents together with ACh. VX, however, did not have such a property. These results indicated that in addition to the well known antiChE activity, these agents possess marked and distinct effects on the post-synaptic receptors of the neuromuscular junction either altering the ACh-activated channels or opening channels as nicotinic agonists. (Supported by U.S. Army Res. & Devel. Command Contract DAMD-17-84-C-4219.)

176.5 COMPARISON OF TWO POTENT, SEMIRIGID NICOTINIC AGONISTS AT THE FROG NEUROMUSCULAR JUNCTION. C.E. Spivak, T.M. Gund*, R.F.-Liang*, and J.A. Waters*. Neuropharmacology Lab., Addiction Research Center, NIDA, Baltimore, MD 21224 and Dept. Pharmacol. & Exptl. Ther., Univ. of Maryland School of Med., Baltimore, MD 21201; Dept. Chem., & Chem. Eng. New Jersey Inst. Technol., Newark, NJ 07102; NIADDK, Bethesda, MD 20205.

Little is known about the relationship between structure and activity of nicotinic agonists. This deficit is due in part to the scarcity of potent, rigid or semirigid representatives. The two agonists described here are cyclic semirigid structures that nearly superimpose. However, they differ greatly in potency. Our objective was to evaluate potency in terms of thermodynamic constants and to relate these to the small differences in structure.

Isoarecolone methiodide (1,1-dimethyl-4-acetyl-1,2,5,6-tetrahydropyridine iodide) (ISO) and 1,1-dimethyl-4-acetylpiperazine iodide (PIP) were synthesized. Potencies were evaluated by isotonic contracture of frog rectus abdominis muscles in assays using carbamylcholine (carb) as a standard. ISO was 50 times more potent than carb (95% confidence interval: 45-56x), making it one of the most potent nicotinic agonists known. PIP was 2.6 times more potent than carb (95% confidence interval 2.4-2.8x). Thus ISO was nearly 20 times more potent than PIP. Intracellular recording of frog sartorius muscles corroborated this finding. For example a 20 mV depolarization was produced by 0.3μ M ISO or by 4.9 μM PIP. Patch clamp studies showed that channel open times could not account for this difference in potency.

Minimum energy conformations of the two agonists were calculated by molecular modeling using a modified version of MM2 with ammonium parameters. The distance from the van der Waals extension of the carbonyl oxygen to the quaternary nitrogen was 6.1Å for both compounds. PIP, however, was more chair-like. Consequently, when the quaternary nitrogens and their alpha substituents were superimposed, the carbonyl oxygen of PIP was displaced upward (on the axis perpendicular to the plane of the rings) by 1.18Å relative to its postion in ISO. Differences in electrostatic potential contours were located using the MNDO method for partial charge distributions. For example, the axial N-methyl group of ISO had a more extended high energy isopotential surface than did the corresponding group of PIP. This finding is noteworthy because this position seems vital for establishing the coulombic bond to the recognition site.

These studies help map the recognition site and the bond types in the nicotinic receptor in both its native and activated conformations.

176.6 GEPHYROTOXIN INTERACTION WITH HISTRIONICOTOXIN BINDING SITES ON THE NICOTINIC ACETYLCHOLINE RECEPTOR COMPLEX OF TORPEDO ELECTRIC ORGAN. L. Narayanan*[1], R.S. Aronstam[1], E.X. Albuquerque[2] and J.W. Daly[3]. (SPON: S.H. Hobbs). [1]Dept. Pharmacol. & Toxicol., Med. College of Georgia, Augusta, GA 30912, [2]Dept. Pharmacol. & Exp. Ther., Univ. of Maryland Sch. Med., Baltimore, MD 21201, & [3]Lab. Bioorganic Chemistry, NIADDKD, NIH, Bethesda, MD 20205.

Gephyrotoxins are a class of tricyclic and bicyclic alkaloids isolated from the skin secretions of the Colombian frogs Dendrobates histrionicus and Dendrobates occultator (Daly, Prog. Chem. Org. Nat. Prod. 41:205, 1982). Gephyrotoxin (GyTX) interferes with neuromuscular transmission through blockade of the receptor complex in the open state and by stabilization of a desensitized conformation of the receptor (Souccar et al., Mol. Pharm. 25: 384-400, 1984). In the present study, we determined the ability of 10 natural and synthetic gephyrotoxins to inhibit [^{125}I]alpha-bungarotoxin (BGT) binding to the acetylcholine receptor of Torpedo electroplax and to inhibit [^{3}H]perhydrohistrionicotoxin ([^{3}H]HTX) binding to sites associated with the receptor-gated ion channel in the presence and absence of receptor ligands.

None of the gephyrotoxins inhibited BGT binding by more than 15% at 50 uM (Carb IC50=2.8 uM). The Ki values associated with GyTX inhibition of [^{3}H]HTX binding are listed below. The natural 1 and synthetic d enantiomers (1 and 2) did not differ in their ion channel affinity, and in the presence of Carb their affinity was increased 3 fold. Structure-activity considerations suggest an important contribution of hydrophobic interactions (e.g., 5 vs 7 or 8 vs 10), although the precise stereoconfiguration was not critical for activity. The data suggest that a GyTX analogue with a longer alkyl chain at C_6 would be very active as an ion channel blocker but would be relatively insensitive to allosteric regulation by receptor agonists.

		Ki, uM		
	Compound	[3H]HTX	[3H]HTX+Carb	R
1	1-gephyrotoxin	2.5	0.79	3.2
2	d-gephyrotoxin	2.0	0.74	2.7
3	dihydrogephyrotoxin	1.2	0.60	2.0
4	dl-perhydrogephyrotoxin	0.22	0.16	1.4
5	(5Z,9E)desamylgephyrotoxin	26	3.9	6.7
6	(5E,9Z)3,5-dibutylindolizidine	1.3	0.63	2.0
7	(5Z,9Z)3,5-dibutylindolizidine	0.85	0.43	2.0
8	(5Z,9E)-5-propyl-3-(2'hydroxybutyl)indolizidine	10	7.9	1.3
9	(5Z,9E)-5-propyl-3-(2'-epihydroxybutyl)indolizidine	4.3	2.8	1.5
10	(5E,9Z)-5-propyl-3-(2'hydroxyethyl)indolizidine	12	10	1.2

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176.7 PHENYTOIN INHIBITS VOLTAGE-DEPENDENT AND DIHYDROPYRIDINE-STIMULATED CALCIUM UPTAKE BY NG108cc15. R.G. Ribares* and R.J. Miller (SPON: C. Houser) Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

Decreases in extracellular Ca++ in the cortex are known to accompany and often precede the onset of seizures in various models of epilepsy. Agents that block postsynaptic Ca++ entry have been shown to prevent seizure activity elicited by various convulsants. Several lines of evidence indicate that diphenylhydantoin (DPH) inhibits Ca++ dependent cellular processes. The present study was undertaken to determine whether DPH can block voltage-dependent Ca++ entry in cultured neuronal cells and to test the possibility of an interaction with the dihydropyridine site.

NG108cc15 were grown as monolayers in EMEM with 10% FBS and 2mM glutamine. After 3 days media was replaced with EMEM supplemented with 10 μM PGE1 and 50 μM IBMX. On the fourth day of this treatment cells were assayed for 45Ca++ uptake by timed incubations with Hepes/EMEM containing 45Ca++, either 5.4mM or 50mM K+, and selected drugs (DPH, carbamazepine, phenobarbital, primidone, valproate, ethosuximide, clonazepam, BAY K8644, lidocaine, 4-aminopyridine). Cells were solubilized and protein content and 45Ca++ were measured. All time course assays and dose response curves were run under both low and high K+ conditions. Low K+(basal) 45Ca++ uptake was subtracted from the high K+ uptake to determine depolarization-dependent Ca++ uptake.

Basal 45Ca++ uptake increased linearly at approximately 300cpm/mg protein/min. High K+ augmented 45Ca++ uptake by 3 to 5 fold within the first 5 to 7 min. DPH had no effect on low K+ uptake but selectively inhibited depolarization-stimulated 45Ca++ uptake with an IC 50 of 4.5 μM, which lies within therapeutic CSF levels. Time course assays showed markedly diminished slope within the first 5 min. Elevated extracellular Ca++ (from 1.3mM to 3.0 and 10.0mM) raised the IC 50 of DPH inhibition from 4.5 to 8.1 and 24 μM, respectively, while its maximal effect decreased only slightly. The dihydropyridine BAY K8644 selectively stimulated voltage-dependent Ca++ uptake in a dose related manner, with maxima of 4 to 5 times control high K+ levels. In the presence of DPH, the dose-response curve for BAY K was shifted to the right and the maximal effect was reduced (to 50% by 10 μM DPH), indicating a non-competitive interaction. None of the other antiepileptic drugs tested inhibited Ca++ uptake at concentrations within their therapeutic range. For those that did inhibit Ca++ uptake, albeit at supratherapeutic levels, inhibitory potency correlated with lipid solubility and differed from DPH in their response to changes in extracellular Ca++, selectivity for depolarization-induced Ca++ uptake, and ability to inhibit BAY K stimulated uptake. Clonazepam showed a biphasic dose-response. In conclusion, DPH appears to inhibit the same voltage-sensitive calcium channels with which the dihydropyridines interact in NG108cc15 cells.

176.8 **INFLUENCE OF AGE ON THE RESPONSE OF BENZODIAZEPINE AND GAMMA-AMINOBUTYRIC ACID RECOGNITION SITES TO DIAZEPAM EXPOSURE.** **Harold L. Komiskey, Kathe L. Mundinger* and Atiqur Rahman*. Department of Biomedical Sciences, University of Illinois, College of Medicine at Rockford, Illinois 61107.**

The effect of age on specific binding of ^{3}H-benzodiazepines and ^{3}H-Gamma-aminobutyric acid (^{3}H-GABA) in the brain has been previously examined in naive animals. In the present experiments, rat brain tissue above the cerebellum was examined for age-dependent changes in ^{3}H-flunitrazepam ex vivo binding and ^{3}H-GABA in vitro binding after an acute injection of a low dose of diazepam. Young (3-4 mo.old), mature (12-15 mo. old) and old (29-31 mo. old) male Fischer 344 rats were injected intravenously with diazepam 180 ug/kg or the vehicle five minutes prior to decapitation.

The acute diazepam injection decreased ^{3}H-flunitrazepam ex vivo binding in only senescent rats. ^{3}H-flunitrazepam (6.3nM) binding at both the BZ_1 and BZ_2 receptor or receptor conformation were significantly reduced in the old rats. In contrast,^{3}H-flunitrazepam binding was unaltered with age in rats injected with the vehicle. The concentration of diazepam, desmethyldiazepam and oxydiazepam in the brain tissue above the cerebellum did not differ significantly among the three age groups at the time of sacrifice.

The in vitro binding of ^{3}H-GABA in well washed brain homogenate showed age-related changes. The affinity of the $GABA_A$ recognition site was significantly less in the old rats compared to either the young or mature after vehicle injections. An acute diazepam injection increased the affinity of the $GABA_A$ recognition site in the senescent rats.

The present findings indicate that a low dose of diazepam elicits within minutes an age-dependent alteration in benzodiazepine and GABA binding. Therefore, age-related modifications in the response of benzodiazepine and GABA recognition sites to diazepam exposure may be involved in the increased sensitivity of the elderly to diazepam.

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176.9 DIAZEPAM REVERSES THE ATROPINE-RESISTANT SECOND POPULATION SPIKE (PS) CAUSED BY DIISOPROPYLFLUOROPHOSPHATE(DFP) IN RAT HIPPOCAMPAL SLICES. A.M. Williamson and J.M. Sarvey. Dept. of Pharmacology, USUHS, Bethesda, MD 20814-4799.

The irreversible organophosphate acetylcholinesterase (AChE) inhibitor, DFP (10 uM), has been shown to produce a second PS in field CA1 in response to stimulation of fibers in stratum radiatum (Williamson and Sarvey, Neurosci. Abst. 10:167.12). While the second PS produced by iontophoretic application of ACh was blocked by bath application of atropine (1-10 uM), the second PS elicited by DFP was resistant to atropine as well as the nicotinic antagonists, hexamethonium, gallamine and dihydro-B-erythroidine (all 10 uM). Additional experiments have shown that when responses to bath applied carbachol (20 uM), muscarine (20 uM) and ACh (200 uM) were blocked by the irreversible muscarinic antagonist, quinuclidinyl benzylate (1 uM), DFP can still produce a second PS. Therefore, a study was conducted to characterize further the nature of the atropine-resistant second PS.

Experiments were performed on rat hippocampal slices (350-400 uM thick) using standard extra- and intracellular techniques.

Intracellular recordings from CA1 pyramidal cells showed that DFP (10 uM) blocked spike afterhyperpolarization (AHP) and accommodation of action potential generation to depolarizing current pulses. These effects were consistent with an increase in the local concentration of ACh following inhibition of endogenous AChE. Both the loss of the AHP and the loss of accomodation were reversible upon exposure to atropine (1 uM). However, the second PS, as measured extracellularly and the corresponding second action potential measured intracellularly, were not affected by atropine.

Extracellular experiments were then performed to evaluate the ability of the anti-convulsant, diazepam, to affect the second PS. Superfusion of diazepam (1 uM) for 20 minutes significantly reduced the amplitude of the second PS elicited by previous exposure to DFP (10 uM) from 56.5 ± 6.2% (mean ± SEM) to 6.4 ± 2.2% (n=8, $p<.05$). Intracellular recordings revealed that diazepam had no effect on the loss of the AHP and accommodation, but did increase the magnitude of the orthodromically generated early IPSP.

Further experiments are now being performed to delineate the origin of the second PS elicited by DFP, but the second PS does not appear to be an indirect effect resulting from inhibition of AChE.

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176.10 EFFECTS OF THE WATER-SOLUBLE DERIVATIVE OF DELTA-9-TETRAHYDROCANNABINOL ON ^{3}H-DIAZEPAM AND ^{3}H-FLUNITRAZEPAM BINDING TO RAT BRAIN MEMBRANES IN VITRO. S. C. Sung and A. Jakubovic. Division of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Over the past several years various biochemical and cellular responses to cannabinoids have been reported. The interference of cannabinoids with the uptake of neurotransmitters into synaptosomes has been ascribed to their action on receptors. Some of the pharmacological activities of the psychotomimetic compounds of cannabis including tranquilizing properties appear to be associated with their affinities for biological membranes. In the present study, the interference of the psychoactive water-soluble derivative of delta-9-tetrahydrocannabinol (1-[4-morpholino)butyryloxy]-3-n-pentyl-6,6,9-trimethyl-10a,6a,7,8-tetrahydrodibenzo[b,d] pyran hydrobromide) (SP-111A) with the ^{3}H-diazepam and ^{3}H-flunitrazepam binding to rat brain membranes was examined. It was found that SP-111A reduced, in a concentration-dependent fashion, the specific binding of ^{3}H-diazepam and of ^{3}H-flunitrazepam to membrane preparations of rat brain cortex. The inhibition of the specific binding of ^{3}H-diazepam by SP-111A was found to be competitive with a K_i value of 3.1 μM. In the presence of 7.5 μM SP-111A the apparent K_d of ^{3}H-diazepam binding increased from 4.3 nM to 12.5 nM, without affecting the B_{max}. The degree of inhibition by SP-111A was dependent on both the concentration of ^{3}H-diazepam as well as the amount of membrane protein. With 6.2 nM ^{3}H-diazepam and 0.082 mg of membrane protein in a final volume of 0.2 ml, the IC_{50} of SP-111A was found to be about 7.5 μM. The inhibition of the specific binding of ^{3}H-flunitrazepam by SP-111A was also competitive; however, the IC_{50} was higher than with ^{3}H-diazepam. Similar inhibition was observed when brain membrane preparations were preincubated for 20 min at 0°C or or 37°C with SP-111A, then repeatedly washed with buffer before incubation with either ^{3}H-diazepam or ^{3}H-flunitrazepam. Comparable concentration-dependent inhibition of specific binding of both ^{3}H-diazepam and ^{3}H-flunitrazepam by SP-111A was observed with membrane preparations either from rat brain cortex or cerebellum or brainstem. Present results indicate that the psychoactive delta-9-tetrahydrocannabinol and other cannabinoids could be competing ligands for benzodiazepine receptors and perhaps other receptor sites in the CNS.

176.11 INTERACTION OF AMINOPYRIDINES WITH [^{3}H]PCP RECEPTORS IN RAT BRAIN. W.S. Lai* and E.E. El-Fakahany. (SPON: A.P. Leccese). University of Maryland School of Pharmacy, Baltimore, MD 21201.

The behavioral effects of phencyclidine (PCP) have been attributed to its ability to block certain potassium channels in brain synaptosomes (Albuquerque et al., Proc. Natl. Acad. Sci. U.S.A. 78, 7792, 1981 and Blaustein and Ickowicz, Proc. Natl. Acad. Sci. U.S.A. 80, 3855, 1983). This hypothesis prompted us to test the ability of 4-aminopyridine, a prototype potassium channel blocker, to interact with [^{3}H]phencyclidine ([^{3}H]PCP) receptors in the brain.

Three analogs of aminopyridine (AP) were tested for their effect on specific [^{3}H]PCP binding in whole rat brain homogenates. For the binding experiments, brain homogenates were incubated with [^{3}H]PCP in 5 mM Tris-HCl buffer (pH 7.4) for 60 min at 4°C. Nonspecific binding was defined using 10 µM PCP and the incubation mixture was filtered over GF/B filters presoaked in 0.05% polyethyleneimine. The rank order of potency in displacing [^{3}H]PCP binding was 4-AP > 3,4-AP > 3-AP. 4-AP displaced [^{3}H]PCP binding with a Hill coefficient close to unity, however, it showed a ceiling effect around 80-85% displacement. When increasing concentrations of [^{3}H]PCP were used in the binding assay, the ability of 4-AP to inhibit the binding was decreased. Dixon plots of such displacement experiements showed an intersection point above the abscissa, suggesting an apparent competitive interaction. In addition, the effects of both 4-AP and unlabeled PCP on the dissociation kinetics of [^{3}H]PCP binding were investigated. Although excess unlabeled PCP failed to alter the rate of dissociation of [^{3}H]PCP-receptor complex induced by dilution, 1 mM 4-AP enhanced the dissociation rate by a factor of three. Our present data suggest that the effect of this class of potassium channel blockers on [^{3}H]PCP receptors in rat brain might not be due to a simple competitive interaction.

176.12 **DOWN-REGULATION OF BETA-ADRENERGIC RECEPTORS (B-AR) WITHIN 24 HR AFTER INTRAVENOUS INFUSION OF ANTIDEPRESSANTS.** V.H.Sethy and J.S.Day, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The down-regulation of B-AR in the cerebral cortex is achieved after chronic administration of antidepressants by either the intraperitoneal or oral route (F.Sulser, J. Clin. Psychiat. 44:14, 1983). The delayed appearance of a reduction in the density of B-AR correlates with the latency of therapeutic effects of antidepressants in patients with depression. However, Saletu et al. (Prog. Neuropsychopharmacol. 1:125, 1977) have reported a significant decline in the Hamilton Depression Score at 48 hr after i.v. infusion of antidepressants to patients with depression. Therefore, investigations were carried out to determine the effect of 24-hr infusion with various antidepressants on B-AR in the cerebral cortex of female rats. Desipramine (1, 3, 10, 60 mg/kg), imipramine (10, 30, 100 mg/kg/day), amitriptyline (3, 10, 30, 60, 100 mg/kg/day), zimelidine (3, 10, 30, 60, 100 mg/kg/day), iprindole (3, 10, 30, 60, 100 mg/kg/day), and U-48753E (1, 3, 10, 30 mg/kg/day) dose-dependently down-regulated B-AR at 24 hr. The lowest dose (mg/kg/day) which produced a significant reduction in B-AR was as follows: desipramine = 3, imipramine = 30, amitriptyline = 3, zimelidine = 10, iprindole = 10, and U-48753E = 3. The time-course studies comparing U-48753E with imipramine, each 10 mg/kg/day, demonstrated that U-48753E significantly down-regulated B-AR at 16 hr, whereas a significant effect with imipramine was observed at 48 hr. Treatment with these antidepressants did not modulate pre- or post-synaptic alpha-adrenergic receptors consistently. The results of these investigations suggest that i.v. infusion of antidepressants rapidly down-regulate B-AR, and this procedure may be of clinical utility for providing a rapid therapeutic effect in patients with depression.

176.13 THE NOVEL ANXIOLYTIC BUSPIRONE ELICITS A SMALL HYPERPOLARIZATION AND REDUCES SEROTONIN RESPONSES AT PUTATIVE 5-HT$_1$ RECEPTORS ON HIPPOCAMPAL CA1 PYRAMIDAL CELLS R. Andrade and R.A. Nicoll. Depts of Pharmacology and Physiology, Univ. of California in San Francisco, San Francisco, CA 94143.

Two main types of serotonin receptors, 5-HT$_1$ and 5-HT$_2$, have been proposed to exist in the central nervous system based upon binding studies. The 5-HT$_1$ type would correspond to binding sites exhibiting high affinity for serotonin agonists and low affinity for antagonists, while the 5HT$_2$ type would correspond to those sites exhibiting high affinity for serotonin antagonists but low affinity for agonists. While this classification has been well established in binding studies, there is at present a paucity of reports concerning the possible functional significance of these binding sites in the brain. In the rat hippocampus, serotonin binding appears to be predominantly of the 5-HT$_1$ type. Therefore we have examined the pharmacology of serotonin responses in this area for possible functional correlates of the postsynaptic 5-HT$_1$ binding site.

Rat hippocampal brain slices were prepared and maintained as previously described. Intracellular recordings were obtained from pyramidal cells of the CA1 region using standard electrophysiological techniques. Serotonin administered either in the bath (300nM - 20µM) or by microiontophoresis elicited a dose dependent hyperpolarization which could be as large as 20 mV and was accompanied by a reduction in input resistance. Administration of the selective 5-HT$_{1A}$ agonist 8-OHDPAT (200nM - 4µM) also hyperpolarized these cells. No depolarizing responses to serotonin were ever observed. The classical serotonin antagonists cyproheptadine and cinanserin were weak at antagonizing responses to iontophoretically applied serotonin with IC50s of 50µM and 100µM respectively, while the selective 5-HT$_2$ antagonist ketanserin did not reduce serotonin responses in concentrations up to 200 µM. The ergot methysergide was found to elicit a small hyperpolarization and to effectively reduce serotonin responses with an IC50 of 30µM. Similarly, the novel non-benzodiazepine anxiolytic buspirone, which has been reported to be a potent and selective ligand at displacing serotonin from hippocampal membranes, was also found to elicit a small hyperpolarization (1-4mV) and to reduce serotonin responses (IC50 = 3µM).

These results are consistent with the suggestion that serotonin receptors in the hippocampus might conform to the 5-HT$_1$ classification and indicate that buspirone behaves as a weak partial agonist at these postsynaptic receptors. Such actions of buspirone could play a role in the mechanism by which this compound exerts its anxiolytic action. Supported by grants MH-38256, MH-00437 and MH09180.

176.14 BUSPIRONE: EFFECT ON BRAIN DOPAMINE AND SEROTONIN RECEPTORS. Tyrone Lee and Gloria Su*, Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

Buspirone, a non-benzodiazepine compound lacking the usual benzodiazepine side effects such as sedation, anticonvulsion or muscle relaxation, has been found to be an effective antianxiety agent in humans. It has also been reported that buspirone possesses some in vivo anti-dopamine actions in animals such as blockage of apomorphine-induced stereotypy, enhancement of striatal dopamine metabolites, elevation of rat plasma prolactin levels and disruption of conditioned avoidance behaviour (Wood et al., Life Sci. 33, 269, 1983). In order to determine if the antipsychotic action of buspirone bears similarities among classical neuroleptics (haloperidol) or atypical neuroleptics (loxapine), its effect on brain dopamine and serotonin receptors was investigated.

Adult male Wistar rats (200 gm) were divided into 6 groups of 8 rats each with daily injection of buspirone (10 mg/kg i.p.) for 1, 2, 3, 5, 7, and 14 days. Another 6 groups of rats received 1 ml of saline i.p. daily for similar duration. All animals were sacrificed 24 hours after the last injection. Brain striatal dopamine (D_2) receptors were measured using ^{3}H-spiperone and frontal cortex serotonin (S_2) receptors were measured using ^{3}H-ketanserine. Scatchard analyses permit the determination of receptor density (B_{max}) as well as receptor affinity (K_d) in saline- and buspirone-treated animals. The results were as follows:

	Buspirone Treatment (Days)					
	1	2	3	5	7	14
B_{max} for D_2	104	101	101	102	126*	130*
B_{max} for S_2	92	94	98	102	105	120

B_{max} values were expressed as % of respective saline-treated group.
*$p < 0.01$, Student's t-test (two-tailed).

Repeated treatment with buspirone for duration of 7-14 days elicited a significant increase in dopamine receptor density with no apparent change in receptor affinity. However, buspirone did not alter serotonin receptors significantly in the rat frontal cortex.

In our present study, buspirone increased dopamine receptor numbers without affecting the serotonin receptors. Atypical neuroleptics such as loxapine or clozapine, however, have been demonstrated to have rapid and potent effect on serotonin receptors but rather weak effect on dopamine receptors (Lee and Tang, Psychiat. Res. 12:277, 1984). Thus, buspirone seems to act more like a classical neuroleptic such as haloperidol. However caution must be taken to interpret these data as buspirone does not cause catalepsy in rats. (Supported by the Clarke Institute of Psychiatry Research Fund.)

176.15 ^{3}H SCH 23390 BINDING IN VIVO: INHIBITION BY ATYPICAL NEUROLEPTIC DRUGS. Peter H. Andersen[1], Erik B. Nielsen[1], Claus Braestrup[1] and Frederik C. Grønvaldt[2] (spon M. Treiman) [1]Depts. of Pharmacology and [2]Med. Chemistry I, NOVO Pharmaceuticals R&D, Novo Allé, 2880 Bagsvaerd, Denmark.

4 µCi of ^{3}H-SCH 23390 was injected into the tailvein of mice. 15 minutes later, the animals were decapitated and within 30 sec the brain (-cerebellum) was homogenized in 14 ml of 25 mM K_2HPO_4 and filtered through Whatman GF/c filters. The binding of ^{3}H-SCH 23390 was saturable, reversible and highly stereospecific. Pharmacological characterization revealed binding of the ligand to D1-receptors since low ED_{50}-values were obtained for drugs known to exhibit D1 affinity: SCH 23390 (0.017 mg/kg), cis-flupentixol (0.4 mg/kg), (+)-butaclamol (0.3 mg/kg), fluphenazine (2.8 mg/kg) and chlorpromazine (7.3 mg/kg). Typical neuroleptics, known to be D2-specific, were almost inactive: haloperidol (72.5 mg/kg), spiperone (168 mg/kg) and pimozide (194 mg/kg). Surprisingly, atypical neuroleptics exhibited high potency in displacing ^{3}H-SCH 23390 in vivo, despite of the fact that most of these compounds were inactive in vitro (below).

	K_i (nM) D1 (in vitro)	ED_{50} (mg/kg) D1 (in vivo)	AMPH cue
SCH 23390	0.14	0.017	0.014
clozapine	600	10.3	1.22
fluperlapine	400	0.8	-
(±)-sulpiride	10.000	90.6	-
(-)-sulpiride	-	-	53.0
thioridazine	21	8	15

Further, the ED_{50}-values obtained for the atypical neuroleptics and SCH 23390 correlated well with ability to block the amphetamin cue in rats. The selectivity of the ^{3}H-SCH 23390 binding was less apparent when applying agonist as inhibitors. These data indicate that ^{3}H-SCH 23390 binds to D1 receptors or a closely associated site. The results obtained with the atypical neuroleptics were striking and indicate that ^{3}H-SCH 23390 binding in vivo, may be a screen for atypical neuroleptic activity.

176.16 BEHAVIORAL EVIDENCE THAT SK&F 38393 IS A PARTIAL AGONIST AT DOPAMINE RECEPTORS. Erik B. Nielsen[1,2] and Peter H. Andersen[1] [1]NOVO Industri A/S, Pharmaceuticals R&D, DK-2880 Bagsvaerd and [2]Sct. Hans Hospital, DK-4000 Roskilde, Denmark.

Rats were trained to discriminate d-amphetamine sulphate (1 mg/kg; AMPH) from saline in a two-lever water-reinforcement operant task. The specific, partial dopamine (DA) agonist SK&F 38393 (5-40 mg/kg) produced only a modest substitution (~50%) for AMPH's cueing effect. The specific DA_2 agonist pergolide (0.2-1 mg/kg) substituted completely for AMPH; on the converse both D-2 specific antagonists (sulpiride, spiroperidol and haloperidol) and the specific D-1 antagonist SCH 23390 blocked completely the AMPH cue.

Partial agonists can sometimes, depending on efficacy, antagonize the effects of agonists. However, SK&F 38393 failed to antagonize the AMPH cue although only low doses (1-5 mg/kg) could be administered due to behavioral disruption. That SK&F 38393 has DA-antagonistic effects was, however, indicated by the ability of the drug (40-80 mg/kg) to antagonize (~80% blocking effect) the unconditioned gnawing behavior elicited by a high dose (60 mg/kg) of methylphenidate in mice. In comparison, low doses of SCH 23390 blocks completely the gnawing behavior induced by methylphenidate. Further, in reserpinized mice, the ability of SK&F 38393 to cause behavioral activation (locomotion, grooming) was increased; indeed, the drug reversed the reserpine-induced behavioral syndrome. In contrast, SK&F 38393 had no consistent effect in normal mice.

The results indicate that SK&F 38393 behaves like a partial agonist; consequently, caution should be exercised when using the drug in studies aimed at determining the role of D-1 receptors in behavior.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT I

177.1 ACETYLCHOLINE SYNTHESIS BY CULTURES OF MATURE ADRENAL CHROMAFFIN CELLS. P. Boksa. Douglas Hospital Research Centre, Depts. of Psychiatry and Pharmacology, McGill University, Montreal, Quebec H4H 1R3 Canada.

Adrenal chromaffin cells normally synthesize and release catecholamines. However recent electrophysiological studies (Ogawa, M. et al, Nature 307:66, 1984) have shown that neonatal rat adrenal chromaffin cells in culture can form functional synapses that are blocked by cholinergic receptor antagonists, suggesting that these cells may be able to synthesize and release acetylcholine (ACh). In the present study, I have shown that cultures of isolated chromaffin cells from the adult bovine adrenal can take up ^{3}H-choline from the extracellular medium and synthesize ^{3}H-ACh. On incubation with 10 µM ^{3}H-choline for 1 h, day 6 cultures synthesized about 4 pmol ^{3}H-ACh/mg protein. The rate of ^{3}H-ACh synthesis was constant in cells maintained in culture from 6 to 15 days and increased from day 19 to day 28, such that ^{3}H-ACh synthesis in day 28 cultures was 2.4 times that in day 6 cultures. Total protein content/culture was constant between days 15 and 28, indicating that there is a relatively specific induction of ACh synthesizing capacity in the cultures. Depolarization of the cells with 60 mM K^+ increased the subsequent synthesis of ^{3}H-ACh, suggesting that ^{3}H-ACh synthesis may be regulated by neuronal activity in a manner similar to that observed in cholinergic neurons. The ability for K^+ to stimulate ^{3}H-ACh synthesis only developed by day 28 in culture.

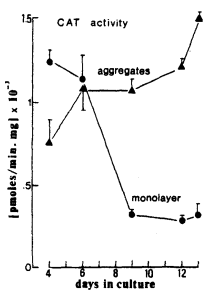
In cholinergic neurons, choline is known to be taken up by a specific high affinity uptake mechanism that is Na^+-dependent and hemicholinium-sensitive, and choline is then acetylated by choline acetyltransferase. In chromaffin cell cultures, ^{3}H-choline was taken up by a single mechanism with a K_T of 8-18 µM. ^{3}H-choline uptake was enhanced by Na^+ omission in day 14 cultures but was 50% Na^+-dependent in day 29 cultures. Hemicholinium-3 (IC_{50} <10 µM) inhibited ^{3}H-choline uptake. Acetyltransferase activity in homogenates of chromaffin cell cultures increased 5-fold after 28 days in culture. Only 5% of the acetyltransferase activity was inhibited by an inhibitor of choline acetyltransferase; however this 5% of acetyltransferase activity was 20 times the amount necessary to produce the observed rates of ^{3}H-ACh synthesis in the intact cell. It is concluded that bovine adrenal chromaffin cells, maintained in culture, are able to exhibit cholinergic properties and this capacity is retained even by the mature cell. Supported by the Medical Research Council of Canada.

177.2 Control of acetylcholine production and release in cultured avian retina cells. F.G. de Mello*, M.C.F. de Mello* and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, Il. 60201.

The differentiation of the cholinergic system of the retina has been investigated in cultured cells from chick embryos. Cells were prepared from 8-day-old embryos and maintained under conditions that favored either monolayer or aggregate cultures. The expression of Acetyl-CoA:choline O-acetyltransferase, EC 2.3.1.6 (CAT) during differentiation of cultured neurons was compared in both culture conditions. CAT activity in monolayer cultures was approximately 1100pmoles/min.mg protein after 4 days of incubation, remaining stable until culture day 6. Thereafter, a 4 fold drop in the activity of the enzyme was observed. When CAT activity was measured in aggregates we observed that after 5 days of incubation the enzyme activity was the same as that observed in monolayer. However,the enzyme activity in the aggregates remained stable in subsequent days. After 12 days in culture CAT activity in aggregates increased further, reaching levels as high as 1500pmoles/min.mg protein. In contrast the enzyme activity of cells in monolayer was less than 400pmoles/min.mg protein after the same incubation period.

The exposure of cultured cells to ^{3}H-choline resulted in the synthesis of ^{3}H-acetylcholine which accumulated in a pool available for release when cells were pulse stimulated either with K^+ (44mM) or L-glutamate (0.5mM). The acetylcholine released by K^+ stimulation was entirely dependent on Ca^{++} ions in the incubation medium, and the L-glutamate promoted Ach release was only partially affected by the absence of Ca^{++}.

The appearance of the evoked release of acetylcholine from cultured neurons followed the same temporal pattern observed for the expression of CAT activity. Moreover, in monolayer cultures that had low CAT activity, the evoked release of acetylcholine was either low or undetectable. Our results indicate that the presynaptic component of the retinal cholinergic system is expressed early during development and suggest that appropriate cellular interaction may be an important factor in maintaining the differentiated state of cholinergic neurons.



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177.3 PHENOTYPIC EXPRESSION OF NEUROTRANSMITTER LEVELS IN 3 TYPES OF DOMESTIC DOGS

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Domestication and continuous selection by man has resulted in the development of many types of dogs that vary along behavioral, tempermental, physiological responsiveness and anatomical scales. The opportunity to correlate certain behavioral differences with neurotransmitter level, and eventually with transmitter receptor levels, may help assess whether such behavioral diversity is reflected in populations of specific brain neurons. Three types of dogs, whose behavioral responses towards a variety of natural stimuli including livestock animals and other "prey" species are being studied to determine the specific behavioral differences among the types and the development of these behaviors. The three types of dogs are: a) Shar Planinetz, a livestock guarding dog that is non-aggressive toward prey; b) Border Collies, a livestock herding dog that displays components of predatory behavior but does not attack livestock; and c) Siberian Husky, a northern type dog that shows full predatory behavior toward livestock and other prey. Besides possible correlation with specific behavioral differences in the three strains, the comparisons of brain neurotransmitter levels will provide an indicator of the variability permitted by natural selection in brain neuronal populations, while still retaining reproductive identification as the same species. Dogs from each type are anesthetized, their brains removed rapidly, and dissected. Brain regions chosen for catecholamine assay were frozen and homogenized later in 0.1 N $HClO_4$ for HPLC separation with electrochemical detection of norepinephrine, dopamine, epinephrine, DOPAC, and 5-hydroxytryptamine. Brain regions chosen for neuropeptide assay were placed immediately into boiling acid, after 20 min. samples were cooled, homogenized and extracts prepared for RIA of enkephalin, B-endorphin, and vasopressin. Region by region comparisons of neurotransmitter content and concentration will be presented, as well as some suggested relationships of particular brain systems to the observed behavioral differences.

177.4 ALTERATIONS IN PRO-OPIOMELANOCORTIN PROCESSING IN THE RAT MEDULLA OBLONGATA DURING DEVELOPMENT. Norman E. Alessi. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Pro-opiomelanocortin (POMC) related peptides Beta-endorphin (B-END) and ACTH have been identified utilizing immunohistochemical techniques in neurons of the nucleus tractus solitarious (NTS) of the rat from embryonic day 17 through the postnatal period into adulthood (Khachaturian et al., Life Sciences, 33 (Suppl. I):61, 1983). Biochemical studies though have been limited to the determination of the alterations in the levels of B-END immunoreactivity (i.r.) in the medulla oblongata during the postnatal period (Alessi and Khachaturian, Neuropeptides, 5:473-476, 1985). No biochemical studies have investigated the processing of POMC in the medulla oblongatas of developing rats. In the present study, levels of B-END i.r. and the processing of POMC were determined in the medulla oblongata during pre- and postnatal development and compared to the adult.

Pregnant (gestation day 21-22) Sprague-Dawley rats were sacrificed by cervical dislocation. The uterus was dissected and placed in a saline-ice bath. The embryonic (E) rats were dissected from the uterus, decapitated, the caudel medulla oblongatas were dissected in block and immediately frozen at -80°C. Also, tissue was collected from postnatal (P) day 1 and adult male rats. B-END i.r. was measured using a previously characterized antibody (Cahill et al., J. Clin. Endocrinol. Metab., 56:992-997, 1983). For molecular sizing the medulla tissue from E21 (65 regions), P1 (55 regions) and adult (15 regions) rats were chromatographied using a 15 mm x 900 mm column with G-50 superfine gel (Sephadex G50-50), and an eluent buffer of 10% formic acid, 0.1% BSA, and 0.1% 2-mercaptoethanol. Percentages of POMC, Beta-Lipotropin (B-LPH), B-END (1-31) and B-END (1-27) were determined for each age group using the results of the chromatography.

Significant changes were noted in the levels of B-END i.r. during development. From E21 to P1 the level of B-END i.r. ($\bar{X} \pm$ S.E.M.) dropped from 14.1 $\pm$ 2.57 fmol/mg to 7.64 $\pm$ 1.30 fmol/mg of protein. Subsequently, B-END i.r. levels increased significantly to 169.3 $\pm$ 19.9 fmol/mg of protein. In addition, chromatographic alterations were noted. At E21, 21% of the total B-END i.r. was demonstrated to be POMC/B-LPH in size, 64% B-END (1-31), and 15% B-END (1-27) increasing to 32%. In the adult POMC/B-LPH concentration decreased to less than 1%, B-END (1-31) increased to 65% and B-END (1-27) remained at the same concentration.

This report demonstrates not only significant changes in the levels of B-END i.r. during development, but changes in the percentages of POMC and related peptides. This data suggests age specific shifts in the processing of POMC in the medulla oblongata with resulting forms of POMC related peptides with markedly different opiate properties. The physiological significance of these changes remain unknown and in need of further study.

177.5 EXPRESSION OF TYROSINE HYDROXYLASE BY CHOLINERGIC CILIARY GANGLION NEURONS IN CULTURE IS REGULATED BY TARGET STRUCTURES. J. Lee*, L. Iacovitti, G. Teitelman, T.H. Joh, V. Albert and D.J. Reis (SPON: J.M. Carroll). Dept. Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

We have previously reported that a few neurons of the parasympathetic ciliary ganglion (CG) of chick embryos contain tyrosine hydroxylase (TH), the first enzyme in the catecholamine (CA) biosynthetic pathway, during pre- and postnatal development in vivo; and that, in vitro, all CG neurons express the enzyme (Teitelman et al., J. Neurosci. 5: 29-39, 1985). In contrast to TH, L-amino acid decarboxylase (AADC), the next enzyme in the CA pathway, is expressed by all CG neurons during development in vivo and in vitro. In this study, we sought to determine whether the expression of TH but not of AADC in CG neurons is regulated by muscle, their target organ. To test this, hearts removed from chick embryos at day 6 of development (E6) were dissociated and plated on collagen-coated dishes at a density of 1 heart/dish. After 24 hrs. in culture, contracting fibers were already abundant. The following day, CG removed from stage 31-34 (E8) embryos were dissociated and added to myocardial cultures. Cultures were grown in media containing horse serum and eye extract as described previously. After 5 days in vitro, the cultures were fixed and processed for immunocytochemical staining. It was found that CG neurons did not contain TH immunoreactivity when cocultured with heart muscle. In these cultures, however, the neurons were easily visualized with antibodies to AADC or to the housekeeping enzyme, neuronal specific enolase (NSE). Sister cultures containing only CG neurons stained darkly with TH antisera as well as with antisera to AADC and NSE. Similar results were obtained when CG neurons were cocultured with skeletal muscle from E11-E12 chick. The effect of muscle on TH expression was tissue specific since CG neurons did not contain TH immunoreactivity when cultured with E7 kidney, E7 lung or E5 liver cell layers.

To test whether the effect of muscle on the appearance of TH in CG neurons was produced by a) a diffusable substance or b) a cell exudate, E8 CG neurons were maintained in vitro either in the presence of heart conditioned medium or in culture dishes containing a substrata of lysed muscle cells. Under both conditions, CG neurons express TH and AADC immunoreactivity after 5 days in vitro.

We conclude that the expression of TH, but not AADC, by CG neurons in culture is regulated by muscle and that this effect is tissue specific. Moreover, the factor(s) controlling the appearance of TH do not diffuse into the media. These results suggest a) that an interaction between CG neurons and living muscle cells is required for the regulation of TH expression and b) that a similar mechanism may exist in vivo. (Supported by NIH Grant HL18974 and NSF Grant PCM-8303019.)

177.6 THE DEVELOPMENT OF PERIPHERAL SEROTONIN-IMMUNOREACTIVE SYSTEMS IN THE CHICK EMBRYO. R.M. Mondragon* and J.A. Wallace (SPON: G. Ballam). Dept. of Anatomy, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131

Serotonin (5-HT) immunoreactive cells have been shown to differentiate from quail neural crest derivatives grown under in vitro culture conditions (Sieber-Blum et al., Dev. Biol., 99:352,'83). In the present investigation we studied the in vivo distribution of peripheral 5-HT immunoreactive cells that appear during development of the chick embryo. 5-HT immunocytochemical staining patterns were examined in chick embryos taken on days 3,4,5,7,9,12,17 and 21 of incubation. A minimum of two embryos at each daily interval were fixed with 4% paraformaldehyde and processed for routine paraffin-embedding. Anti-5-HT immunostaining was visualized on paraffin sections by the peroxidase ABC staining method. The earliest 5-HT immunoreactivity was observed within APUD cells located in the early forming dorsal pancreas of embryos at 3 days of incubation (E3). By E5, stained cells were also found in all primary and secondary sympathetic ganglia, particularly at more rostral embryonic levels. The immunoreactive cells in each ganglia were relatively small in number comprising approximately between 5 and 10% of the ganglionic cell population and were located primarily in clumps at the periphery of the ganglia. By E7, 5-HT-stained cells were observed in all secondary sympathetic (paravertebral) chain ganglia throughout the length of the embryos. In addition, at E7 staining was noted in various developing prevertebral ganglia, especially in positions ventral and lateral to the dorsal aorta. At this age, intense anti-5-HT staining was also observed in numerous cells of the adrenal medulla and within the forming carotid bodies. From E9 to E12 there was a gradual loss of immunoreactive cells found in the sympathetic chain ganglia starting rostrally and progressing caudally to ganglia found in the lumbro-sacral region. At E17 and E21 (hatching) 5-HT-stained perikarya were only rarely found in sympathetic chain ganglia in caudal portions of the embryos, although stained varicose fibers were occassionally encountered in ganglia at all embryonic levels. In contrast to this decrease in immunoreactivity that occurs in sympathetic chain ganglia, intense staining was found in cells of the carotid bodies, adrenal medulla, pancreas and in numerous scattered prevertebral ganglia throughout the period of embryonic development in the chick. At this point, it is impossible to discern whether the loss of anti-5-HT staining in the sympathetic chain ganglia represents cell death, the switching of transmitter phenotypes or the migration of the cells to other embryonic locations. Supported by NSF BNS 82-08433 and NIH NS 20039 and RR 08139.

177.7 TYROSINE HYDROXYLASE MESSAGE LEVELS INCREASE DURING INDUCTION OF CHOLINERGIC PHENOTYPE. K.L. O'Malley*, A. Maniotis* and M. I. Johnson, Dept. of Anat. & Neurobiol., Washington Univ. School of Medicine, St. Louis, MO 63110.

Previous studies have shown that the adrenergic neurons of the neonatal rat superior cervical ganglion (SCG) can be induced in vitro to develop cholinergic traits. It has been a matter of debate whether they do so at the expense of their adrenergic properties (i.e., whether catecholamine pathway enzymes are still maintained after the cells develop the cholinergic phenotype). We decided to re-examine this issue using a cloned cDNA for rat tyrosine hydroxylase (TH). Dissociated SCG neurons were prepared from neonatal rats and non-neuronal cells were eliminated. Dishes of 40,000 neurons were treated with either 20 mM K^+ in order to maintain an adrenergic phenotype, or human placental extract and chick embryo extract to induce a cholinergic phenotype (Iacovitti et al., 1981). At 1, 3 and 5 weeks in vitro, cultures were harvested and used to prepare total RNA and to determine TH and choline acetyltransferase (CAT) enzyme activities. Three dishes of neurons were pooled at each time point and 10 µg of total RNA recovered, electrophoresed in a formaldehyde gel and transferred to nitrocellulose by blotting. Filters were then probed with a high specific activity TH RNA probe. The medium used here to induce cholinergic function resulted in the persistance of TH mRNA at equal or greater levels than that seen in starting ganglia or cultures treated for 10 days with high amounts of K^+. TH enzyme activity followed suit with a 2-3 fold increase over starting ganglia in cholinergically induced cultures. In contrast to the de-sheathed E_{21} ganglia and the cultures treated with 20 mM K^+, a steady linear increase in CAT activity was measured over the 5-week time course in cholinergic inducing medium. These results demonstrate that SCG cultures are capable of maintaining and expressing both adrenergic and cholinergic phenotypes. Moreover, there is a 2-fold increase of TH message levels and enzymatic activity during the cholinergic induction.

Iacovitti et al., J. Neurosci. 1: 685, 1981.

177.8 THE EFFECT OF VARYING NUMBERS OF STRIATAL TARGET CELLS ON DOPAMINE AXONAL PROLIFERATION IN ROTATION-MEDIATED REAGGREGATE TISSUE CULTURE. P. Kontur, A. Heller and P.C. Hoffmann, Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637

Rotation-mediated reaggregate tissue cultures containing embryonic mouse dopamine neurons of the rostral mesencephalic tegmentum show a developmental increase in dopamine levels with increasing time in culture which is associated with the increasing development of axonal processes revealed by fluorescent histochemistry (Kotake et al., J. Neurosci. 2, 1307, 1982).

We have now investigated the effect of varying the proportion of target cells to which dopamine neurons project in such reaggregates upon the extent to which dopamine axonal processes develop as assessed by quantitative measurements of dopamine levels. The ratio of rostral mesencephalic tegmentum cells to striatal target cells was varied over the range of 1:2 to 1:20 in 5 step increments. This was accomplished by varying the number of rostral mesencephalic tegmental cells (between 0.5×10^6 and 5×10^6) added to cultures containing a constant number (10×10^6) of striatal target cells. A constant total number of cells per flask (15×10^6) was maintained by adding an appropriate number of non-target tectal cells. After 14 days in culture, random pairs of flasks of similar combinations were pooled. Dopamine levels were measured by high performance liquid chromatography in 0.1N perchloric acid extracts from sonicated aggregates.

Dopamine levels were found to be linear functions of the increasing number of rostral mesencephalic tegmental cells present in the cultures (r=.99). This linear relationship held for aggregates maintained in culture for an additional 7 days as well.

These results suggest that increasing the relative target cell numbers for dopamine neurons does not result in an increase in dopaminergic axonal proliferation as estimated by dopamine levels. Rather the axonal field per added dopaminergic neuron appears to be relatively constant. (Supported by MH-28942 and the Brain Research Foundation. P.K. supported by MH-14274 Training.)

177.9 NEUROPEPTIDE DEVELOPMENT IN STRIATE AND PRESTRIATE CORTICES. L. A. Benevento and S.K. Itaya. Dept. of Anatomy, University of Illinois College of Medicine, Chicago, Il 60680.

We compared the neuropeptide development of striate and prestriate cortices and attempted to correlate transient neuropeptide distribution with the development of cortical extrinsic vs. intrinsic connections. Here we report on vasoactive intestinal polypeptide (VIP) and somatostatin (SRIF) immunoreactivity (Immunonuclear lot nos. 8403015 & 8344015) determined with fluorescent (Rhodamine) and Avidin-Biotin peroxidase techniques in the rat. Each animal also received true blue retrograde tracer (TB) injections in one parieto-occipital cortex. On postnatal days 3 to 4 (PND=3-4), areas 17, 18a & 18 contained a sparse number of VIP cells in suplate layer V which was heavily labeled with transported TB from contralateral cortex. By PND=6-7 VIP cells and processes were found in layers II to V (mainly II-III) where ipsilaterally (III) and callosally (III & V) projecting cells are now well labeled with TB. This VIP pattern remains in the adult. Although VIP and TB were in the same layers, there were no double labeled cells. Since the onset and location of VIP intrinsic neurons conincides with those of developing corticocortical projecting neurons, the relationship may be due to VIP cells becoming active when "local regulation of energy metabolism" (Peptides 5:213-8,'84) is required by cortical layers which are forming axonal circuitry. The distribution of VIP cells in areas 17, 18a & 18 was similar at each age. This was not true for SRIF containing cells. By PND=3-4, the heaviest SRIF immunoreactivity was noted in in layers V and VI of area 18. In area 17 there were a few cells in layer VI which were somewhat more numerous at the borders with 18a and 18. Area 18a appeared like 17, but had a few more cells in VI . As development continued this relative pattern of SRIF cell density between 17, 18a & 18 persisted. By PND=6-7 area 18 had a large number of SRIF cells in layers V and VI and a moderate amount in III-IV. A moderate number of SRIF cells was seen in layers V and VI in both 17 and 18a with an occassional cell in layer IV. Again, SRIF cells in area 17 seemed more concentrated at the borders with 18 and 18a. By PND=11-12 SRIF cells increased noticeably in layers II-III of areas 18 and 18a and moderately in II-III of area 17. Area 18 still contained the most cells. This is the developmental pattern which continues into adulthood. In the adult there is a decline in SRIF cells; prestriate and striate areas are no longer as noticeably different as during the first two postnatal weeks. Double labeling revealed some SRIF neurons belong to corticocortical systems. However, the diversity of SRIF cell types and layers indicate that SRIF cells can belong to a number of cortical systems, both intrinsic and extrinsic. The different rates and patterns of SRIF cell development among areas 17, 18a & 18 may refect the different types of connectional organization developing within each area's cortical layers. (Supported by NS21021 and BRSG 8309).

177.10 SPECIFIC BINDING OF ^{3}H-SPIROPERIDOL IN POSTHATCH CHICK BRAIN. S. A. McDougall*, J. L. Neisewander*, M. T. Bardo, and J. F. Zolman. Dept. of Psychology and Dept. of Physiology and Biophysics, University of Kentucky, Lexington, KY 40506.

Previous studies have shown that dopamine agonist and antagonist drugs produce age-dependent differences in the young chicks' behavior during the first posthatch week. Since little is known about the distribution of receptors which are activated by dopaminergic drugs in chicks, we assessed specific binding of the dopamine antagonist ^{3}H-spiroperidol in different regions of 1-, 4-, and 16-day-old chick brain.

Chicks were decapitated and their brains were dissected and frozen at -70° C until assay. Tissue samples were homogenized in 50 mM Tris buffer and washed twice by centrifugation (40,000 x g, 10 min). The samples were then suspended in 100 vols of 50 mM Tris containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 10 µM pargyline and 0.1% ascorbic acid (pH 7.7). Brain tissue, preincubated at 37° C for 5 min, was then incubated with 4 nM ^{3}H-spiroperidol (NEN, 23 Ci/nmol) for 15 min in either the presence or absence of 1µM haloperidol. Following incubation, samples were washed under vaccuum pressure onto a Whatman GF/B filter and the radioactivity was counted by liquid scintillation spectrometry.

Specific binding of ^{3}H-spiroperidol in 1-day-old chick cerebellum was 1.38 fmol/mg wet weight. At 4 and 16 days of age, cerebellar binding of ^{3}H-spiroperidol was increased by 26% and 96%, respectively. Specific binding of ^{3}H-spiroperidol in the roof of the chick forebrain was minimal at 1 and 4 days of age (< 1 fmol/mg wet weight), but increased significantly by 16 days posthatch (1.37 fmol/mg wet weight). In contrast, specific binding of ^{3}H-spiroperidol in the base of the chick forebrain, where the paleostriatum is located, was 1.18 and 1.95 fmol/mg wet weight at 1 and 4 days, respectively. A Scatchard analysis of ^{3}H-spiroperidol binding (0.1 - 40 nM) indicated that the age-related increase in binding in the 1- and 4-day-old forebrain base may reflect an increased number of low-affinity receptors (K_d > 10 nM).

177.11 FUNCTIONAL SENSITIVITY OF DORSAL RAPHE NEURONS DURING POSTNATAL DEVELOPMENT: EFFECTS OF 5HT, GABA AND THE BENZODIAZEPINES. D. A. Smith and D. W. Gallager, Depts. of Psychiatry and Neuroanatomy, Yale Univ. School of Med., New Haven, CT 06508 and Dept. of Psychology, Oberlin College, Oberlin, Ohio 44074

To assess potential differences in the functional responsiveness of serotonergic (5HT) and GABAergic receptors during postnatal development, extracellular recordings were made in in vitro midbrain slices cut through the dorsal raphe nucleus (DRN). Slices were superfused with artificial CSF containing 2.5μM phenylephrine which induces pacemaker activity analagous to spontaneous activity in vivo. GABA (.001M) and 5HT (.004M), made up in .1M NaCl, were applied microiontophoretically. Only those cells that showed inhibitory responses to both 5HT and GABA were included for analysis. Drugs were ejected for one minute at regularly spaced intervals. Dose-response relationships were determined by measuring the mean rate of firing during the minute of drug ejection and comparing it with the mean rate during the preceeding minute. Logit and regression procedures were used to obtain the IC_{50} for current. No significant changes in sensitivity to 5HT were observed at any time during postnatal development; (Mean IC_{50}: Adults, 15.0 ± 3.6nA; 8-30 days old, 18.0 ± 3.6nA; 1-7 days old, 11.0 ± 1.7nA; $F(2,30) = 1.25$, $p<.30$). Similarly, a comparison of the sensitivities of DRN neurons to GABA at 4 different ages (1-7 days old, 8-15 days old, and 16-30 days old, adults) showed no significant differences in GABA sensitivity between the age groups ($F(3,78) = 1.43$, $p<.240$), (Mean IC_{50}: 1-7 days of age = 6.4 ± 1.6nA; 8-15 days= 3.6 ± 0.8nA; 16-30 days 3.9 ± 0.9nA; adults= 5.7 ± 1.0nA).

In contrast, preliminary data suggests that cells in slices obtained from new born rats (1-11 days of age) are more sensitive to benzodiazepine mediated enhancement of GABAergic inhibition. The application of the benzodiazepine, clonazepam, in the perfusion fluid at doses between 10^{-8} and 10^{-7}M, produced a 50% increase in GABA inhibitory effects (ED_{50}) at a lower dose in tissue from young animals (1-11 day old: ED_{50} 3.18×10^{-8}M) as compared to adult tissue (7.32×10^{-8}M; $F(1,9) = 11.80$, $p<0.01$). This increased potentiation of GABA effects by benzodiazepines is consistent with previous literature showing enhanced coupling of the GABA-BZ complex at early postnatal ages.

Supported by USPHS NS 16995 and NS07522 & State of CT.

177.12 PRENATAL EFFECTS OF 5 METHOXYTRYPTAMINE AND FLUOXETINE ON SEROTONIN UPTAKE AND BEHAVIOUR IN THE NEONATE. A. Shemer*, A. Ramirez*, P. Whitaker-Azmitia, E. Azmitia (SPON: I. Fand, MD). Department of Biology, New York University, New York, NY 10003.

Serotonergic neurons are one of the first CNS systems to form. It has been proposed that serotonin (5HT) plays a role in development and in the regulation of growing neurons. Furthermore, in tissue culture, it has been demonstrated that 5 Methoxytryptamine (5MT, a 5HT agonist) can inhibit the outgrowth of serotonergic neurites. For these reasons we looked into the in vivo effects of drugs affecting the 5HT system by administering them to pregnant rats and looking at behaviour and high affinity uptake of 5HT in the offspring.

Pregnant Sprague Dawley rats were injected i.p. daily from day 12 of gestation until the day of birth. They received either 5MT 1 mg/kg, or Fluoxetine FL (an uptake blocker specific for 5HT) 10 mg/kg, or saline. On day 1 and 2 after birth pups were sacrificed and their brains removed. ^{3}H-5HT specific high affinity uptake (10 nM) was measured in both brainstem and frontal tissue. Nonspecific uptake was defined as that occurring in the presence of 10 uM Fluoxetine.

Both 5MT and FL administration during gestation lead to greater ^{3}H-5HT uptake on both day 1 and 2. However, this effect was more pronounced in the brainstem with 5MT and more pronounced in the frontal section with FL.

In the adult, 5HT has been shown to have an effect on both memory and on general activity levels, we therefore looked at spontaneous alternation, the retention of a passive avoidance response, as well as activity, at 18 and 15 days old respectively. A subgroup of treated offspring were given 1 trial to acquire a passive avoidance response with 0.16 ma. shock to the feet. The 5MT offspring showed significantly less retention when tested the following day, than either the controls or the FL offspring. Furthermore 5MT offspring showed significantly less spontaneous alternation than control offspring. In the general activity test treated offspring from both drug groups were less active than controls.

Preliminary results show a clear effect of both 5MT and FL administration during gestation on both the biochemistry and behaviour of the offspring. Further research is currently underway to explore these phenomena in detail.

177.13 EFFECTS OF MATERNAL TREATMENT WITH PCPA ON THE DEVELOPMENT OF HIGH AFFINITY SEROTONIN RECEPTORS IN RAT BRAIN. P.M. Whitaker-Azmitia and J.M. Lauder. Dept. of Psychiatry and Behavioral Science, SUNY, Stony Brook, NY 11794, and Department of Anatomy, University of North Carolina, Chapel Hill, NC 27514.

Serotonin plays a role in neuronal development prior to assuming its role as a neurotransmitter in the mature brain. We have shown that serotonin receptors occur in fetal rat brain. Furthermore, using a tissue culture model, we have shown that these receptors may regulate the direction and extent of axonal growth (Whitaker-Azmitia and Azmitia, 1984). We now wish to further characterize these fetal receptors by studying their ability to respond to changes in levels of serotonin.

In the mature rat brain, p-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, has been shown to increase serotonin receptor number (Fleisher et al., 1979). To test the effect of PCPA on developing receptors, pregnant Sprague-Dawley rats were treated as follows: 300 mg/kg PCPA methyl ester given IP on gestational day 8 and 100 mg/kg PCPA methyl ester IP on days 9-17. High affinity serotonin receptors (S_1) were studied in the forebrain and brainstem of pups on day 1, 30 and 60 post-natal. Receptor kinetics were determined from Klotz plots using at least eight different concentrations of ^{3}H-serotonin (0.5 to 20 nM) in triplicate. Five animals were examined at each time point.

Preliminary results indicate a significant increase in the number of S_1 receptors in newborn forebrain (control = 24.5 ± 2.6 fmoles/mg protein: treated = 33.5 ± 3.3 fmoles/mg) and brainstem (control = 47.0 ± fmoles/mg; treated = 72.4 ± 8.3 fmoles/mg) in PCPA-treated rats. However, this effect was not observed at 30 and 60 days postnatally. There were no changes in the apparent K_D of the receptors in either brain region at any time point.

Our results indicate that fetal serotonin receptors may respond to changes in brain serotonin concentrations in the same way as mature serotonin receptors. Furthermore, this finding implies that fetal serotonergic receptors are "functional" and may be capable of playing a role in development.

177.14 EFFECTS OF SEROTONIN AND 5-METHOXYTRYPTAMINE ON GABA AND 5-HT IMMUNOCYTOSTAINED CELLS FROM HIPPOCAMPUS AND MESENCEPHALIC RAPHE GROWN IN TISSUE CULTURE. M. I. Davila-Garcia, P. Alvarez, P. M. Whitaker-Azmitia, A. C. Towle*, and E.C. Azmitia. Dept. of Biology, New York University, New York, NY 10003 and* University of North Carolina, Chapel Hill, NC 27154.

Serotonin has been implicated as a differentiation signal in early neurogenesis (J. M. Lauder and H. Krebs, 1978, Dev. Neuro. Sci. 1:15-30) and as an inhibitor of neurite outgrowth (P. G. Haydon et. al. 1984, Science 226:561-564). Previous studies in our laboratory have shown that 5-methoxytryptamine (5-MT, a specific serotonergic agonist) at low concentrations inhibits growth of serotonergic neurons in vitro (E. C. Azmitia and P. M. Whitaker-Azmitia, 1984, Neurosci. Absts. 10:663).

In this study we examined the morphological characteristics of neurites and growth cones of specific immunocytochemically identified cells from hippocampus (GABA immunoreactive, GABA-IR) and mesencephalic raphe (5-HT immunoreactive, 5-HT-IR). The effects of serotonin (5×10^{-7}M) and 5-MT (5×10^{-7}M) were assessed by light microscopy. The cells were dissociated and plated in 8 well chamber/slides (Lab-Tek). After 18 hours of incubation the cells were immunocytostained for GABA (hipp), 5-HT (raphe), and GABA or 5-HT (co-cultures).

Our results indicate that after 18 hours of plating GABA-IR cells have one or multiple neurites when grown alone or in the presence of raphe cells. The growth cones of these cells are fan-shaped, contain many filopodia, and display a dense immuno reactivity. The 5-HT-IR cells grown alone or in co-cultures are limited to one or two long neurites which sometimes display small varicosities. Bulbous growth cones and small filopodia are common in these cells. When low concentrations of serotonin and 5-MT are added to the cultures they inhibit growth cone elongation of 5-HT-IR cells but have no effect on GABA-IR cells.

Further details on the effects of these chemicals are being investigated by electron microscopy.

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177.15 ONTOGENY OF α-BUNGAROTOXIN BINDING IN RAT NEOCORTEX. J.L. Fuchs. Laboratory for Neurobiology GR 4.1, The University of Texas at Dallas, Richardson, TX 75083

The developmental distribution of an acetylcholine binding site was studied using the ligand [^{125}I]α-bungarotoxin (α-Btx). Subjects were 2 or 3 male hooded rats from each of the following age groups: P0, P2, P4, P6, P8, P10, P12, P14, P17, P20, P31 and adult. Coronal sections of unfixed brain tissue were cut on a cryostat. Tritium-sensitive Ultrofilm autoradiographs were prepared from in vitro labeled brain sections. The sections were later stained with cresyl violet.

At ages P0 and P2, high levels of α-Btx binding were present in the subplate (laminae V-VI) and in lamina I. Subsequently (P4-P10), a more complex laminar pattern emerged. Dense label generally appeared in I,IV, V and deep V to upper VI, although regional variation was present. Only the bands in I and in deep V to upper VI remained prominent in adults. The autoradiogaphs also revealed a radial organization which appeared as early as P2 and became more distinct through P10. At least in some cortical areas, the more dense label was in those regions identifiable as "granular", thalamic recipient zones. The radial pattern became progressively less distinct from P10 to adult. The unfolding of laminar and radial α-Btx patterns coincides with or slightly precedes the development of thalamocortical innervation and the segregation of thalamic and callosal afferents into alternating radial zones (Wise & Jones, 1978). These observations suggest a possible role for α-Btx binding sites in forming stable thalamocortical connections during development.

177.16 POSTNATAL DEVELOPMENT OF B_1- AND B_2-ADREDERGIC RECEPTORS IN THE RAT: AN IN VITRO AUTORADIOGRAPHIC STUDY. D. Lorton, and J.N. Davis. Vet. Admin. Med. Ctr. and Depts. of Med. (Neurology) and Pharmacol., Duke Univ., Durham, NC 27705.

Radioligand binding has made it feasible to study a- and B-adrenergic receptors in the brain during postnatal development. We have used in vitro autoradiography to compare the development of B_1- and B_2-adrenergic receptors to our previous studies of a_1-adrenergic receptor development in the rat brain.

Male Sprague-Dawley rats were decapitated on postnatal days 1, 5, 10, 15, 23, 33, and 45. The brains were rapidly removed and frozen. Cryostat cut horizontal and coronal sections of brains from each age group were processed for autoradiography using [^{125}I]-cyanopindolol with either 50 or 100 nM of the B_1 and B_2 antagonists ICI 89,406 and ICI 118,551, respectively. 1 uM (+)-Propranolol was used to determine nonspecific binding.

At birth, levels of B_1- and B_2-receptors were low in all brain regions. With brain development, the levels of B_1- and B_2-receptors in various brain regions developed at different times and rates. In some regions, such as, globus pallidus and substantia nigra, both B_1- and B_2- receptors increased very early in development (between postnatal day (PND) 1 and 5). However, in other regions, such as, cerebral cortex and caudate-putamen B_1- and B_2-receptors levels were low between PND 1 and 10 and then increased rapidly between PND 10 and 15. In the cerebellum, B_2-receptors of the molecular layer increased rapidly between PND 10 and 23 to the high levels normally observed in the adult rat. In contrast, B_1-receptor levels in the cerebellum molecular layer increased gradually from PND 1 to 23.

These studies provide evidence that B_1 and B_2 subtypes may have different developmental patterns and differ from the patterns of a_1-adrenergic receptor development. Since all three receptors respond to norepinephrine, the differing patterns of development suggest that expression of receptors may not be determined by the apperance of neurotransmitter but rather is determined by the development of the cell expressing that receptor. When the cellular localization of noradrenergic receptor subtypes is better understood, it may be that the appearance of receptors can be predicted by the developmental stage of the cell.

(supported by VA and NIH AG00007)

177.17 DEVELOPMENT OF DRUG AND NEUROTRANSMITTER BINDING SITES IN FETAL RAT BRAIN: M. Schlumpf, J.M. Palacios+, R. Cortes+*, A. Pazos+*, A. Bruinink* and W. Lichtensteiger. Inst. of Pharmacology, Univ. of Zürich, CH-8006 Zürich, and Sandoz AG+, CH-4002 Basel, Switzerland.

Information on the prenatal ontogeny of receptor sites and their regional distribution pattern in brain is essential for developmental pharmacology. Qualitative and quantitative autoradiographic methods are especially suited to follow the developmental course of individual binding sites that may change rapidly both in density and distribution. For these studies, frozen fetal rat brains were cut on a cryostat; 10 um sections were incubated with tritiated ligand and apposed to tritium sensitive film (Ultrofilm). Microdensitometry of binding sites was based on Amersham tritium standards and standards prepared from fetal brain homogenates.

Binding sites for the muscarinic cholinergic ligand H3-N-methylscopolamine appear at gestational day (GD) 14-15 in spinal cord and lower brainstem. Subsequently, labeling increases in brainstem and spreads to di- and telencephalon, neocortex being labeled in late fetal life. Microdensitometry revealed a predominance of the M2 receptor type in lower brainstem, while the ratio of M1/M2 varies with time and brain region. In contrast, beta-adrenergic (H3-dihydroalprenolol) binding sites are characterized by their early presence in olfactory bulb and neocortex, where the distribution pattern is quite similar to catecholamine fiber innervation. During late fetal stages this distinct neocortical pattern changes into a more uniform dense distribution of binding sites. Specific binding of H3-serotonin, which represents S1 type receptors in adult brain, is found at an early fetal stage (GD 16) restricted to the chorioid plexuses. The same structure is also labeled by the peripheral benzodiazepine ligand H3-methyl-Ro-5-4864. Through further development 5 HT-1 binding sites appear in lower brainstem (GD 18), the septal nuclei, the basal ganglia and in deep layers of the developing neocortex at GD 20, a time when S2 sites are also well documented by biochemical studies (Bruinink et al., J. Neurochem. 40, 1227, 1983). The development of benzodiazepine binding sites corresponds to the caudo-rostral maturation pattern of the fetal CNS (Schlumpf et al., J. Neurosci. 7, 1478, 1983). These distinct differences in the ontogenetic pattern of individual binding sites suggest that drug sensitivity of different brain regions may vary considerably in the course of fetal development.

177.18 PRE- AND POSTNATAL ONTOGENY OF BRAIN NEUROTENSIN RECEPTORS: AN AUTORADIOGRAPHIC STUDY. A. Pazos[1*], J.M. Palacios[1], M. Schlumpf[2] and W. Lichtensteiger[2]. (SPON:R.MAURER) [1]Preclinical Research, Sandoz Ltd., Basle and [2]Inst. of Pharmacology, University of Zürich, Zürich, Switzerland.

In the rat brain neurotensin (NT) has been detected by immunohistochemistry as earlier as day 16 of gestation (GD16) and seen to increase steadily postnatally in several brain regions (Bissette et al., J. Neurochem. 43, 283, 1984). Receptors for NT have been characterized in the mammalian brain membranes by binding assays and visualized by autoradiography (Young and Kuhar, Brain Res. 206, 273, 1981).

Using in vitro receptor autoradiographic techniques we have analyzed the pre- and postnatal development of NT receptors in the rat brain. NT receptors were labeled in mounted tissue sections from animals of ages ranging from GD14 until the postnatal day 21 (PN21) as well as young adult animals. ^{3}H-NT was used as a ligand and unlabeled NT was added to the incubation mixture to produce blanks. ^{3}H-Ultrofilm was used to generate autoradiograms. Autoradiograms were analyzed and quantified using a computer-assisted image analyzer.

Very low densities of NT receptors were visualized on GD's 14 and 15. Between GD16 and GD18 a marked increase in the density of NT receptors was seen in the developing neocortex. Densities in other brain areas, particularly midbrain and brainstem were much lower than cortical densities. The density of NT receptors in the cortex increased throughout the last part of the gestation and the earlier postnatal life with a peak at the end of the first postnatal week. After that, NT receptor binding decreased dramatically to reach the relatively low densities seen in the adult animal towards the end of the third postnatal week. Development of NT receptors in other brain areas followed very different time-patterns. For example, NT receptors associated with dopaminergic cell-bodies in the midbrain were seen first at GD18 and increased slowly with development to reach adult levels at about the second week of postnatal life. NT receptors in the hippocampal formation presented a postnatal development being detected first at PN5 and showing a developmental peak around the second week.

The main finding of these experiments are the clear differential regional ontogenetic patterns for NT receptors. The very high densities present in the cortex even in very early fetal states suggest that NT could play a role as a signal in the growth of the brain. This role has also been suggested for other peptides in other models of cell division and development.

177.19 NEUROPEPTIDE RECEPTORS ARE PRESENT IN FETAL NEOCORTICAL TRANSPLANTS.

T.W. Moody, R.L. Getz, M.M. Shaffer and J.M. Rosenstein. Depts. Biochemistry and Anatomy, The George Washington University School of Medicine, Washington D.C. 20037.

Neural transplantation provides an opportunity to study neuronal development and growth characteristics. Many cell biological parameters may be responsible for extensive growth properties exhibited by transplants of fetal CNS. These might include induction of vascularization or peptide or transmitter elaboration. We have transplanted fetal cerebral cortex from E15-19 donors into the IV ventricle of young adult recipients in order to determine if certain neuropeptide are present. In this atraumatic system, the fetal tissue grows remarkably to fill the ventricular space. Host rats were sacrificed between 1 and 4 months postoperatively. While the anatomical correlates of neocortical transplants have been described, their neurochemical properties remain to be elucidated. Here we report that neuropeptide receptors are present in these transplants in high density.

Specifically, neuropeptide receptors for Vasoactive Intestinal Peptide (VIP) and Bombesin-like peptides (BN) and Substance P (SP) were detected using the in vitro autoradiographic methods described previously (Zarbin et al. J. Neuroscience 5:429, 1985). VIP is a vasodilator in the neocortex and putative neuromodulator. Receptors for VIP which may mediate brain blood flow to the transplant were present in moderate concentrations. The density of VIP receptors in the transplant, however, was lower than some areas of the host brain such as the area postrema or normal cerebral cortex (Shaffer and Moody, Soc. Neurosci Abs. 14:553). In contrast high densities of receptors for BN were present in the transplant in contrast to the normal receptor complement in such areas as nucleus tractus solitarius and cerebral cortex. Only low densities of SP receptors were present in the transplant areas. Since BN peptides function as growth factors in normal and malignant cells, these receptors may facilitate the growth and/or differentiation of the transplant. Thus certain neuropeptides and their receptors such as VIP and BN may play an important role in the continued survival of neocortical transplants as well as possible modulation of blood flow and barrier properties.

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MORPHOGENESIS AND PATTERN FORMATION I

178.1 SELF-ORGANIZATION OF EPENDYMA IN REGENERATING TELEOST SPINAL CORD. M.J.Anderson, S.G.Waxman and Chi Choy*. Dept. of Neurology, Stanford University Sch. of Medicine and the VA Medical Center, Palo Alto, CA 94304.

Within spinal cord of the teleost Sternarchus albifrons which is regenerating after amputation of the tail, multiple ependymas, each surrounding a lumen, are sometimes encountered. We have documented two modes of formation of such secondary ependymas. In one mode, secondary ependymas are created by branching from the main (original) ependyma and central canal of the spinal cord. In the second mode, self-organization occurs within a mass of undifferentiated cells given off from the original ependyma. In this second mode, no continuity exists between the original central canal and the lumen of the newly-formed secondary ependyma. In the caudal region of regenerating cord, clusters of cells are frequently seen lateral to the main ependyma, apparently having arisen from it. Within such masses of undifferentiated cells, self-organization of ependymal structure is accompanied by a reduction in electron density of the cytoplasm, the establishment of numerous desmosomes and close junctions between neighboring cells, and the appearance of cilia. Subsequently, some of the central cells in the mass may break down and extracellular space opens up to form a lumen in the center of the new ependyma. The capacity of ependymally-derived cells to spontaneously form the structure of an ependyma correlates with the high degree of developmental potential already documented for Sternarchus ependyma. It has previously been shown that some cells of Sternarchus spinal cord, presumably those of the ependymal layer, remain pluripotent throughout adult life and can generate new neurons and non-neuronal cells both in vivo and in vitro (Anderson and Waxman, Cell Tiss. Res. 219:1, 1981; Anat. Rec. 208:9A, 1984; and Dev. Brain Res., in press, 1985). The present results indicate that not only can adult Sternarchus ependyma generate new neurons and glia, but that cells derived from it can spontaneously self-organize into the complete ependymal structure. Supported by the Veterans Administration and NS 15320.

178.2 DEVELOPMENT OF RETICULOSPINAL NEURONS OF THE ZEBRAFISH. B. Mendelson. Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

The development of identified reticulospinal (RS) neurons of the zebrafish (Brachydanio rerio) was studied in order to learn how neuronal features including size, position and axonal pathway are correlated with the time of cellular development.

The times of origin (birthdays) of the RS neurons were determined by combining ^{3}H-thymidine autoradiography with horseradish peroxidase (HRP) histochemistry. Individual types of RS neurons were born at characteristic developmental times. Dorsally located neurons always had earlier birthdays than more ventral cells located at the same axial level of brain. Often larger and more lateral neurons, located at one axial level, were generated before smaller and more medial ones. There was no overall longitudinal gradient of RS neuronal generation.

The sequence of axonal outgrowth and the early cell body positions of the RS neurons were determined by filling the cells retrogradely with HRP from their growing axons. The lesions were located in the spinal cord and were made at a series of developmental stages. Individual types of RS neurons grew axons into the rostral spinal cord in a similar sequence to that of their birthdays. Some RS neurons grew axons across the midline of the brain while others extended axons directly caudally at nearly the same time and from somata located at similar locations in the hindbrain. All RS somata were initially observed along the ventral surface of the brain and subsequently were found at more dorsal locations. This dorsal displacement could be a passive one, by growth of the ventral hindbrain, which would explain the dorsal to ventral sequence of time of origin. As a population, the RS axonal projections developed in two distinct waves separated by about 10 hours and, where examined, all RS neurons elongated axons at a constant rate of 100 μm/hr through the spinal cord. If the time that an axon reaches the area of its target is important in the determination of specific synaptic contacts, then it is possible that neurons which project axons in the two waves of RS development differ in their synaptic connectivity in the spinal cord. (Supported by NIH grant NS 17963 and GM 07257).

178.3 SEGMENTAL PATTERNS OF DEVELOPMENT IN THE ZEBRAFISH CNS. Bill Trevarrow and Eric Hanneman*. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The CNS of developing zebrafish contains individually identifiable neurons that comprise segmentally homologous sets (Kimmel et al., JCN 205 112, 1982; Myers, JCN 237 in press, 1985). Early labeling of cells for acetylcholine esterase (AChE) activity or for the antigen of the zn-1 monoclonal antibody demonstrated that: 1) similar or identical populations of cells were stained by the two different histochemical methods, 2) the cells stained by these techniques were among the earliest appearing neurons, and 3) some of the labeled cells correspond to individually identifiable neurons. These early appearing neurons seemed to follow a common program of cellular differentiation consisting of: 1) cessation of cell division, 2) AChE expression, 3) axogenesis, and 4) zn-1 antigen expression.

AChE staining was observed beginning at 15 and 18 hours postfertilization (h) ventrolaterally in the hindbrain and spinal cord respectively. The first cells stained were bilaterally located single neurons at one neuromere intervals. The length of the hindbrain and spinal cord neuromeres were identical and corresponded to the length of a somite. Later, single cells in the same locations were the first to stain for the zn-1 antigen. In the spinal cord, these cells were identifiable as primary motoneurons, because of their stained motor axons.

As development proceeded, more cells stained for both AChE activity and zn-1. In the spinal cord, clusters of cells were stained where only a single AChE positive cell was found previously. In the hindbrain, single cells staining darkly for the zn-1 antigen, were now present at half-neuromere intervals. By 28 h, in each neuromere, an alternating pair of large and small clusters of cells were found in similar locations, by both staining techniques. Among the cells in these clusters were identifiable reticulospinal cells.

These results support the hypothesis that both the vertebrate hindbrain and spinal cord are segmentally organized and suggest a common developmental basis for segmentation of the hindbrain and spinal cord. (Supported by NIH grants NS17963 & NS21132, and Med. Res. Foundation of Oregon.)

178.4 SEGMENTAL PATTERNING OF MORPHOLOGICAL TYPES OF RETICULOSPINAL NEURONS IN LARVAL ZEBRAFISH. Walter K. Metcalfe, Charles B. Kimmel, and Bruce Mendelson. Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have examined the morphology of identified reticulospinal neurons in larval zebrafish by retrogradely labeling them with HRP. We describe the position, axon pathway, and dendritic morphology of twenty-seven different types of reticulospinal neurons found in the hindbrain five days after fertilization. Nineteen of these types are present as single identified neurons on each side of the brain; the others are present as pairs or small groups of cells.

The reticulospinal neurons form a bilaterally symmetric pattern of cells and are arranged in seven clusters spaced at regular intervals along the neuraxis. Each cluster contains 2-5 different types of reticulospinal neurons. We observe that cells with similar morphological features are found within adjacent clusters, although the composition of each cluster is unique. By considering cell position within the cluster and axon pathway, nearly all of the cells can be assigned to one of about seven serially repetitious classes. Independent features of the cells (size, dendritic morphology) support the same classification.

We propose that the neurons of the same class that are present in the different clusters are segmental homologues. Thus these clusters represent the primitive neuromeres from which the vertebrate hindbrain is thought to be derived. Assuming that this series evolved by successive duplications and divergence of primitive neuromeres, we can analyze the changes that occurred during the evolution of a new segment. The results suggest that most cells are conserved, alhtough occasionally some individual features of cells have been modified. For example, changes between ipsilaterally and contralaterally projecting axons may have occurred several times during the evolution of the series. In addition, cells may have been added or deleted. (Supported by NIH grant NS17963, and the Medical Research Foundation of Oregon.)

178.5 DORSAL ROOT AXONS IN THE DEVELOPING TAIL SPINAL CORD OF XENOPUS. D.M.Awwiller* and R.H.Nordlander. (SPON:J.Kriegler). Dept. Oral Biology, Case Western Reserve University, Cleveland, OH 44106.

As in other lower vertebrates, the spinal cord of Xenopus characteristically displays a distinct set of primitive, or primary, neurons that are responsible for the earliest behavior of the animal. Later, a new set of neurons, the secondary neurons, are superimposed upon these and, for the most part, completely replace them in the adult. This study is concerned with development of the central processes of dorsal root ganglion cells and their relationships to primary neurons and to the dendrites of motor neurons within the tail spinal cord.

Dorsal root ganglion cells were filled with horseradish peroxidase (HRP) applied to epidermis and segmental muscle at tail levels of Xenopus embryos and larvae. After dissection and reaction for HRP, spinal cords were mounted whole in glycerine and viewed with the light microscope.

Dorsal root ganglion cells begin to appear in the tail during early larval stages. One to six cells per hemisegment come to sit on each ventral root, and their central afferent processes travel to the spinal cord along the root. From the root entry point, they travel diagonally just beneath the surface of the cord until they reach the dorsolateral fascicle in which they travel rostrally along with ascending processes of primary sensory neurons. During their diagonal traverse, dorsal root axons do not fasciculate but travel independently. In these preparations, the course of an individual central dorsal root process can be followed over its entire length and its relationships with motorneurons labeled from the same region of the periphery ascertained. Growth cones have been observed at the lead of ascending dorsal root processes, as well as the tips of their developing collaterals. A description of these and other growth cones appears elsewhere in these proceedings.

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178.6 QUANTITATIVE ANALYSIS OF THE DEVELOPMENT OF DESCENDING PROJECTIONS IN THE BULLFROG. G.R. Davis and P.B. Farel. Dept. Physiol., Univ. N. Carolina Sch. of Med., Chapel Hill, NC 27514.

In many vertebrate classes, neurons projecting to the spinal cord at early stages of development and in more mature animals are located in similar brainstem regions. However, the number of brainstem neurons projecting to spinal cord increases over development. We are interested in (1) the developmental course of this increase and (2) whether the increase is distributed proportionally among all brainstem regions.

HRP was applied bilaterally to the lumbar enlargement of bullfrog (Rana catesbeiana) tadpoles (st. IV to XX) and juvenile frogs. After survival periods of 3-7 days, the perfused CNS was removed, sectioned at 40-um on a cryostat, and processed with the Hanker-Yates reagent. The locations and numbers of retrogradely labeled cells were determined in every section of brainstem.

Between st. IV (prior to the formation of myotubes in the hindlimb bud) and st. XX (when the forelimbs emerge from the body wall, marking the onset of metamorphic climax), the number of brainstem neurons retrogradely labeled from the lumbar enlargement increased from approximately 1200 to 2000. This developmental span takes 1-2 years in the wild. In contrast to this relatively slow increase, between st. XX and the completion of metamorphosis at st. XXV (requiring about 2-4 weeks) the number of retrogradely labeled brainstem neurons increased to approximately 3300. Thus, the rate of accumulation of descending inputs is not constant with respect to either developmental stage or time.

Retrogradely labeled neurons were located in the same brainstem regions throughout development. However, particular regions showed a disproportionate increase in numbers of projecting neurons: especially the vestibular nucleus (at the level of the facial nucleus) and the inferior reticular formation.

The pronounced increase in the number of retrogradely labeled brainstem neurons between st. XX and the completion of metamorphosis has implications for the interpretation of previous studies (J. Neurosci. 2:654, 1982). Spinal cord transection prior to the onset of metamorphic climax was followed by the restoration of descending pathways only if the operated animals survived until the completion of metamorphosis. These results suggest that the projections restored to the spinal cord from the brainstem may be formed, at least in part, by newly developed axons rather than regeneration of previously transected axons.

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178.7 NEUROGENESIS IN THE REPTILE CORTEX: H-3-THYMIDINE AUTORADIOGRAPHIC STUDY. A.M. Goffinet, Ch. Daumerie*, B. Langerwerf*, C. Pieau* (+) Devpt. Neurobiol.,Univ. Louvain Sch. Med. B-1200 Brussels, Belg. (+) Univ. Paris, France.

Histogenesis has been studied in forebrain cortical structures in two widely separated reptilian species, Emys orbicularis and Lacerta trilineata, using tritiated thymidine autoradiography. Four areas were considered for analysis, namely the medial (hippocampal), the dorsal (general) and the lateral (pyriform) cortices, and the dorsal ventricular ridge (DVR).

Following a single injection of the tracer at an early developmental stage, all neurons are labeled, indicating that thymidine remains available at least throughout the period of neurogenesis. With the exception of the medial part of the hippocampus, where it lasts longer, the bulk of neurogenesis in the four cortical areas proceeds within a short period of 8-9 days, between developmental stages 15 and 18 in Emys, and stages 32-34 in Lacerta. Lateral to medial as well as anterior to posterior tangential gradients of histogenesis are present in all cortical fields in both species. Radial neurogenetic gradients are directed from outside to inside, with the exception of the medial, hippocampal cortex of Lacerta, where no radial gradient is seen.

This pattern of timing and of areal, tangential variations in histogenesis of the reptile cortex is comparable to that found in mammals. In contrast to the mammalian cortex, however, which develops according to an inside to outside, "inverted" gradient, radial neurogenesis in reptile cortex follows an outside to inside pattern.

These observations show that the inside-out gradient of neurogenesis has been acquired during cortical evolution from reptiles to mammals. The appearance of the "inverted" gradient of cortical histogenesis might be related to the evolution of radial cortical organization (Goffinet, JCN 215:437-452, 1983).

178.8 DEVELOPMENT OF SPINOCEREBELLAR PROJECTION IN THE CHICK. N. Okado and M. Yoshimoto*. Dept. of Anat. Univ. of Tsukuba, Inst. Basic Med. Sci., Ibaraki 305, Japan.

This study was undertaken to dermine: (1) the termination field of spinocerebellar fibers in the posthatching chick and; (2) the development of spinocerebellar projection in the chick embryo. Mossy fiber terminals were anterogradelly labelled by injections of lectin horseradish peroxidase in the lumbar spinal cord.

In the posthatching chick (two months) the terminals were found to be distributed throughout the entire depth of lobules I-V, whereas in lobules VI and IX they were concentrated in the apical part of the lobules. Labelling in lobules II-IV showed distinct clusters of terminals in the mediolateral dimension, consisting of three sagittal "strips" of aggregations of labelled terminals in the lateral half of cerebellum. The midpoints of the three strips were located 85 µm, 800 µm and 2140 µm lateral to the midline. The width of the medial-most strip was 110 µm, while the intermediate and lateral strips were 690 µm and 860 µm, respectively. The strips were most distinct in lobule II, whereas they were not so clear in the lobule IV. Labelling was found throughout the entire mediolateral extent of lobule I; labelled terminals were most numerous in lobule I compared to other lobules.

Ontogenetic development of the spinocerebellar projection was examined with special reference to the "strip" formation of mossy fibers found in the adult cerebellum. Axons from the lumbar spinal cord projected to the cerebellar plate as early as embryonic day (E)8, a stage when the mossy fiber targets (internal granular layer) had not yet formed. All the labelled axons projecting from the lumbar spinal cord to the cerebellar plate were contained in one distinct bundle, indicating that gross projection errors do not occur during development of spinocerebellar fibers. A dense accumulation of labelled fibers were first found in the granular layer of lobules III-VI on E10; some labelled mossy fibers were also found in the molecular layer. Labelled fibers first began to penetrate into lobules I and II on E12. The initial axon terminals in the granular layer were already distributed in "strips" at this time. Although a few labelled fibers were found in the regions between strips on E12-E14, the basic segregation of terminals into distinct "strips" was basically similar to that found in the posthatching chick.

178.9 DEVELOPMENT OF THE SEROTONERGIC SYSTEM IN THE CHICK EMBRYO, WITH SPECIAL REFERENCE TO SPINAL PROJECTIONS. H. Sako*[1], T. Kojima*[1] and N. Okado[2]. (SPON: Y. Katayama) [1]Dept. of Anat., Nihon Univ. Sch. of Med., Tokyo 173 and [2]Dept. of Anat. Univ. of Tsukuba, Inst. of Basic Med. Sci. Ibaraki 305, Japan.

The ontogenetic development of the central serotonergic system in the embryonic as well as posthatching chick was studied with the PAP method using an antisera to serotonin (5-HT).

On embryonic day (E)4, a distinct cluster of immunoreactive cell bodies were first found in the primitive raphe nuclei of the metencephalon as well as in the upper rhombencephalon, whereas single reactive cells were occasionally observed in the outer boundary of the ventricular zone of the lower rhombencephalon. After a rapid increase in the number of immunoreactive cell bodies around E6, the basic pattern of 5-HT nuclei found in the posthatching chick appeared on E8.

A few 5-HT immunoreactive cell bodies were also found throughout the rostrocaudal extent of the spinal cord. These 5-HT positive spinal cord cells, which first appeared on E8, were also present in adult spinal cord.

5-HT immunoreactive fibers first appeared in the marginal layer of upper cervical spinal cord on E6 and in the marginal layer at lumbar levels on E8. In general, 5-HT fibers were first observed to penetrate into the mantle layer on E10, when they were found in laminae V, VII, VIII and X. 5-HT fibers in lamina IX, which developed slightly later compared to the other laminae in the basal plate, were first observed in the cervical spinal cord on E10. By E16 5-HT positive fibers were found in lamina IX of the lumbar spinal cord as well as in the dorsal horn. A dense accumulation of 5-HT fibers and varicosities were observed around cell bodies in lamina IX of posthatching chicks. Thus 5-HT fibers and "terminals" fully differentiated in the spinal cord during late embryonic (>E16) and early posthatching stages.

The development of the descending 5-HT fiber system in the spinal cord can be divided into three stages: (1) the projection of axons from the brainstem to cervical (E6) and lumbar (E8) regions within the prospective white matter; (2) penetration of fibers into different laminae of the mantle layer of the spinal cord (E8-E16) and; (3) the formation of terminal connections (E16-posthatching).

178.10 CHARACTERIZATION OF SEROTONIN UPTAKE MECHANISMS IN THE REGION OF THE CAUDAL NEUROPORE OF THE EARLY CHICK EMBRYO. J.A. Wallace, S. Lilly* and R.R. Maez*. Dept. of Anatomy, Univ. of New Mexico School of Medicine, Albuquerque, NM 87131.

We previously reported on the occurrence of serotonin (5-HT) uptake mechanisms in the early chick embryo (Am. J. Anat., 165:261, '82). Cells accumulating 5-HT were visualized by formaldehyde-induced fluorescence as well as by anti-5-HT immunocytochemistry, and were localized in the notochord and in the floor plate of the neural tube in the region of the caudal neuropore. Over several stages, there was an apparent movement of these sites that accumulate 5-HT that was correlated spatiotemporally with the caudal advance of neural tube closure. For these reasons, we have undertaken studies to ascertain if 5-HT has a role in closure of the caudal neural tube. As part of these overall investigations, we first examined the characteristics of the embryonic 5-HT uptake mechanisms with respect to their sensitivity to several pharmacologic agents that act as 5-HT uptake inhibitors on adult neurons. Chick embryos between stages 11 and 14 were cultured in vitro for 2 hours with 0.1 uM 5-HT and 10 uM pargyline as controls. Sites of 5-HT accumulation were visualized in paraffin sections by anti-5-HT immunocytochemistry utilizing the peroxidase ABC staining method. Experimental embryos were treated similarly to controls except that they received one of the following compounds at either 10 or 50 uM concentration: amitriptyline, fluoxetine, imipramine or chlorimipramine. Based upon comparisons of staining intensity (scored as either dark, light or no peroxidase staining observed) with controls, all drugs tested at the 50 uM level completely blocked 5-HT accumulation in the caudal notochord and neural tube. At the 10 uM level, all of the compounds either sharply reduced or eliminated 5-HT staining but with a ranked order of efficiency of chlorimipramine>fluoxetine> amitripytline>imipramine. In addition, we tested two 5-HT receptor blockers (cyproheptadine and methysergide) for their affects on embryonic 5-HT uptake mechanisms. At doses 2 to 5 times above those reported to produce 50% inhibition of 2.5 nM 5-HT binding to receptors on adult neurons (Science, 212:827, '81), these drugs did not reduce 5-HT accumulation. From these studies, we now plan to expose chick embryos to these 5-HT uptake inhibitors at the doses shown to inhibit embryonic 5-HT uptake mechanisms in order to determine their potential for producing neural tube closure defects. Supported by NIH grants NLS19712 and RR08139.

178.11 SHAPING OF NEUROEPITHELIAL CELLS DURING BENDING OF THE CHICK NEURAL PLATE. J.L. Smith, D. Folsom* and G.C. Schoenwolf, Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, Ut 84132.

Three selective points of attachment are formed between the neuroepithelium and adjacent tissues. These comprise a median attachment either to the prechordal plate mesoderm at forebrain levels or to the notochord more caudally and paired dorsolateral attachments to the surface ectoderm of the neural folds. These three areas anchor the neuroepithelium and provide hinge-points for bending. Bending involves elevation of the neural folds (associated with the median hinge-point) and convergence of the folds (associated with dorsolateral hinge-points). Neuroepithelial cells change shape from spindle-like to wedge-like within these hinge-points. The present study evaluates two proposed mechanisms for controlling cell shape: constriction of apical bands of microfilaments and alteration of cell cycle length. Neurulating chick embryos were treated in ovo with cytochalasin D for 1-24 hours to disrupt microfilaments and assess their role in cell shape changes. Elevation of the neural folds continued after cytochalasin treatment, and median neuroepithelial cells were predominantly wedge-shaped despite the absence of apical microfilaments. Although elevation occurred, complete convergence and fusion of the folds were blocked consistently, producing dysraphic neural tube defects. In cases where some convergence occurred, dorsolateral hinge-points formed and wedge-shaped cells could be identified in these areas. Frequently, the neural folds seemed to be pulled laterad; this effect may be due to inhibition of surface ectoderm expansion. Collectively, these results suggest that intact microfilaments are not required for maintaining the wedge-shaped configurations of neuroepithelial cells but may act in epithelial expansion, convergence, and fusion. To identify possible regional differences in the lengths of cell cycles, other neurulating chick embryos were treated in ovo with colchicine for 1-3 hours, and percentages of mitotic figures were determined in the median hinge-point and adjacent, non-bending areas. Preliminary results suggest that median cells have a longer cell cycle than more lateral cells. This could be important because interkinetic nuclear migration occurs during the cell cycle and the nucleus is contained within the widest part of the cell. Thus, the shape of the cell changes as the nucleus migrates. Also, previous studies have suggested that the nucleus is positioned basally during most of the length of the cell cycle. Hence, it would be expected that populations of cells with long cycles would contain a greater percentage of wedge-shaped cells than those with shorter cycles. Autoradiographic experiments on embryos treated with tritiated thymidine are under way to explore this possibility further. Supported by grant nos. NS 18112 and HD 18143 from the NIH.

178.12 THE ROLE OF CELL ELONGATION IN NARROWING OF THE CHICK NEURAL PLATE. G.C. Schoenwolf and M.L. Powers*. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, Ut 84132.

The chick neural plate undergoes striking changes in shape prior to and during its bending into a neural tube. The purpose of this study is to define some of the mechanisms involved in this shaping. During Hamburger-Hamilton stages 4-6, the overall shape of the plate changes from spade shield-like to band-like. This band continues to narrow and elongate during stages 7-11 as bending of the plate occurs. Overall, during stages 4-11, the width of the plate decreases 50%, its length increases 773%, its height increases 28%, and its volume increases 394%. Narrowing of the plate could be due to: increase in the heights of neuroepithelial cells without increase in their volumes, decrease in the volumes of neuroepithelial cells, reduction of the number of cells within the width of the plate, or stretching of the plate. To test the role of cell elongation in narrowing of the plate, microtubules were disrupted by incubating embryos at 4°C for 2 to 24 hours. Neuroepithelial cells in cold-treated embryos remained partially elongated, reducing their heights to those of stage-4 levels. Concomitantly, the width of the plate increased proportionately, providing experimental evidence for a role for cell elongation in narrowing of the neural plate. The effects of cold treatment were readily reversible--within 2 hours of reincubation at 38°C, microtubules had reformed, neuroepithelial cells had re-extended to their former heights, and the width of the plate had decreased to its normal value. The numbers of neuroepithelial cells in cross sections through the future mesencephalon and cranial spinal cord levels of the plate were counted to determine whether cells are lost from the width of the plate during its narrowing. The number of cells within the width of the plate gradually increased during its narrowing and bending, demonstrating that cell loss from the width of the plate is not responsible for these processes. In summary, our results show that over half of the decrease in width of the plate is due to increase in neuroepithelial cell heights without increase in their volumes, and narrowing of the plate is not due to a decrease in the number of cells within its width. Examination of additional mechanisms involved in shaping of the neural plate is in progress. Supported by grant nos. NS 18112 and HD 18143 from the NIH.

178.13 NEUROANATOMICAL, NEUROVASCULAR, AND FUNCTIONAL RELATIONSHIPS OF TRANSPLANTED VASOPRESSIN NEURONS. F.E. Marciano* D.M. Gash and S.J. Wiegand. (SPON: G.J. Thomas) Department of Anatomy, University of Rochester Medical Center, Rochester, N.Y. 14642.

Our laboratory group has shown that fetal vasopressin (VP) neurons can survive and function appropriately in adult Brattleboro rats with congenital diabetes insipidus and neurohypophysectomized (NPOX) rats. The present study was conducted to examine the effect of lesions of the host magnocellular neurosecretory system on transplant growth and survival. One or two weeks prior to the transplantation of fetal anterior hypothalamus to the third ventricle, young adult male Long Evans rats received either an anterior hypothalamic deafferentation (AHD), NPOX, or sham lesions. Approximately 40 days after transplantation the animals were perfused intracardially with Zamboni's fixative followed by 2% gelatin-ink and the brains were processed immunocytochemically for the peptides VP and oxytocin and/or their associated neurophysins.

Following the AHD, the animals exhibit approximately a 50% reduction in urine osmolality, a sensitive indicator of peripheral VP activity, and concurrent increases in water consumption. Bilateral AHD lesions cause almost a complete loss of pituitary projecting magnocellular neurons in the host. This lesion appears to cause a regression or atrophy of the fenestrated capillaries of the median eminence. The grafts in AHD hosts display a vascular density comparable to the neighboring parenchyma of the host hypothalamus. Few magnocellular elements are present within the grafts and are usually located at the graft-ventricular or graft-host brain interface. In grafts that are juxtaposed to the host median eminence, there is an apparent innervation of this structure by the few surviving magnocellular elements. The physiological response of these animals following transplantation was not remarkable. Following NPOX, there is an immediate polydipsia and polyurea as a result of both the loss of pituitary VP and of a portion of pituitary projecting magnocellular elements, an effect that is partially restored. In contrast to AHD animals, NPOX rats present with a proliferation of the fenestrated capillaries in the median eminence. The grafts, when in apposition the underlying median eminence, display an enriched vascular density compared to the parenchyma of the host hypothalamus. Magnocellular neurons, when present, send axonal processes toward this hypertrophied vascular target. Ealier studies have shown that NPOX animals with grafts in juxtaposition to the median eminence respond functionally. Transplants into non-denervated animals display characteristics similar to the AHD animals.

These results suggest that the availability of an appropriate neurovascular target is an important factor for the viabilty, growth and function of transplanted neuroendocrine cells. (Supported by NIH grants NS 19900 and NS 15109)

178.14 DIFFERENTIATED HUMAN NEUROBLASTOMA CELLS GRAFTED INTO THE HIPPOCAMPUS: STUDIES ON RODENTS AND NONHUMAN PRIMATES. D.M. Gash, M.F.D. Notter, J.H. Kordower, A.L. Kraus* and S.H. Okawara*. Dept. of Anatomy, Lab. Animal Medicine and the Neurological Division of Surgery. University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Recently, our research group has begun to examine the potential of using cultured neuroblastoma cells as donor tissue in neural implants into the damaged mammalian brain. In the present study, human neuroblastoma cells were first rendered amitotic by chemical pretreatment. Cultured IMR-32 cells were labelled with H^3-thymidine and then treated with either mitomycin C and 5-Bromodeoxyuridine or prostaglandin E_1 and dibutyryl adenosine 3',5' cyclic phosphate. The differentiated cells were grafted into the hippocampus of medial septum lesioned rats or fornix-lesioned African Green monkeys. Graft survival in the rodent studies was analyzed at 1, 2, and 4 week intervals following implantation while in the five monkey graft recipients were allowed to go for periods ranging from 51 to 270 days. Host animals were perfused with a phosphate buffered 1.0% paraformaldehyde-1.25% gluteraldehyde solution and frozen sections prepared. Alternate series were stained for acetylcholinesterase (AChE) activity, Nissl stained, or processed for light microscopic autoradiography (ARG).

Grafts in the rodent hippocampus could be identified by H^3-thymine ARG and Nissl staining features. The cells were found in the parenchyma of the brain in a radial pattern spreading out for distances of up to 1.5 mm from the transplantation site. The grafted cells tended to cluster around blood vessels and were also prominent in the ependymal layer of the ipsilateral lateral ventricle. Little AChE staining activity was present in the grafted cells, even at 4 weeks following transplantation into the rodent brain.

A similar distribution pattern of grafted IRM-32 cells was seen in two monkeys receiving implants. Numerous grafted cells were present in animals which survived 240 and 270 days, respectively, after implantation. The grafted cells in these two hosts had been pretreated with mitomycin C and 5-Bromodeoxyuridine. Very few implanted cells could be identified in the other three monkeys which received grafted cells pretreated with prostaglandin E_1-cAMP. The major difference seen between grafts into rodents and monkeys was that cells grafted into the primate hippocampus often stained intensely for AChE and processes could followed from the grafted cells into the host brain. No evidence of tumorous growth of grafted cells was found in either the rodent or primate hosts.

Supported by the Brain Fund.

178.15 ORGANIZATION AND EFFERENT PROJECTIONS OF SUPRACHIASMATIC NUCLEI CONTAINED IN INTRAVENTRICULAR TRANSPLANTS OF FETAL ANTERIOR HYPOTHALAMUS. S.J. Wiegand and D.M. Gash. Dept. of Anatomy, University of Rochester, NY 14642.

Anterior hypothalamic anlage obtained from normal Long Evans rat fetuses at 17 days post conception were transplanted to the lateral, third, or fourth ventricles of adult male Brattleboro rats homozygous for diabetes insipidus (DI). Host animals were sacrificed 3 to 6 weeks post-transplantation. Half of the graft recipients received an intravascular (iv) injection of horseradish peroxidase (HRP, 200 mg/kg) 12-24 hrs. prior to perfusion with a mixed aldehyde fixative. The remaining animals were perfused with a gelatin-ink solution and the brains were post-fixed in Zamboni's fixative. Alternate series of frozen sections were processed for immunocytochemical localization of arginine vasopressin (AVP), oxytocin (OT), neurophysins (NP), or vasoactive intestinal polypeptide (VIP). For animals receiving iv HRP, additional series were processed for histochemical localization of HRP and according to a double labeling procedure for simultaneous localization of HRP and AVP- or NP-immunoreactive (ir) neurons.

Fetal hypothalami survive transplantation to all intraventricular sites and usually contain numerous NP-ir and AVP-ir neurons. OT-ir neurons are noted less frequently. The AVP-ir populations of the transplants are heterogeneous and both magnocellular and parvicellular types can be clearly identified. Parvicellular AVP-ir neurons comprise the predominant subtypes in nearly all transplants, and are frequently organized into well defined clusters that exhibit many of the anatomical relations characteristic of the AVP-ir neurons of the suprachiasmatic nucleus (SCN). Like the AVP-ir neurons of the SCN, and unlike vasopressinergic cell populations in the paraventricular and supraoptic nulei, these parvicellular cell clusters exhibit only a moderate capillary density. These cells, but not other AVP-ir neurons within the transplants, are associated with VIP-ir neurons of a similar small size. AVP-ir fibers originating from these cell clusters are of very fine caliber and ramify extensively among the cell bodies of origin and in their immediate vicinity. In addition to innervating other portions of the graft, these AVP-ir fibers also project to areas of the host brain normally innervated by the vasopressinergic component of the SCN, such as the periventricular hypothalamus, paraventricular thalamic nucleus and the host SCN itself. However, these parvicellular VP-ir neurons generally do not project to areas of the brain that normally receive an extensive vasopressinergic innervation from sources other than the SCN (eg. lateral septum, dorsal medulla and neurohypophysis).

These observations demonstrate that the fetal anlage of the SCN not only survive transplantation to the brain of adult host animals, but also retain or develop many of the intrinsic neural and vascular relations characteristic of the SCN in situ, and are capable of innervating appropriate areas of the host brain.

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178.16 GRAFTS OF DISSOCIATED FETAL CEREBELLUM DEVELOP TRILAMINAR CORTICAL STRUCTURES BY RECAPITULATING NORMAL ONTOGENETIC EVENTS. Richard Schmidt and R.K. Bhatnagar, Dept. of Pharmacology, Univ of Iowa, Iowa City, IA 52242.

We have reported that grafts prepared by injecting trypsin-dissociated fetal rat cerebellum into the forebrain of adult hosts developed regions of trilaminar cortex (Neurosci. Abst. 9:849, 1983). In this study we used ^{3}H-thymidine autoradiography and immunohistochemical (IHC) staining for glial fibrillary acidic protein (GFAP), 5-HT and cGMP-dependent protein kinase (cGK, a Purkinje cell marker) to further investigate the structure and development of these grafts.

Grafts prepared from 15-17mm CRL fetal cerebella dissociated with trypsin (Schmidt et al., in Seil, Nerve Organ and Tissue Regeneration, 1983) were injected into adult rats with one deposit within the parenchyma of the dorsal hippocampus, and a second deposit in the ambient cistern ventral to the hippocampus. For autoradiography, 5 hosts received 10-20 µCi ^{3}H-thymidine in the cistern, medial to the graft 18 days post-transplantation. 9-day-old rat pups received isotope via the cisterna magnum as positive controls. Tissues were processed 7, 20 and 72 hrs later.

All the intracisternal grafts had areas of trilaminar organization, and in the larger grafts microfoliation was apparent. Grafts within the parenchyma of the hippocampus were disorganized except for small areas of cortical organization which was invariably associated with blood vessels or cystic spaces.

At 18-21 days post-transplantation, a distinct external granule layer labelled heavily with ^{3}H-thymidine. Over time, label began to appear in granule cells in the IGL, especially in cells near the IGL-PC interface. This pattern of labeling corresponded with that seen in the normal 9 day-old cerebellum, indicating a normal pattern of mitosis and migration.

IHC staining for GFAP revealed the presence of radial glial fibers wherever regular laminar organization had developed, both in the 3 week old grafts as well as at later time. Radial fibers were not seen in the disorganized regions of the intraparenchymal grafts, however they were present in those portions which had developed laminar organization around vessels or cysts.

IHC staining for cGK revealed that PC dendrites were present and were oriented toward the molecular layer. Although 5-HT fibers were clearly present within the adjacent hippocampus, they rarely crossed into graft itself.

This study demonstrates remarkable similarities between the dissociated grafts and normal cerebellum with respect to their structure and development. Several factors appear to contribute to the development of cortical organization within the grafts: the preservation of normal granule cell migration, the presence of a surface upon which to organize, and the presence of radial glial fibers. (Supported by USPHS #GM 22365 and U.I. Coll. of Med).

178.17 AN ANALYSIS OF THE DENDRITIC FORM OF HIPPOCAMPAL NEURONS IN DISSOCIATED-CELL CULTURES GROWN AT DIFFERENT CELL DENSITIES. A.B. Waxman and G A. Banker, Department of Anatomy, Albany Medical College, Albany, New York 12208

Hippocampal neurons in culture develop a class of processes which display many of the features characteristic of dendrites in vivo. In order to assess how cell interactions affect dendritic development, we have analyzed the form of dendrites which develop in cultures prepared at low, intermediate, and high plating densities (1,000, 6,000, and 16,000 cells per cm^{2}, respectively). At higher cell densities the frequency of contacts between developing dendrites and afferent axons is greatly increased. Cells were studied after growth for 24 days in culture, using a monoclonal antibody against Microtubule Associated Protein 2 to selectively stain their dendrites.

The dendrites of cells in high density cultures were significantly longer and more highly branched. Although the number of primary dendrites was fairly constant in the three culture conditions, averaging about 5 per cell, the number of dendritic segments was twice as great in high density cultures. On average the length of individual segments was about 30% less at high cell density, but because of the increased branching the total dendritic length was about 45% greater. Under all conditions, terminal dendritic segments were consistently longer than nonterminal segments, as is true of pyramidal cells in vivo.

These studies establish that cell-cell interactions have an important influence in determining the extent of dendritic arborization in culture. In this regard these findings are consistent with the conclusions drawn from many studies of the role of innervation on dendritic development in situ. Unlike studies in situ, manipulation of cell density in culture involves only a quantitative change in the opportunity for cell interaction. It does not alter the relative numbers of different afferent populations nor does it involve cell degeneration. Nerve cell culture may provide an amenable model for studying the cell biological mechanisms which govern the development of dendritic form.

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179.1 ENERGY-DEPENDENT UPTAKE OF IMIPRAMINE IN NEURONS CULTURED FROM RAT CEREBELLUM. A. Novelli, P.G. Lysko* and R.C. Henneberry*. Lab. of Molecular Biology, NINCDS, NIH, Bethesda, MD 20205.

We have characterized the uptake of imipramine in neurons from an 8 day old primary culture of rat cerebellar granule cells.

In our model the uptake of imipramine was time, temperature and pH dependent and was reduced in the presence of inhibitors of cellular energy metabolism such as dicyclohexylcarbodiimide or in the absence of glucose. The maximal cellular capacity for imipramine before the appearance of cytotoxic effect was about 50 nmoles/mg. of protein. The uptake of imipramine was competed by propranolol but not by serotonin. It was also inhibited by chloroquine. Ionophores such as monensin, known to dissipate the proton gradient, greatly reduced the uptake of imipramine. The uptake was sodium independent but required the presence of calcium.

These data show that an energy-dependent uptake of imipramine occurs in neurons in analogy to previous reports on uptake of exogenous basic amines in other cell types.

179.2 CONTINUOUS RELEASE OF INHIBITORY AMINO ACIDS IN RAT HIPPOCAMPUS: GABA AND TAURINE RECOVERY IN PUSH-PULL PERFUSATES. L.P. Hudson* and R.J. Reiffenstein. Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Perfusion-dialysis techniques (Lerma et al., Neuropharmacol., 23: 595, 1984) have demonstrated very low extracellular recovery of gamma-amino butyric acid (GABA) in rat hippocampus; such low extracellular levels of this amino acid are thought to reflect a precise regulation of inhibitory neural transmission in this brain region (Ben-Ari et al., Neuroscience, 6: 2445, 1981). In vivo estimation of taurine, however, is substantially higher, and may point to a neuromodulatory role for this compound (Lehmann et al., Neurosci. Lett., 52: 341, 1984).

We have attempted to demonstrate consistent changes in the release of these compounds in response to physiological and neurochemical manipulations, using the local tissue perfusion method described by Bliss et al., (J. Neurosci. Meths., 7: 353, 1983). Male Sprague-Dawley rats (180-220 g) were anesthetized with urethane (1.25-1.5 g/Kg), and a bipolar stimulating electrode was placed within entorhinal cortex. A stainless steel push-pull cannula with attached recording electrode was placed in CA1 or CA3; placement of the tip of the assembly within the pyramidal cell layer was confirmed by reaching the maximum amplitude of the field response evoked by perforant path stimulation (10V, 1 msec, 1/30 sec). Local tissue perfusion was done at 20 µl/min; 10-min samples were stored at -80°C. Amino acid analysis was performed using reverse-phase, fluorometric HPLC with precolumn OPA derivitization.

In the presence of 5 mM nipecotic acid, an antagonist of GABA uptake, we have demonstrated a substantial increase in GABA and taurine recovery. Although perforant path stimulation (10V, 1 msec, 40 Hz) evoked an increased in taurine recovery under these conditions, a corresponding increase in GABA recovery was not observed. Pretreatment of animals with ethanolamine-o-sulfate, an irreversible GABA transaminase antagonist (Fletcher and Fowler, Biochem. Pharmacol., 29: 1451, 1980) produced a significant further elevation in GABA recovery in the presence of nipecotic acid. These findings suggest that control of mechanisms responsible for the removal of GABA may be necessary in order to realistically appraise GABA release in vivo under changing physiological conditions.

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179.3 EVIDENCE FOR THE PRESENCE AND CO-RELEASE WITH NORADRENALINE OF MET-ENKEPHALIN FROM ADRENERGIC NERVE TERMINALS. W.De Potter*, E.Coen* and R.De Potter* (SPON:O.H.Viveros).Dept.of Pharmacology, Universitaire Instelling Antwerpen,Universiteitsplein 1, B2610 Wilrijk,Belgium.

The opioid peptides Leu-enkephalin (Leu-Enk.)and Met-enkephalin (Met-Enk.)are present in some neurons of the central and peripheral nervous system and in chromaffin cells of the adrenal medulla.A role in neuromodulation and/or neurotransmission has been suggested and their release from brain nerve terminals and chromaffin cells has been shown.Using the immunogold technique we could visualize Met-enkephalin-like immunoreactivity in the large dense cored vesicles of bovine vas deferens adrenergic nerve terminals,whereas the small dense cored vesicles did not show any Met-Enk.immunoreactivity.By means of differential and density gradient centrifugation we have confirmed a recent report (Neuman,B. et al.,Neurosci.,13:921, 1984)that Met-Enk.is present in large dense cored noradrenaline-containing vesicles.

Release of radiolabelled noradrenaline and Met-enkephalin immunoreactivity was studied using a superfusion technique.The vasa deferentia were preincubated for 1 hr at 37°C in aerated Krebs-Ringer medium containing $3x10^{-7}$ M of (-)-7-^{3}H-noradrenaline,specific activity 14.1 Ci $mmol^{-1}$ (NEN Chemicals).In preparations superfused (1 ml min-1)with control medium,electrical stimulation (rectangular pulses of 200 mA,2ms duration,4 Hz) during 10 min caused an overflow of ^{3}H and Met-Enk.immunoreactivity (S1).After cessation of the stimulus,the release returned to prestimulation levels.A second stimulation period (S2),after 60 min.,evoked an overflow of ^{3}H and Met-Enk.which reached mainly the same level as S1 for both ^{3}H and Met^{5}-Enk.(89.3 ± 11.28% and 89.3 ± 9.90% respectively).Upon adding the adrenergic neuron blocking drug guanethidine (4×10^{-5}M),both the evoked release of total ^{3}H and Met-Enk.was potently inhibited (20.7 ± 6.01% and 27.4 ± 9.63% respectively).Superfusions with Ca^{2+}-free medium nearly abolished the electrically evoked release of ^{3}H and Met-Enk.

The present study,using the bovine vas deferens as a model, gives additional biochemical and morphological evidence for the presence of enkephalins in large dense cored vesicles of peripheral sympathetic neurons.More important,from a physiological point of view,is the demonstration that the neurotransmitter(s) noradrenaline and Met-Enk.are co-released from peripheral sympathetic neurons in a Ca^{2+}-dependent way.

179.4 ION DEPENDENCY PROFILES OF SIX Na^{+}-DEPENDENT TRANSPORT SYSTEMS. R.P. Shank and C.R. Schneider*. Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477-0776.

Ion substitution studies have revealed that the synaptosomal uptake (membrane transport or translocation) of many neurotransmitters and other substrates is, in addition to being Na^{+}-dependent, markedly affected by changes in K^{+} and Cl^{-} concentrations. The design of experiments to study specific effects of ions on membrane transport systems is complicated by indirect effects of ions on uptake due to changes in the membrane potential and permeability, and the possibility that the substitute ion (eg., Li^{+} for Na^{+}) exerts a direct effect on the transport system being studied. Consistent with previous observations we have found that uptake as a function of K^{+} concentration is bimodal in that for all systems studied (norepinephrine, NE; dopamine, DA; serotonin, 5-HT, and GABA) the rate of uptake was greatly enhanced as the concentration of K^{+} was increased from 0 to 2 mM, but as the concentration was increased above 5 mM the rate decreased almost linearly as a function of the log of $[K^{+}]$. For NE (other substrates were not studied) changes in K^{+} affected primarily the V_{max}, although high K^{+} concentrations did increase K_{m}. These results are consistent with the concept that effects of K^{+} on transport are due predominantly or exclusively to changes in membrane potential and permeability.

Our Na^{+} substitution studies revealed widely different profiles for uptake as a function of Na^{+} concentration. The uptake of NE, DA and 5-HT was highly sigmoidal as a function of Na^{+} concentration; virtually no Na^{+}-dependent uptake occurred at Na^{+} concentrations below 25 mM. In contrast the uptake of GABA increased linearly (non-saturating) as a function of $[Na^{+}]$ between 0 and 128 mM. For glutamate and ∝-ketoglutarate uptake increased in hyperbolic fashion (saturating) between 0 and 25 mM Na^{+}, but at higher concentrations the uptake increased almost linearly in proportion to the increase in Na^{+}. For NE (other substrates were not studied) changes in $[Na^{+}]$ affected both the K_{m} and V_{max}. Our results are consistent with the view that Na^{+} activates uptake by exerting direct biochemical effects on the transport systems (eg., by increasing substrate affinity) and by increasing the electrochemical potential that drives the uptake process. The relative proportion of these two effects may differ greatly among the various substrates.

Substituting isethionate for Cl^{-} inhibited the uptake of NE, DA, 5-HT and GABA, whereas glutamate uptake was unaffected, and ∝-KG uptake was enhanced. Although Cl^{-} may affect uptake by a direct action on the transport systems, its effect might arise either partly or entirely, from alterations in the membrane potential caused by changes in the Cl^{-} equilibrium potential.

179.5 CHARACTERIZATION OF MgATP-DEPENDENT PROTON PUMP IN GLUTAMATERGIC SYNAPTIC VESICLES FROM BOVINE CEREBRAL CORTEX AND PROPOSAL OF ITS ROLE. J. Shioi* and T. Ueda. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

L-glutamate is now generally accepted as a major excitatory neurotransmitter in the central nervous system. Recently, Naito and Ueda (1983 J.B.C. 258:696) reported MgATP-dependent glutamate uptake into highly purified synaptic vesicles and suggested synaptic vesicular storage and release of this neurotransmitter.

In an effort to show that the uptake is mediated by proton motiveforce (pmf), which consists of chemical potential difference of H^+ (ΔpH) and electrical potential difference ($\Delta\Psi$) across the membrane, we measured ΔpH and $\Delta\Psi$ using fluorescent dye, 9-aminoacridine, and $\Delta\Psi$-sensitive dye, oxanol XI, respectively. Synaptic vesicles were prepared from bovine cerebral cortex. It was further purified by immunoprecipitation using anti-Synapsin I for some experiments. A pre-existing small ΔpH (inside acidic) of synaptic vesicles was detected, but no additional significant contribution by MgATP to ΔpH was observed. In contrast, a substantial magnitude of $\Delta\Psi$ (inside positive) was generated upon addition of MgATP. It was collapsed by KSCN, a $\Delta\Psi$ dissipator, or carbonyl cyanide p-nitrofluoromethoxyphenylhydrazone (FCCP), a pmf dissipator. Correspondingly, there were a small but measurable amount of glutamate uptake in the absence of ATP and a substantially large uptake in the presence of MgATP. The uptake was also sensitive to FCCP. β,γ-Methyleneadenosine 5'-triphosphate, a nonhydrolyzable analog of ATP, did not substitute for ATP in either $\Delta\Psi$ generation or glutamate uptake. Ca^{2+} could not substitute for Mg^{2+}, but Mn^{2+} was more potent than Mg^{2+} in both systems. The results support the hypothesis that a proton pump generates pmf in synaptic vesicles while hydrolyzing MgATP, and the pmf thus formed, whose major component is $\Delta\Psi$, provides a driving force for the vesicular glutamate uptake. The proton pumping activity was further characterized. The $\Delta\Psi$ generation by ATP hydrolysis was resistant to vanadate, ouabain and oligomycin but inhibited by N-ethylmaleimide, quercetin, trimethyltin, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole and 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid. The results indicate a difference of this proton pump from those in mitochondria, chloroplast and plasma membrane and a similarity to those in chromaffin granule, lysosome and platelet granule.

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179.6 VESICLE CALCIUM PUMP FROM BOVINE BRAIN: SUBSTRATE KINETICS AND PHARMACOLOGY. Steven C. King* and Stanley M. Goldin. Department of Pharmacology, Harvard Medical School, Boston, MA 02115.

Starting with a membrane preparation from lysed synaptosomes (PNAS 76: 3708), our laboratory has reconstituted and purified by transport specific fractionation, a calcium pump from the bovine cerebral cortex. The pump is immunologically distinct from those found in the erythrocyte or sarcoplasmic reticulum (J. Neurosci. 4: 1468). We now report that the purified, reconstituted vesicles (PRV) contain a calcium-translocating ATPase with properties which suggest: i) that it may function to regulate the Ca^{2+} concentration in nerve terminals, and ii) that it is not derived from the plasma membrane.

$^{45}Ca^{2+}$ transport into the PRV depends upon the concentration of ATP in a complex manner, possibly reflecting the presence of two ATP binding sites with different values for $K_{0.5}$ (5 and 500μM). Maximum rates of $^{45}Ca^{2+}$ transport occur with 1mM ATP. Although at lower rates, GTP, carbamylphosphate, and other energy rich phosphate compounds also support calcium transport.

In solutions containing ATP, EDTA, and 2mM free Mg^{2+}, both ATP hydrolysis and $^{45}Ca^{2+}$ transport, measured under initial rate conditions, were activated over the narrow range of Ca^{2+}concentrations (0.1 to 1μM) which are expected to occur in nerve terminals during neurotransmitter release.

The ratio of transported Ca^{2+} to ATP hydrolysed is at least 1:1, indicating that the calcium-translocating ATPase is well coupled and has been purified away from other ATPases present in the starting material. After solubilization in Triton X-100, the ATPase is not inhibited by 500μM ouabain, indicating that the Na/K ATPase does not significantly contaminate the PRV preparation.

$^{45}Ca^{2+}$ transport is inhibited by quercetin, vanadate, and La^{3+} with $K_{0.5}$ values of 1, 10, and 5μM, respectively. Vanadate and La^{3+} also inhibit the ATPase activity at similar concentrations. On the other hand, Sr^{2+}, Ba^{2+}, Cd^{2+}, and Ni^{2+} did not inhibit the ATPase, raising the possibility that some of these ions might be transported by the pump.

In general, calcium pumps derived from the plasma membrane are: i) unable to utilize energy from sources other than ATP to drive calcium transport, and ii) are sensitive to vanadate in the 0.1 to 1μM range (Calcium and Cell Function, Vol. 4, p. 100. Academic Press, 1983). Our observations are not consistent with these generalizations. On the other hand, our results are more consistent with the properties of calcium pumps from intracellular membrane systems. Inasmuch as secretory granules purified from either adrenal chromaffin tissue or the Torpedo electric organ are known to transport Ca^{2+} in an ATP-dependent manner, we are presently investigating the possibility that the PRV calcium pump is derived from synaptic vesicles.

179.7 **UPTAKE AND RELEASE OF [^{3}H]GABA BY CHICK SPINAL CORD AND BRAIN CULTURES.** J. J. Celentano* and D. H. Farb. (SPON: J. Ranck). Department of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn., N.Y. 11203.

It has been suggested that cells which possess high affinity GABA uptake sites also release GABA as a neurotransmitter. We have examined the uptake and release of [^{3}H]GABA, comparing 1 and 3 wk spinal cord and brain cultures. We find a decrease in the number of neurons labeled by autoradiography (ARG) and a decrease in K^+-stimulated [^{3}H]GABA release by spinal cord cells, consistent with a time dependent decrease in the use of GABA as a neurotransmitter in spinal cord cultures. For uptake, cells were exposed to [^{3}H]GABA (60nM, 20 min, 37°C), washed, fixed with glutaraldehyde, rinsed, and processed for ARG (exposure time: 1 wk, 4°C). Labeled spinal cord cells decreased from 18±2% (n=6; 1700 cells counted) to 3.2±0.7% (n=6; 900 cells counted), but labeled brain cells did not change (20±1%, n=4, 1000 cells counted, and 20±4%, n=4; 900 cells counted). At both ages 55% of the GABA taken up was fixed by glutaraldehyde, and there was little change in K_m (6 and 3uM) or V_{max} (18 and 11 pmol/min/cm^2). L-DABA inhibited 30nM [^{3}H]GABA uptake more potently than B-alanine at both ages, suggesting that uptake is mostly neuronal. We have also investigated the release of [^{3}H]GABA. Cells were loaded with [^{3}H]GABA as for ARG, and the rate of release was determined at 21°C. After the last time point cultures were solubilized (0.05% deoxycholate, 1 N NaOH) to determine the cpm remaining. Cumulative [^{3}H]GABA released (as % of [^{3}H]GABA present initially) followed an initial burst and then occurred linearly for up to 1hr. In 1 wk cultures the rate of release was 0.40±0.04%/min (n=3). 100mM K^+ increased release more than 2-fold to 0.96±0.15%/min (n=3). If Ca^{++} was replaced by Mg^{++} the rate decreased to 0.6±0.1%/min (n=3). This implies that K^+-stimulated release is Ca^{++}-dependent. Three wk cultures showed a slightly higher rate of release, 0.7±0.1%/min (n=3), that was not increased significantly by high K^+: 0.7±0.1%/min (n=3). In 1 wk brain cultures, release was 0.22±0.03%/min and 0.6±0.1%/min in the absence and presence of high-K^+, respectively (n=3). In 3 wk brain cultures, the rates were 0.53±0.08%/min and 1.1±0.1 (n=3). These results suggest that in spinal cord cultures, significant changes occur in the uptake and release of GABA during the first 3 weeks in culture, which may prove to be relevent to the factors that regulate the use of GABA as a neurotransmitter during early neuronal development.

179.8 EFFECTS OF MEDIA CONDITIONS ON THE EXPRESSION OF GLUTAMATE UPTAKE IN PURIFIED NEURONAL CULTURES. C.H. Zambrano* and A.G. Hyndman (SPON: A. Feldstein), Rutgers Univ., Department of Biological Sciences, Piscataway, N.J. 08854.

Previous work has shown that media conditions can effect neuronal survival following glutamate exposure (Zambrano and Hyndman, Dev. Brain Res., 1983). Hyndman and Adler (Dev. Brain Res., 1982) have reported that in a purified neuronal culture, cells with glutamate high affinity uptake (GHAU) appear to be resistant to glutamate toxicity. We now examine the effect of different media conditions on the expression of GHAU, and its relationship to glutamate toxicity. Purified neuronal cultures from 8 day embryonic chick retinas were grown in one of the following medium supplements: CI (catalase, insulin), CIT(CI plus transferrin), N1C(CIT plus progesterone, putrescine and seleniun), Ht and OL (N1C plus heart or optic lobe extract from 14 day chick embryos, respectively). At 48h, cultures were treated with 5mM, NaCl(control) or glutamate (glu). At 72h, cultures were exposed to 3H-glutamate (1x10-7M) for 5min, fixed and processed for autoradiography. Cultures were examined for neuronal survival and the presence of GHAU.

	% of cells with GHAU		survival x10^3 cells/dish		
	NaCl	glu	NaCl	glu	% of control
OL	28.9	23.6	26.1	25.3	96.9
Ht	15.7	5.4	45.0	16.5	36.7
N1C	18.9	5.7	41.0	40.2	98.0
CI	8.8	3.0	28.5	13.7	48.1
CIT	8.1	2.0	33.8	28.9	85.5

Our results show that media conditions can effect the expression of neuronal GHAU. A desensitization of GHAU is seen in N1C toxin treated cultures, a condition in which neurons are resistant to glutamate toxicity. However, this desensitization is not noted in the case of cultures grown in OL. Our data suggest that cells which lose their ability to express GHAU, still remain resistant to glutamate toxicity as shown in N1C-cultures. In future studies, we will attempt to return GHAU expression to control levels in cultures that were exposed to glutamate. Also, since different media conditions yield different levels of GHAU expression, we will further identify which media components may serve to enhance the expression of neuronal GHAU.

179.9 **FURTHER STUDIES OF XYLAMINE BINDING TO SYNAPTIC PLASMA MEMBRANE PROTEINS.** S.D. Cushing*, E. Mullieg* and A.K. Cho, Dept of Pharmacol. and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

We have further characterized the binding of Xylamine [(XYL), N-2-chloroethyl-N-ethyl-2-methyl benzylamine], an irreversible inhibitor of catecholamine uptake, to striatal membrane peptides. Rat striatal synaptosomes (P_2) were incubated with ^{3}H-XYL (0.5μM, S.A. = 19 Ci/mmol) under various conditions. Following ^{3}H-XYL exposure the P_2 was lysed and synaptic plasma membranes (SPMs) were recovered by centrifugation at 45,000g. The SPMs were analyzed by SDS polyacrylamide gel electrophoresis followed by fluorographic detection of radioactivity.

Recently Jacobson and Wilkinson found that 1μM XYL displaced 50% of the ^{3}H-naloxone which was specifically bound to rat hypothalamic tissue [TIPS 6(1):17 (1985)]. We have, therefore looked at naloxone's ability to protect against XYL labeling of striatal synaptosome membrane peptides. With our protocol, no differences were seen in peptide labeling from striatal synaptosomes which had been incubated with ^{3}H-XYL in the absence or presence of 50μM naloxone.

In previous studies we showed that ^{3}H-XYL prominently labeled eleven intrinsic membrane peptides in striatal synaptosomes, and that the labeling of many of the peptides could be blocked by coincubating with various dopamine (DA) uptake inhibitors [Soc. Neurosci. Abstr. 10, part 1: 363 (1984)]. When striatal synaptosomes were exposed to ^{3}H-XYL in the absence of Na^+ or in the presence of gramacidin (a Na^+ ionophore) only two of the eleven peptides were labeled. These peptides correspond to molecular weights of 45K and 31K daltons. Also, ^{3}H-DA uptake was equally inhibited in striatal synaptosomes which were preincubated with 0.5μM XYL in the absence or presence of Na^+. These data imply that either the 45K or 31K peptide or both peptides are functionally involved in the inhibition of DA uptake.

We have recently exposed CHAPS solubilized SPMs to ^{3}H-XYL and found essentially the same labeling pattern as that found for intact synaptosomes. However, binding to the 45K peptide was increased relative to the other peptides. (Supported by USPHS grant MH23839).

179.10 SURFACE AND INTRACELLULAR ALTERATION OF ACETYLCHOLINESTERASE HISTOCHEMISTRY IN CULTURED MYOTUBES AFTER EXPOSURE TO CALCIUM AND IONOPHORE A23187. S. Bursztajn and L.W. Schneider*. Departments of Neurology and Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The enzyme acetylcholinesterase (AChE) becomes localized at the neuromuscular junction during development and is present intracellularly and on the surface of cultured skeletal muscle. We have shown that the organization of the AChR clusters is calcium (Ca^{2+}) concentration dependent in cultured myotubes (Bursztajn et al., J. Cell Biol., 98:507-517, 1984). Since AChRs and AChE are present in high concentrations at the neuromuscular synapse, we have investigated the role of Ca^{2+} concentration on AChE secretion. Cultured chick myotubes were incubated with various concentrations of Ca^{2+} (10^{-2}-10^{-3}M) and the Ca^{2+} ionophore A23187 (10-200 nM). The intracellular AChE activity and the AChE activity secreted into the medium was assayed spectrophotometrically, and the distribution of AChE histochemistry was examined by electron microscopy. Nonspecific cholinesterase was assayed with butyrylthiocholine as a substrate and accounted for less than 5% of the intracellular AChE. Cells incubated with high Ca^{2+} containing medium showed a significant increase in AChE release; and this increase was not observed with equal concentrations of Mg^{2+}, Ba^{2+}, Mn^{2+}, or K^+ ions. Scanning electron microscopy of cells exposed to high Ca^{2+} concentration revealed large amounts of extracellular matrix surrounding each muscle fiber. This matrix was absent in control myotubes. To determine whether this extracellular matrix was associated with AChE histochemistry, cultures were processed for ultrastructural localization of AChE. For this purpose we used thiocyanate as a capturing agent and acetylthiocholine chloride as a substrate which reacts with the cupric ions to form insoluble mercaptides. This procedure produced a small uniform reaction product which was essential for the intracellular localization of AChE. The specificity of the AChE histochemical localization was tested by using inhibitors specific for AChE and others specific for pseudocholinesterase. The AChE histochemistry was found associated with the extracellular matrix in myotubes exposed to high Ca^{2+} concentration and ionophore A23187. In control cells only small amounts of scatter reaction product were observed. Intracellular AChE histochemistry was observed in the perinuclear space, the sarcoplasmic reticulum, the Golgi cisternae, and coated vesicles. Our results indicate that increasing Ca^{2+} concentration and ionophore A23187 induces the secretion of the extracellular matrix, and this extracellular matrix is associated with AChE histochemistry. (Supported by NIH grant NS17876 and a RCDA to S.B.).

179.11 INCREASE IN ACETYLCHOLINESTERASE SECRETION FROM CULTURED CHICK MYOTUBES IS CALCIUM CONCENTRATION DEPENDENT. L.W. Schneider* and S. Bursztajn. (SPON: S.A. Berman). Departments of Neurology and Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Calcium (Ca^{++}) has been shown to have an effect on many important cellular processes. Previous work in this laboratory has shown that Ca^{++} and the Ca^{++} ionophore, A23187, regulates acetylcholine receptor (AChR) cluster formation and increases the number of AChR aggregates in cultured rat muscle cells. Since both the AChR and the enzyme acetylcholinesterase (AChE) are found in high concentration at the synapse we have examined in detail how Ca^{++} may exert its effect on AChE secretion. Chick myotubes were incubated with various Ca^{++} concentrations (5-15mM) and Ca^{++} ionophore A23187 (25-100nM) for periods of 3h - 72 h. The specificity of Ca^{++} concentration on AChE release was determined by substituting other monovalent and divalent ions for Ca^{++}. Intracellular and secreted AChE activities were measured spectrophotometrically using acetylthiocholine iodide as a substrate and radiometrically with ^{3}H-acetylcholine in order to quantitate AChE amounts in fractions obtained from sucrose gradients. To determine the amount of Ca^{++} influx, cells were incubated with ^{45}calcium and intracellular uptake measured by scintillation counting after washing and sonication of the cells. Incubation of cells with high Ca^{++} concentration did not increase AChE secretion during the first 24 h, however after 48 and 72 h a 40 - 50% increase in AChE activity over controls was observed. This 24 h lag time was not observed when cells were incubated with Ca^{++} ionophore A23187. In this case a 40% increase in secreted AChE activity was observed 24 h after addition of the ionophore, and a 70% increase after 48 h of incubation. Treatment of cells with high Ca^{++} concentration and ionophore simultaneously resulted in further increase in secreted AChE activity, but the amount of AChE secreted did not appear to be additive. The intracellular AChE activity did not change significantly under these incubation conditions. $^{45}Ca^{++}$ influx determinations showed that in the presence of high Ca^{++} concentration in the media, myotubes increased their intracellular Ca^{++} content 10 - 100 fold that found in control cells. Other divalent or monovalent ions had no effect on AChE secretion and only Sr^{++} was found to be capable of substituting for Ca^{++}. Sucrose density gradient profiles of secreted AChE forms after 24 hours of treatment with ionophore revealed a minor shift of AChE to a smaller molecular weight (6.5S) than that seen in control cultures. However in both cases AChE activity was distributed as broad peaks. Our results indicate that Ca^{++} and A23187 increase AChE but with different rates of response. (Supported by NIH Grant NS17876 and a RCDA to S.B.)

179.12 UPTAKE OF ASCORBIC ACID BY THE NEUROBLASTOMA X GLIOMA HYBRID CELL LINE NG108-15. E. Bachar* and Z. Vogel. Dept of Neurobiology, Weizmann Institute of Science, Rehovot 76100 Israel.

Ascorbic acid is present at high concentrations in the nervous system where it might play a role as a neuromodulator. For example, it serves as a cofactor for dopamine β hydroxylase (Kaufman, S. and Friedman, S., Pharmacol.Rev.,17:71,1965) and stimulates acetylcholine release from brain synaptic vesicles (Kuo, C.H. and Yoshida, H., Japn.J.Pharmacol. 30:481,1980). Moreover, we have shown that ascorbic acid stimulates collagen deposition and aggregation of acetylcholine receptors on the surface of cultured muscle cells (Kalcheim, C., et al PNAS, 79:3077,1982). In support of the suggestion that ascorbic acid is a neuromodulator we report here that ascorbic acid is actively accumulated by the neuroblastoma x glioma hybrid cell line NG108-15.

Cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Uptake of [^{14}C]ascorbate was assayed at 37°C in Ringer solution containing 2mM thiourea (to protect ascorbate from oxidation). The uptake exhibited Michaelis-Menten kinetics with K_m of 43μM and Vmax of approximately 100pmol ascorbate/min/mg protein. A similar K_m value (39μM) was obtained for NG108-15 cells that were differentiated by 5 day treatment with 1mM theophylline and 10μM prostaglandine E_1. The uptake of ascorbate was temperature dependent; the rate of uptake at 0°C was 2.5% of that obtained at 37°C. Similar to the observation made with adrenomedullary chromaffin cells (Diliberto, E.J. et al J.Biol.Chem. 258:12886,1983) the uptake into NG108-15 cells was dependent on external Na^+ ions. When Na^+ concentration was reduced to 10mM (Li^+ salts added instead) the rate of uptake was reduced by 8 fold. The uptake was not affected by glucose but was inhibited by several ascorbate related compounds. Dehydroascorbate reduced uptake by 16 and 75% at 1 and 10mM respectively. D-isoascorbate, a sterioisomer of L-ascorbate inhibited uptake by 59 and 92% at these concentrations. HPLC analysis showed that more than 90% of the labeled material which accumulated in the cells following 90 min of uptake coeluted with authentic ascorbate. Allmost all (>95%) of the accumulated ascorbate was found in the cytosolic soluble fraction. The [^{14}C]ascorbate taken up by the cells was spontaneously released with a half life of about 10h.

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179.13 **DIFFERENTIAL EFFECTS OF N-ETHYLMALEIMIDE ON NOREPINEPHRINE TRANSPORT, RESERPINE BINDING AND MG ATPASE ACTIVITY OF BOVINE CHROMAFFIN GRANULES** J. D. Deupree and J. J. Hitchcock* Dept. Pharmacology, University of Nebraska Medical Center, Omaha, NE, 68046

Catecholamines are translocated into storage granules by an active transport process requiring a transport molecule and the generation of an electrochemical gradient produced by a Mg ATPase. How this electrochemical gradient is used to drive catecholamines across the storage vesicular membrane is not well understood. We have examined the effects of N-ethylmaleimide (NEM) on the different steps in this transport process using chromaffin granule membranes isolated from bovine adrenal glands. The effects of NEM on the generation of the electrochemical gradient was examined by measuring the effects of NEM on the Mg ATPase activity. The effects of NEM on the transport molecule was studied by looking at the effects of NEM on both ^{3}H-norepinephrine transport into the granules and binding of ^{3}H-reserpine to the chromaffin granule membrane. The IC50 values for NEM inhibition of the Mg ATPase, norepinephrine transport and ^{3}H-reserpine binding was 39 mM, 0.6 μM and 2 μM, respectively. Since there is a 0.5 to 104 fold difference between the IC50 for inhibition of Mg ATPase, norepinephrine transport and reserpine binding, the data suggest that NEM is acting on different sulfhydryl groups when blocking each of these functions. Since norepinephrine and reserpine appear to bind to the same site on the chromaffin granule membranes (J.D. Deupree and J.A. Weaver, J. Biol. Chem. 259:10907-10912, 1984), these results would suggest that the thiol group with a IC50 of 2 μM may be associated with the binding site for reserpine and norepinephrine on the transporter. The thiol group having the highest affinity for NEM may be associated with the transporter but with a site which is more involved with the transport of norepinephrine than with the binding of norepinephrine. (This research was supported by a grant #NS 15187 awarded by NINCDS.)

179.14 STRIATAL DOPAMINE RELEASE: A METHODOLOGICAL STUDY. Jill B. Becker, Department of Psychology and Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, MI 48104.

To study the release of dopamine (DA) from neural tissue, most experimenters have preloaded tissue with either radioactively labelled DA or its precursor tyrosine (TYR). The implicit assumption is made that the efflux of the endogenous neurotransmitter and the efflux of the labelled compound are proportional. Last year I reported that the release of preloaded [^{3}H]DA was not always proportional to the release of endogenous DA. The experiments reported here were conducted to examine whether the release of [^{3}H]DA synthesized from [^{3}H]TYR is proportional to the release of endogenous DA.

Striatal tissue slices were incubated with shaking for 15 min in 5 ml Krebs-Ringer-phosphate at 34° C. Slices were then transferred to beakers containing 3 ml medium with 80 μM [^{3}H]TYR (100 μCi) and the tissue was incubated with shaking for an additional 30 min. The tissue was then washed and transferred to superfusion chambers (Becker et al., J. Neurosci.Meth., 11: 19, 1984). Effluent samples were collected on ice over 5 min intervals. Dihydroxybenzylamine (20 ng in 25 μl 0.05 N $HClO_4$) was added to each tube as an internal standard. Catecholamines were extracted from the effluent by alumina extraction. The amount of [^{3}H]DA in each sample was determined by liquid scintillation counting. The total amount of DA in each sample was determined by high performance liquid chromatography with electrochemical detection. The efflux of [^{3}H]DA and endogenous DA were expressed as a percent of their respective total pools. The effects of various treatments on [^{3}H]DA vs. endogenous DA efflux were examined, including: 1.) temperature dependence; 2.) the role of DA synthesis on in vitro DA release was examined by infusing either [^{3}H]TYR or α-methyl-ρ-tyrosine (MPT) with the superfusion medium; 3.) the influence of MPT on amphetamine (AMPH)-stimulated DA release; and 4.) the influence of timing and duration of AMPH infusion on stimulated DA release.

The results of these experiments indicate that the use of [^{3}H]DA synthesized from [^{3}H]TYR is not always a valid index of endogenous DA release. Under some experimental conditions the release of endogenous and [^{3}H]DA are proportional. However, only minor changes in the experimental procedures can have differential effects on endogenous vs. [^{3}H]DA release. It is suggested that one cannot assume that the release of [^{3}H]DA synthesized from [^{3}H]TYR is proportional to endogenous DA release without first validating the assumption under the specific experimental conditions used.

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179.15 METHYLXANTHINE-INDUCED CAUDATE DOPAMINE (DA) RELEASE AS MEASURED BY IN VIVO ELECTROCHEMISTRY IN THE FREELY MOVING RAT. M.E. Morgan and R.E. Vestal*. Clinical Pharmacology Unit, VA Medical Center, Boise, ID 83702 and Dept. of Med., Univ. of Washington, School of Medicine, Seattle, WA.

Methylxanthines (MX) are found in numerous foods and over-the-counter medications. Caffeine (C) is one of the most highly consumed psychoactive drugs. Theophylline (T) is extensively used in the treatment of asthma and chronic obstructive pulmonary disease. MX stimulates the central nervous system. However, the mechanism of stimulation by MX is unknown. Therefore, we now report the time dependent and drug dependent relationship between C, T and caudate DA release using in vivo electrochemistry in freely moving rats. Male Sprague-Dawley rats (250-350 gm) were anesthetized with chloral hydrate (150 mg/kg i.p.) and ketamine (50 mg/kg i.p.). Stearic acid carbon paste electrodes (250μ) were implanted stereotaxically into the left and right caudate. Ag/AgCl reference and auxiliary electrodes were implanted through separate bur holes. The entire apparatus was cemented into place with cranioplastic cement. The animals were allowed a 24 hr recovery period. Electrochemical oxidations were made with a Bioanalytical Systems DCV-5 voltammeter with the electrochemical output processed by semidifferentiation. After achieving a steady basal release, animals were injected with C or T (250 μ moles/kg i.p.). Caudate DA release was monitored for 150 min.

Per Cent Control of Average Baseline Peak Heights (Mean ± SEM)

Drug	0 min	30 min	75 min	150 min
Caffeine†	100±4	69±5*	84±8*	90±8
Theophylline†	100±3	115±5*	125±6*	109±9

† dose: 250 μmoles/kg; * $p < 0.05$ vs 0 min (paired comparison) n = 5-7 experiments per drug

C significantly decreased caudate DA release 30 min after administration with a gradual return to baseline release over 150 min. There was no signifcant difference in C-induced DA release from the left or right caudate. These studies confirm our previous findings in the urethane anesthetized rat (Soc. Neurosci. 9:482, 1983) and indicate that with this paradigm there is no significant anesthetic effect on C-induced DA release. T increased caudate DA release (peak at 75 min) with gradual return to baseline (150 min). At this dose, the data indicate a differential response of MX-induced caudate DA release. Since C is more lipid soluble than T such an effect may be one of MX caudate distribution.

179.16 CHRONIC HALOPERIDOL AND CLOZAPINE ALTER DOPAMINE AND ACETYLCHOLINE RELEASE FROM LIMBIC AND STRIATAL SLICES. David R. Compton and Kenneth M. Johnson. Dept.Pharm.,Univ.Tx.Med. Branch,Galv.,TX,77550.

Chronic treatment of rats with haloperidol (HAL) or clozapine (CZP) has been reported to:1) reduce the number of spontaneously active dopamine (DA) cells, via depolarization block, in the A9 and A10 regions (HAL), or in the A9 region only (CZP); and 2) reduce in vivo DA release in the respective terminal fields. The depolarization block of A9 cells, but not A10 cells, is reversed by transection of the nerve path between the cells and the terminal fields. This report shows that chronic drug treatment also alters some transmitter release mechanisms and/or local neuronal controls of release at the nerve terminal in vitro.

Tissue slices (300 microns) from the striatum (Stri) or nucleus accumbens (NA) were placed into slice superfusion chambers after loading with 3H-DA and C-14 choline. Standard dual label techniques for assessing each isotope were utilized to measure DA and ACh release, respectively. Release was induced by either amphetamine (Amph) or amfonelic acid (AFA) at 1μM (1min), or by electrical field stimulation (60 pulses; 1,5,10 Hz).

ACh release decreased with increasing Hz in both brain areas. DA release decreased slightly in the Stri, but release increased in the NA by 23% from 1Hz to 10Hz ($p<0.05$). The table shows the results from ex vivo experiments (as % of control) using rats injected (s.c.) with HAL on stimulated transmitter release. However, no effect was seen on electrically-stimulated release of DA or ACh in either region after acute or chronic CZP treatment. Also no changes occurred in the ability of Amph or AFA to stimulate DA release in NA after chronic treatment with HAL or CZP. However Amph (but not AFA)-induced DA release in Stri was enhanced to 144% by chronic CZP (but not HAL). CZP could also potentiate Amph-induced DA release in vitro (133% at 10μM, 222% at 30μM, $p<0.05$).

HAL dose x days		0.5mg/kg x 1			0.5mg/kg x 21		
		1Hz	5Hz	10Hz	1Hz	5Hz	10Hz
ACh	Stri	NS	NS	NS	219%	220%	224%
	NA	NS	NS	NS	135%	119%	132%
DA	Stri	NS	NS	NS	NS	NS	NS
	NA	146%	154%	142%	138%	134%	144%

NS=not significant vs control (100%); $p<0.05$ for all values.

These results indicate: 1) basic differences exist in electrically-stimulated DA release between Stri and NA, and the effects of HAL on these regions; 2) chronic HAL enhances ACh release, which may be related to the development of extrapyramidal symptoms; 3) CZP affects the mechanisms of Amph (but not electrically or AFA)-induced DA release in the Stri; and 4) HAL and CZP induced decreases in DA release in vivo are not due to changes in release mechanisms at the nerve terminal. Supported by the Scottish Rite Schizophrenia Research Program, NMJ, USA.

180.1 ANATOMICAL CORRELATES OF LATERALITY AND ENDOCRINE STATE IN A CANARY SONG CONTROL NUCLEUS. R. P. Clower and T. J. DeVoogd, Psychology Department, Cornell University, Ithaca, NY 14853

The hypoglossal nucleus (nXII) is a relay center which is essential for the production of avian song (Nottebohm, et al., 1976). Motoneurons project from it to the tongue and syrinx, the avian vocal organ. All of the syringeal motoneurons appear to accumulate testosterone (Arnold, 1980). The canary nXII is larger on the left than the right (Nottebohm and Arnold, 1976), an anatomical asymmetry associated with the dominance of the left hypoglossal nerve and syrinx in song production. We now report cellular and ultrastructural correlates of lateral asymmetry and hormone induced plasticity.

The brainstems of normal female canaries and female canaries which had received testosterone as adults were sectioned at 100μ, lightly Nissl stained, and photographed. nXII was dissected from the slices and processed for electron microscopy. Cell sizes and distributions were measured on the photographs of the Nissl stained slices. nXII was 8-10% larger on the left than on the right in both T-treated and control females. T-treatment resulted in a 16% increase in the size of the nucleus and a 12% increase in cell size. There was no lateral difference in cell size.

Electron microscopy reveals that more than 90% of nXII synapses occur directly on dendritic shafts, occasionally even wrapping about the shaft. Preliminary results indicate that there are about 15% more synapses per unit area on the left than on the right in both groups of animals. Both Gray type I and II synapses occur in nXII. A striking consequence of T-treatment was seen in the Gray type I: Testosterone results in a sixfold increase in the incidence of axonal processes with very large numbers of round vesicles (200-1000+). In T-treated tissue, vesicles in such synapses sometimes occur in tightly packed paracrystalline arrays.

Thus, these anatomical correlates of functional laterality are not dependent on either androgens or experience with song. In this nucleus as in other song control nuclei, testosterone results in major structural changes. The very high numbers of vesicles seen in nXII synapses after testosterone may give the capacity for the sustained firing needed in producing prolonged bouts of song.

Supported by the Sloan Foundation.

180.2 INDUCED MATERNAL BEHAVIOR AND CONCOMITANT ULTRASTRUCTURAL CHANGES IN THE RAT NEUROHYPOPHYSIS. B.K. Kohn*, A.K. Salm, and G.I. Hatton (SPON: Q. Z. Yang). Neuroscience Prog. and Dept. of Psychology, Michigan State Univ., E. Lansing, MI 48824-1117.

Previous work has shown that the peptide oxytocin promotes the rapid onset of maternal behavior in virgin female rats (Science 216:648, 1982). Oxytocin is produced by some of the magnocellular neurosecretory cells (MNCs) of the paraventricular nucleus and supraoptic nucleus (SON) of the hypothalamus. Many of the axons of these MNCs terminate in the neurohypophysis where under conditions not requiring enhanced hormone release, some are completely surrounded by the cytoplasm of astrocytic glial cells, i.e. pituicytes. Under conditions such as parturition and lactation which involve increased hormone release the number of enclosed axons decreases significantly (Brain Res. Bull. 8:205, 1982). The present study was prompted by recent evidence from our laboratory that ultrastructural changes occurred in SON neurons concomitant with pup-induced maternal behavior in virgin female rats (Salm et. al., this meeting), suggesting that their neurohypophyses might undergo changes also.

Briefly, virgin female rats were exposed to rat pups until exhibiting the behaviors of pup retrieval, anogenital pup licking, crouching in a nursing posture and nesting. On the 3rd day of exhibiting maternal behavior the "maternal behavers" (MBs) were sacrificed by ether anesthesia and prepared for electron microscopy. Controls were similarly housed virgin females, but without direct physical contact with pups. Fourteen neurohypophyses from the rats used in the SON study (7 from the MB group and 7 controls) were sampled.

Without the investigator being aware of treatment group membership, fifteen pituicytes from each neurohypophysis were randomly sampled with an electron microscope (4500X). The total number of axonal processes completely enclosed by the fifteen pituicytes per animal was obtained. The mean number of enclosed axons per animal decreased 43% for the MBs when compared to controls. A t-test showed this difference to be statistically significant ($p<.035$, t-test; 3.2+.56 vs. 5.8+.82 respectively). The group means for number of axons enclosed per pituicyte were .22+.04 for MBs vs .39+.05 for controls, a significant ($p<.035$, t-test) decrease of 43% for the MBs.

These results corroborate the evidence for ultrastructural changes in the SON coincident with induced maternal behavior. Both studies suggest that the hypothalamo-neurohypophysial system may play a role in the induction of maternal behavior in the rat.

The technical assistance of Inge Taubitz is gratefully acknowledged. Supported by NIH NS09140.

180.3 PUP-INDUCED MATERNAL BEHAVIOR IS ACCOMPANIED BY ULTRASTRUCTURAL CHANGES IN THE SUPRAOPTIC NUCLEUS OF RATS. A.K. Salm, B.K. Kohn*and G.I. Hatton. Neuroscience Prog. and Dept. of Psychology, Michigan State Univ., E. Lansing, MI 48824-1117

Evidence exists that the peptide oxytocin is a potent elicitor of maternal behavior (Science 216:648, 1982). The supraoptic nucleus (SON), which contains magnocellular neuroendocrine cells (MNCs) that produce oxytocin, has been shown to exhibit ultrastructural plasticity during conditions which stimulate the release of this peptide (Peptides 5 (Suppl 1):121, 1984). In particular, directly apposed MNC somata (without an intervening glial process), and the occurrence of dendritic bundles (2 or more dendrites in direct membrane apposition) have been reported to increase. In this study, the SON ultrastructure of virgin female rats in which maternal behavior had been induced by exposure to rat pups was examined to determine if like changes occurred.

14 virgin female rats, ages 102-138 days, were divided equally into control and "maternal behaver" (MB) groups. MBs were each exposed to 2 different rat pups each day for 5-16 days prior to exhibiting the 4 behaviors of pup retrieval, anogenital pup licking, crouching in a nursing posture and nesting behavior. On the 3rd day of exhibiting maternal behavior MBs were sacrificed by ether anesthesia and prepared for electron microscopy. The somatic region (6-19 micrographs, $\bar{X}$=13) and the ventral dendritic zone of the bilateral SON in its entirety were sampled from each rat. Electron micrographs (7590X) were then scored for the incidence of somatic apposition and extent of dendritic bundling. Since bundles of 1-4 dendrites had been previously reported to occur in normal rats (Neuroscience 13:769, 1984) bundles were categorized into groups of 1-4, 5-8, or 9-12 dendrites. Differences in the mean number of dendrites per bundle in each category were assessed with a 2-way ANOVA followed by post-hoc comparisons. An overall difference ($p<.025$) between MBs and controls was found for the mean number of dendrites per bundle. Whereas no differences were detected between groups for bundles of 1-4 or 5-8 dendrites, the MBs had more dendrites in bundles of 9-12 (Tukey's, $p<.010$). No differences were found in the total number of dendrites observed in each group. However significantly more micrographs (median test, $p<.0005$) were obtained from the dendritic field of the MB group, suggesting that this area was greater in these rats. At the level of the somata the situation was found to be the reverse, i.e., MNCs of MBs were seen to exhibit fewer (Mann-Whitney U, $p<.04$) direct appositions with other MNCs (19.45% vs 28.88% respectively) relative to controls.

These data point to a differential activation of SON MNC somata and dendrites during maternal behavior and a rapidly emerging picture of a labile SON which may be enlarged to include responses to environmental stimuli as well as possible mediation of a reproductive behavior. The technical assistance of Inge Taubitz is gratefully acknowledged. This work supported by NIH NS09140.

180.4 ADRENAL SEX STEROIDS SPARE MALE SPROUTING RESPONSE FOLLOWING CASTRATION. J.K. Morse, S.T. DeKosky and S.W. Scheff. Depts. of Anatomy and Neurology, Univ. of Kentucky and V.A. Medical Center, Lexington, KY 40536

We have previously reported that sex steroids play a role in a sexually dimorphic sprouting response observed in the hippocampal dentate gyrus. Untreated male and female rats demonstrate equivalent amounts of axon sprouting. Ovarectomized females display significantly decreased sprouting, while castrated males are equivalent to controls. Ovarectomized females with either estrogen (E) or testosterone (T) replacement are found to be equal to female and male controls while castrated males with E or T showed no change in axon sprouting. In the female, gonadal steroids appear to play an important role in CNS reactive outgrowth, while the role of these steroids in males is unclear. Since the adrenal glands also produce testosterone and other androgens, it may be that these adrenal androgens are sufficient to protect the male sprouting response. The present study tested the hypothesis that the adrenal gland sex steroids may be involved in the male sprouting response.

Sprague-Dawley rats 90 days of age of both sexes were randomly assigned to one of two groups: 1) gonadectomy only; and 2) gonadectomy plus adrenalectomy. One week after initial surgery (gonadectomy or gonadectomy + adrenalectomy) the rats were subjected to a unilateral ablation of the entorhinal cortex. Fifteen days after the entorhinal ablation all animals were killed. Changes in axon sprouting were determined by assessing the reactive outgrowth of the hippocampal commissural-associational fiber plexus.

Preliminary results show: a) males in group 2 (N=5) significantly decrease their sprouting response from control levels and in fact match that of ovarectomized females; b) females of group 2 (N=4) show outgrowth equivalent to that of controls. These results indicate a possible role for adrenal sex steroids in the male sprouting mechanism. It appears that estrogen may be sufficent but not essential to the female sprouting response, however, testosterone would seem to be essential for the male response. Adrenal sex steroids may play a role in the sexually dimorphic sprouting response seen in the rat hippocampus following an entorhinal cortex lesion.

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180.5 REGENERATION AND RECOVERY OF FUNCTION IN THE LESIONED HYPOTHALAMIC NEUROSECRETORY SYSTEM IS BLOCKED BY ADMINISTRATION OF VASOPRESSIN. J.P. Herman, F.F. Marciano, S.J. Wiegand, M. Matise and D.M. Gash. Department of Anatomy, University of Rochester, Rochester, NY 14642

Rats show considerable capacity for regeneration of the hypothalamic neurosecretory system (HNS) following neurohypophysectomy (NPOX), as evidenced by marked post-surgical recovery of antidiuretic function. Following NPOX, rats exhibit plasma volume depletion and hyperosmolarity and deficiencies in ion regulation, condition believed to signal vasopressin (VP) release and stimulate VP neurons. These physiological stimuli may provide information important for regeneration of the HNS. In this experiment we sought to determine whether elimination of stimulatory physiological information could have deleterious effects on recovery and regeneration of the HNS following NPOX. Fisher 344 rats received NPOX and immediately after surgery were implanted subcutaneously with Alzet miniosmopumps containing 0.9% saline (NPOX-SAL) or 1ug/ul arginine VP (NPOX-VP). The pumps delivered contents at a rate of 0.48ul/h continuously for 2 weeks. The pharmacological dosage of VP insured that plasma entering the renal circulation possessed maximal antidiuretic activity, promoting water retention and therefore putatively eliminating physiological stimulation of VP release. Water consumption and urine osmolality were monitored throughout the experiment. Six weeks following NPOX, rats were perfused with Zamboni's fixative, brains sections at 50um and stained for Nissl substance or VP-specific neurophysin.

Following surgery NPOX-SAL rats showed high normal levels of water consumption and excreted dilute urine. Over the post-operative period water intake remained stable while urine osmolality gradually increased to approximately half of normal levels (1160 mOsm/kg). NPOX-VP rats curtailed water intake and excreted concentrated urine during the period of VP administration. Following cessation of VP delivery NPOX-VP rats were afflicted with a profound diabetes insipidus which lasted for the remainder of the experiment, drinking 133% of their body weight daily and excreting urine with an osmolality of only 170 mOsm/kg. Histological analysis revealed a marked paucity of magnocellular VP-immunoreactive cells in the supraoptic and paraventricular nuclei of NPOX-VP rats relative to NPOX-SAL rats. Marked hypertrophy and increased density of VP staining was evident in the median eminence of NPOX-SAL rats relative to NPOX-VP animals.

The data suggests that administration of VP during the two week period following NPOX blocks the normal regeneration of the neurosecretory system. It is apparent that peripheral stimuli for VP release are necessary for magnocellular VP neurons to survive and sprout following extirpation of the normal target region.

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180.6 MORPHOLOGICAL ADAPTABILITY OF HYPOTHALAMIC NEUROSECRETORY ENDINGS AT THE PERIVASCULAR CONTACT ZONE OF THE RAT NEUROHYPOPHYSIS. Charles D. Tweedle and Glenn I. Hatton, Anatomy Dept. and Neuroscience Program, Michigan State Univ., East Lansing, MI 48824.

It was previously reported [Anat. Embryol. (1974) 146:157] that 3 days of water-deprivation increased the size of hypothalamic neurosecretory endings in the rat neurohypophysis and thus led to their greater coverage of the basement membrane of the perivascular contact zone (PCZ) where the hormones oxytocin (OX) and vasopressin (VP) are thought to be released. Would other acute and chronic stimuli leading to OX and/or VP release have a similar effect? Neurohypophyses were prepared for electron microscopy from rats in the following treatment groups (all rats 100-120 days old): untreated female controls [4]; pre-partum (day 21 gestation) [4]; post-partum (2-24 hr after birth) [4]; female rats dehydrated by being given 2% saline for 10 days [4]; female rats similarly dehydrated, then rehydrated by being given tap water again for 14 days [4]. Twelve-fourteen electron micrographs (12,000X) per animal were analyzed for percentage of neuronal (vs. glial) coverage of the PCZ and the average length of individual axonal contacts with the PCZ. Coverage of the PCZ by neurosecretory axons was statistically determined to increase following both birth (vs. pre-partum) and saline treatment (vs. control) ($P < .02$). length of individual neurosecretory axon contacts also was increased by both stimuli ($P < .02$). This measure, but not the total percentage of neuronal coverage of the PCZ went back to normal following rehydration. These data suggest that changes in neuronal coverage at the PCZ of the neurohypophysis can occur rapidly (e.g., at parturition) and may persist for some time following chronic stimuli. One interpretation of the data is also that the number of neurosecretory terminals at the PCZ increases with chronic dehydration. Supported by N.I.H. Grant NS 09140.

180.7 POSSIBLE MECHANISM OF SYNCHRONY AMONG SUPRAOPTIC (SON) NEURONS IN HYPOTHALAMUS OF LACTATING RAT: EVIDENCE FOR ENHANCED DYE COUPLING. Q.Z. Yang, P. Cobbett and G.I. Hatton. Neuroscience Program, Michigan State Univ., E.Lansing MI 48824-1117.

During suckling, the oxytocinergic magnocellular neurons of rat hypothalamus probably fire in relative synchrony to produce the rise in blood oxytocin required for the milk ejection reflex. The mechanism(s) producing this synchrony are not known, but we have hypothesized that electrotonic coupling among these cells may be one of the factors involved. According to this hypothesis, coupling among neurons is labile and for oxytocin-producing cells in particular, would be expected to be more extensive in lactating than in non-lactating rats. As an initial test of this hypothesis we have compared the incidence of dye coupling (as an indicator of at least strong electrotonic coupling) in the SON of virgin female and 8-12 day lactating, actively suckling animals. Slices of hypothalamus were prepared and maintained as in our previously published studies. Magnocellular neurons in the anterior and dorsal (predominantly oxytocinergic) parts of the SON were injected with Lucifer Yellow CH using appropriate precautions to prevent artefactual dye filling and incorrect counts of dye coupling. Fixed, cleared slices were examined microscopically using epifluorescence to determine the number of filled neurons. In each group the Dye Coupling Index (DCI) was calculated as the ratio of the number of dye filled coupled neurons to the total number of dye filled neurons. Tissue containing dye filled neurons is being analyzed immunocytochemically to determine the hormone contents of the coupled neurons, in particular. Results to date are as follows. The DCI for virgin females = 0.231 based upon 45 injections yielding 40 single and 12 coupled neurons. In lactating rats, the DCI = 0.453 based upon 48 injections yielding 35 single and 29 coupled neurons. Comparison of these two groups gave a $\chi^2 = 6.21$, $p < 0.02$. These data indicate that the incidence of dye coupling in the SON is different under the two physiological conditions which immediately preceded the in vitro preparation of the tissue and that this difference persisted. The latter indicates that the enhanced incidence of dye coupling outlasts the suckling stimulus conditions for at least several hours after removal of the suckling stimuli while the brains are maintained in vitro. The physiological importance of these results should become clearer if the immunocytochemical analyses reveal that the majority of coupled neurons are oxytocinergic.

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180.8 DYE COUPLING AMONG MAGNOCELLULAR PARAVENTRICULAR (PVN) NEURONS IN SLICES OF RAT HYPOTHALAMUS: EFFECT OF CASTRATION P. Cobbett, Q.Z. Yang and G.I. Hatton. Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824-1117.

Previous in vitro work has shown that some magnocellular PVN neurons are dye coupled and that changes in physiological state induced in vivo may alter the number of dye coupled cells. In males, 2% saline drinking for 8 days has been shown to affect the incidence of dye coupling in PVN and to reduce seminal vesicle weights. These data suggested that testicular hormones influence coupling of magnocellular neurons. To assess such a possibility, we have now determined the incidence of dye coupling among PVN neurons in castrated and sham castrated control rats. Adult male rats were either castrated or sham castrated under methoxyfluorane anesthesia 8 days prior to being used in recording experiments. Slices were prepared and maintained as in our previously published studies. Neurons in the magnocellular region of the PVN were injected with Lucifer Yellow CH using appropriate precautions to prevent artefactual dye filling of neurons and incorrect counts of dye coupling. Fixed, cleared slices were examined microscopically using epifluorescence to determine the number of dye filled neurons. In each group the Dye Coupling Index (DCI) was calculated as the ratio of the number of dye filled coupled neurons to the total number of dye filled neurons.

The DCI for sham castrated rat was 0.327 and was based upon a total of 48 injections producing 39 single and 19 coupled neurons. Since untreated male controls in our previous studies (J. Neurosci. 1984, 4, 3034) yielded a DCI of 0.333, it appears that the sham castration had no detectable effect upon dye coupling. Castration produced a decrease in this index. Based upon 51 injections, 48 single and 6 coupled neurons were found for a DCI of 0.111. Comparison of the sham and castrate groups gave a $\chi^2=7.56$, $p < 0.01$. Interestingly, roughly one half of these data were collected independently by each of two experimenters and each data subset yielded nearly identical DCIs (0.313 vs 0.346 and 0.148 vs 0.074).

We conclude that in the absence of testicular hormones (e.g. testosterone) there is a reduced incidence of dye coupling among magnocellular PVN neurons. This may be a direct effect of reduced testicular hormones which normally support a higher incidence of coupling. Conversely, a suppression of coupling may be caused by other factors (other hormones or transmitters) whose effects become manifest in the absence of testicular hormones.

Research supported by NIH grant NS16942. P.C. is currently a Beit Memorial Fellow, AFRC Inst. Animal Physiol., Babraham, Cambridge, U.K. Technical assistance of Kevin Grant is gratefully acknowledged.

180.9 BRAIN GRAFTS REDUCE THE OBESITY PRODUCED BY VENTROMEDIAL HYPOTHALAMIC LESIONS. G.A. Mickley, H. Teitelbaum and P.J. Reier. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145; NIH, Bethesda, MD 20205; Departments of Neurological Surgery and Neuroscience, University of Florida, College of Medicine, Gainesville, FL 32610.

Bilateral lesions of the rat ventromedial hypothalamus (VMH) produce a syndrome which includes hyperphagia and obesity. (Brobeck, J.R. et al., Yale J. of Biol. Med. 15:831, 1943). We attempted to alter these reactions by transplanting embryonic hypothalamic tissue into VMH lesion sites of adult rats.

Bilateral electrolytic lesions completely destroyed the VMH of female Sprague-Dawley rats (200-250g). Immediately following the lesions, some rats were stereotaxically implanted with pieces of hypothalamic tissue dissected from E14-E16 day fetuses. Other similarly-lesioned subjects were implanted with brain tissue from one of a variety of non-hypothalamic regions of the embryonic CNS (including cerebral cortex, spinal cord and hippocampus). Still other VMH-lesioned rats received no brain grafts. In some cases, neurons within donor tissue were labeled with 3H-Thymidine in order to radiographically differentiate graft and host. We recorded both body weight and food consumption for at least 50 days after surgery. Animals were included or excluded from the data analysis after "blind" histological determination of graft viability and lesion locus.

Total VMH destruction produced a reliable hyperphagia and obesity. Over time, surviving hypothalamic brain grafts shortened slightly the duration of the "dynamic phase" of this syndrome and resulted in significantly less total weight gained. Several of these rats lost weight following an initial dramatic gain and plateau. None of the brain grafts produced an immediate reduction of the lesion-induced weight gain. Transplantation of non-hypothalamic brain tissue caused a modest attenuation of the VMH lesion effect.

This experiment suggests that brain grafts can alter the dynamics of weight gain following VMH lesions. The precise neuroanatomical and neurochemical substrates of these findings have not been investigated.

180.10 **A ROLE FOR SEROTONERGIC NEURONS IN THE REGULATION OF SPECIFIC ELECTRICAL SYNAPSES IN HELISOMA.** **A.I. Mercier, P.G. Haydon, and S.B. Kater. Department of Biology, University of Iowa, Iowa City, IA 52242.**

Neurotransmitters have been implicated in playing various roles, ranging from chemical signalling during synaptic transmission to the regulation of neurogenesis. Recently, it has been demonstrated that serotonin can regulate the process of neurite outgrowth and, thereby, determine whether specific neurons will form electrotonic synapses (Haydon et al, Science 226: 561, 1984). Furthermore, experimental manipulation of the serotonin content of Helisoma embryos significantly affects the strength of electrotonic coupling between specific neurons when measured in the adult (Goldberg et al, Soc. Neurosci. Abstr. 1985).

The buccal ganglia of Helisoma receive defined serotonergic inputs from a single pair of neurons whose cell bodies lie in the cerebral ganglia. The present study tests the hypothesis that the presence of such serotonergic inputs can regulate the degree of intercellular communication through mature electrotonic synapses. We injected the serotonin-analogue, 5,7 dihydroxytryptamine, into adult snails at dosages shown previously to functionally remove these serotonergic inputs for up to 30 days (Gadotti et al, Soc. Neurosci. Abstr. 10: 689, 1984). Subsequently, the strength of electrical coupling between selected pairs of neurons was determined from measurements of the electrotonic coupling coefficient. Within the first week following injection, there was no change in the strength of coupling between the left and right buccal neurons 19. By three weeks post-treatment, however, the 19-19 coupling coefficient was 46% higher in serotonin-depleted snails compared to control animals. By contrast, coupling strength between a separate pair of neurons, buccal neurons 4, was unaffected. This indicates that the presence of endogenous serotonin in normal ganglia maintains the strength of a specific electrical synapse (19-19) at a reduced level. As a complement to the serotonin-removal experiments, we determined whether the addition of exogenous serotonin could reduce the strength of the 19-19 synapse. Acutely solated buccal ganglia which were exposed to 5×10^{-5} M serotonin for four hours showed a 53% decrease in 19-19 coupling strength, while the 4-4 electrical synapse was unaffected. Thus, the amount of information flow through a specific electrotonic synapse can be selectively set by the presence of serotonin within the buccal ganglia. In view of our ongoing studies with Helisoma embryos, these results lend support to a general principle that neurotransmitters play a role in determining the degree of intercellular communication among neurons throughout the life of an organism.

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180.11 INTRACEREBRAL AUTOGRAFTS OF ADRENAL MEDULLA CHROMAFFIN CELLS IN ADULT CATS WITH UNILATERAL FRONTAL CORTEX ABLATIONS (UFCA): BENEFICIAL EFFECTS ON RECOVERY OF LOCOMOTOR AND TACTILE PLACING (TP) ABILITIES. R.L.Sutton, D.A.Hovda, D.M.Feeney and W.G.Dail. Departments of Psychology, Physiology and Anatomy, University of New Mexico, Albuquerque, NM 87131.

Amphetamine (AMP) treatment produces an enduring acceleration of recovery of beam-walking ability (1) and temporarily (12hr) restores contralateral forelimb TP ability (2) in cats with UFCA. In this study, after 1 week of baseline assessment on beam-walking and TP responses, the right frontal cortex was removed by suction ablation in 6, male or female, adult cats (see 1 and 2 for methodological details). For 3 cats, testing resumed on days 4,8 & 12 postinjury. On day 12 these animals (Group 1) were reanesthetized, a right adrenalectomy (AX) was performed, and chromaffin cell "pellets" were placed adjacent to the striatum and lateral ventricle in the re-exposed cortical wound. In the other 3 cats, testing was conducted on days 4, 8,12,16 & 20 postinjury, with AX and chromaffin cell autografts performed on day 21 postablation in 2 cats (Group 2) while the last cat served as an AX control. Testing (once every 4th day) was resumed on day 33 postablation for all animals.

Behavioral testing is still being conducted, with cats currently 2 1/2 - 4 1/2 months postgrafting or postAX. The Group 1 cats and the AX control animal show slower recovery of beam-walking ability as compared to Group 2 cats who show a postgraft recovery similar to that obtained in UFCA cats given multiple treatments of AMP (1). The Group 1 and AX control cats have shown a complete absence of TP responses since UFCA, while the 2 cats in Group 2 have shown a partial (35-70%), yet "permanent" (now at 128 days postgrafting), restoration of TP responses. These data suggest the later grafts are surviving and acting as "endogenous catecholamine mini-pumps", alleviating a catecholamine depression which occurs after cortical injury and allowing intact neural systems to function such that behavioral recovery occurs. Cats will be sacrificed at 5-6 months posttransplant and brains examined for surviving graft tissue by catecholamine histofluoresence. Supported by a grant from the UNM Research Allocation Committee.

1. Hovda & Feeney, Brain Res., 298, 358-361, 1984.
2. Feeney & Hovda, Psychopharmacol., 79, 67-71, 1983.

180.12 FINE STRUCTURE AND COMPOSITION OF ADRENAL MEDULLARY GRAFTS IN BRAIN STEM REGIONS INVOLVED IN PAIN MODULATION G.D. Pappas[1], J. Sagen[1], and M.J. Perlow[2] [1]Department of Anatomy and [2]Neurology, University of Illinois College of Medicine, the West Side V.A. Hospital and Augustana Hospital, Chicago, IL 60612.

We are presently studying changes in pain perception in the rat following the transplantion of adult rat adrenal medullary tissue into the periaqueductal gray, nucleus raphe magnus, and the dorsal spinal cord - regions known to be involved in pain modulation. Preliminary findings indicate that these transplants produce significant alterations in pain sensitivity. Electron microscopic studies were undertaken to correlate these behavioral changes with the neural interactions of the host and graft tissues. The transplanted tissue consists of small pieces of adult rat adrenal medulla dissected free from cortical tissue and placed stereotaxically into the parenchyma or adjacent ventricular spaces.

Eight weeks following transplantation, catecholamine-containing chromaffin cells can be identified by fluorescence microscopy. We have also analyzed the catecholamine contents of the grafts using HPLC and found more norepinephrine (NE) than epinephrine (E) as well as low amounts of dopamine. In contrast, the normal, in situ values show much greater E compared to NE and less dopamine. The changes in the concentration ratios of E and NE suggest that the removal of the medullary tissue from the steroid secreting adrenal cortex and placing it into a new intracranial environment alters the relative concentrations of amines. Support for this hypothesis comes from fine structural studies of the chromaffin cells in the grafts which are characterized by granular inclusions morphologically typical to the norepinephrine type (i.e. dense core, often elongate) in contrast to the larger evenly dense appearing epinephrine granules commonly found in the in situ chromaffin cells.

Another striking change found 8 weeks after transplantation is that pronounced myelination has taken place both in the graft and in the host tissue. The new myelin formation in the graft has the typical appearance of PNS myelination and, in the host the appearance of CNS myelination.

By 8 weeks, the graft becomes heavily encapsulated with collagen, while the host CNS tissue shows pronounced gliosis. Chromaffin cells can be found protruding into the host CNS tissue and forming synapses with presumably the host neuronal processes. Occasionally neuronal processes, abutting onto chromaffin cells and containing clusters of clear vesicles suggest presynaptic endings. Other processes have post-synaptic specializations, such as a distinct cytoplasmic density next to the junctional membrane. Thus, chromaffin cells appear to participate as both postsynaptic and presynaptic components of synaptic junctions.

180.13 ALTERATIONS IN PAIN SENSITIVITY FOLLOWING THE TRANSPLANTATION OF ADRENAL MEDULLARY TISSUE INTO THE SPINAL CORD J. Sagen[1], G.D. Pappas[1], and M.J. Perlow[2] [1]Department of Anatomy and [2]Neurology, University of Illinois College of Medicine, West Side V.A. Hospital and Augustana Hospital, Chicago, IL 60612.

Recent studies have demonstrated that adrenal medullary tissue transplanted into the CNS survives and produces alterations in behavior. Adrenal chromaffin cells contain and release pharmacological agents known to alter pain sensitivity in the spinal cord (e.g. met- and leu-enkephalin, norepinephrine, and epinephrine). Furthermore, the release of these substances from chromaffin cells can be induced by nicotine. The purpose of the present study was to determine whether adrenal medullary tissue transplanted to the spinal cord can produce alterations in pain sensitivity when stimulated in vivo by nicotine.

Baseline pain sensitivity and pain sensitivity following nicotine administration was assessed in female rats using the tail flick, hot plate and paw pinch tests. Following laminectomy, 6-8 pieces of dissected adult adrenal medulla were inserted into the subarachnoid space through a slit in the dura. Control animals received an equal amount of either heated killed adrenal medullary tissue or sciatic nerve tissue.

Pain sensitivity was again assessed following an 8 week recovery period. Pain sensitivity was determined at 2, 10, 20, and 30 minutes following a subcutaneous injection of nicotine (0.1 mg/kg).

Without nicotine, the animals with grafts did not exhibit any alteration in pain sensitivity. However, the injection of nicotine induced potent analgesia in animals with adrenal medullary transplants as assessed by all three analgesiometric tests. Tail flick latency was elevated from 4.0 $\pm$ 0.3 sec to 9.2 $\pm$ 0.6 sec, hot plate latency was elevated from 7.9 $\pm$ 0.2 sec to 24.5 $\pm$ 3.3 sec, and paw pinch threshold was elevated from 9.6 $\pm$ 2.0 to 15.4 $\pm$ 1.1. This analgesic response was apparent 2 minutes following the nicotine injection and remained elevated at 10 and 20 minutes, tending toward baseline by 30 minutes. This dose of nicotine had no affect on pain sensitivity in animals with control transplants. Preliminary studies indicate that the observed analgesia induced by nicotine in transplanted animals is reversed by the opiate antagonist naloxone.

Both light and electron microscope studies show that the transplants were attached to the dorsal spinal cord within the subarachnoid space and contained typical chromaffin cells.

Results of this study indicate that it is possible to reduce pain responsiveness in experimental animals following the transplantation of adrenal medullary tissue into the spinal cord.

180.14 PLASTICITY IN CROSSED NIGRO-STRIATAL PROJECTIONS TEN DAYS AFTER HEMI-VIBRISSECTOMY IN THE RAT. J.P. Huston, K. Lange *, S. Morgan *, H. Steiner *. Institute of Psychologie III, University of Düsseldorf, D-4000 Düsseldorf, Fed. Rep. Germany.

There is evidence for plastic changes in the crossed efferents of the substantia nigra (SN) about one week after a unilateral peripheral or central lesion in the CNS. This is manifested by an increase in the number of cells in the SN, labeled by horseradish peroxidase (HRP) injected into the contralateral thalamus or caudate nucleus (CN). These alterations temporally coincided with a decrease in behavioral asymmetries (turning) induced by the lesion. Preventing the animals from turning after a unilateral SN lesion suppressed both the behavioral recovery and the change in the interhemispheric nigro-thalamic projection. These results led to the hypothesis that such apparent changes in crossed efferents from the SN could be part of a morphological correlate of learning to compensate for lesion-induced asymmetries. However, in these previous experiments the possibility that the changes occured in response to large scale central denervation could not be ruled out. Thus, we decided to perform a unilateral intervention with minimal damage to the nervous system. Hence, we examined the effect of unilateral vibrissectomy on the crossed nigro-caudate projection.

Normal male rats had their vibrissae cut unilaterally, and recut on every second day to eliminate regrowth. Ten days after the initial removal, HRP was applied iontophoretically to the CN either ipsilateral or contralateral to the side of vibrissa removal. The SN on both sides of the brain was examined for labeled cells. When the HRP was applied contralateral to the vibrissectomy there were more labeled cells in the SN of the opposite hemisphere than when it was applied ipsilaterally. (Supported by Grant Hu 306/3-2 from the Deutsche Forschungsgemeinschaft).

180.15 INCREASED HOST FIBER PLASTICITY AFTER CORTICAL TRANSPLANTATION IN MICE WHICH RECEIVED BASAL FOREBRAIN LESIONS. C.F. Hohmann, F.F. Ebner and J.T. Coyle. Dept. of Neuroscience, Johns Hopkins Univ. School of Med., Balto., MD. 21205 and Center for Neuroscience, Brown University, Providence, R.I. 02912.

Cerebral cortical plasticity in mammals, the ability of connections to rearrange in response to a modified environment, has been proposed to be regulated by systems diffusely projecting from brainstem and basal forebrain to cortex. Norepinephrine (NE) and Acetylcholine (ACh) have been suggested as possible regulators of plasticity. Studies of the influence of NE on cortical plasticity have yielded contradictory results. The potential role of ACh has not been explored thoroughly. The present experiments investigated the effect of a lesion of basal forebrain cholinergic nuclei on the morphological rearrangement of cortical connections in a transplantation paradigm.

Embryonic neocortex was transplanted into the neocortex of either normal adult mice or mice that had received electrolytic lesions in the ventromedial globus pallidus area prior to transplantation. The lesions decreased cortical ChAT activity by 50-70% and nearly abolished AChE staining. The ability of thalamocortical afferents to invade the transplant tissue was assessed by anterograde tracing methods. Injections (3mm below 0, 1.5mm right of midline, 2.4mm posterior to 0) of 0.25ul (5%) WGA-HRP were made with stereotaxic guidance into the ventrobasal complex of the thalamus. Thalamocortical fibers in host and transplanted cortex were visualized with the TMB enzyme reaction product for HRP. Thalamocortical fiber ingrowth, although variable, could be detected in transplants of all mice with basal forebrain lesions but not the unlesioned mice (N=15). Basal forebrain lesions thus affect the plastic response of adult host thalamocortical fibers to a transplant.

To assess the specificity of the response to cholinergic deficits, the effects of similar electrolytic lesions in basal forebrain on ChAT activity, NE and 5-HT levels and β-adrenergic as well as serotonin type 2 receptor binding were monitored 1 week, 1 month and >2 months after the lesion in a parallel group of mice. ChAT activity always decreased in cortex ipsilateral to the lesion. NE and 5-HT levels decreased in some mice but not in others in cortex ipsilateral to the lesion. Receptor binding changes were not consistent. In conclusion, the most consistent correlate of the basal forebrain lesion on thalamocortical fiber plasticity is a reduction in the levels of presynaptic cholinergic markers. However, it is quite possible that involvement of CA or 5-HT fibers due to the lesion has an impact on the extent of the plastic response. This could account for the variability in the amount of host fiber ingrowth into transplants.

180.16 RELATION BETWEEN FUNCTIONAL RECOVERY AND BRAIN ACETYLCHOLINE CHANGE IN RATS WITH UNILATERAL AND BILATERAL ROSTRAL RETICULAR LESIONS. T. Yamamoto,* Y. Katayama,* S. E. Robinson, C. E. Dixon, B. G. Lyeth, M. L. Giebel,* H. H. Stonnington,* D. P. Becker and R. L. Hayes. Division of Neurosurgery, Department of Pharmacology, and Department of Rehabilitation Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0001.

Bilateral destruction of the reticular activating system in cats can produce profound, irreversible behavioral suppression resembling coma. When such lesions are made unilaterally in two stages, minimal behavioral disruption results if a sufficient period of time intervenes (Adametz, J. Neurosurg., 16:85-97, 1959). Little is known about changes in neural function associated with this phenomena. Many cholinergic neurons in the reticular formation (RF) comprise a large ascending system with extensive projections to forebrain areas. Thus, we examined relationships between changes in behavioral disruption and levels and turnover of acetylcholine (ACh) in specific brain regions at various times after unilateral and bilateral lesions of the RF employing the rat.

Bilateral electrolytic lesions of the RF including the dorsal tegmental ascending system (10 mA, 5-sec, bipolar coagulation) produced pronounced behavioral suppression in all rats (n=10). Animals displayed no purposeful or spontaneous activity (locomotion, eating, drinking) and did not survive longer than seven days without supplemental feeding. Unilateral lesions produced only transient (<3 days) contralateral motor disturbances. Following unilateral lesions, 40 rats received staged bilateral lesions 1, 5, 15 or 30 days later. Such lesions made at 15 or 30 days did not produce significant behavioral disturbances.

Employing the method of Zsilla et al. (Neuropharmacol., 16:25-30, 1977), ACh levels and turnover were calculated for the diagonal band and medial septum, frontal cortex, thalamus, hypothalamus, amygdala and hippocampus. Initial results indicate that within seven hours following bilateral lesions (n=4) ACh levels and turnover decreased in all areas studied (>50% decrease from unlesioned controls, n=4) with the exception of the hypothalamus.

Within 24 hours, unilateral lesions (n=2) produced a decrease in ACh levels and turnover of >30% in areas ipsilateral to the lesion. A smaller decrease in levels and turnover was seen in contralateral areas. Results of other experiments indicate recovery of ACh levels and turnover in regions ipsilateral to the lesion within 15 days. These results suggest that lesions of the RF which include the dorsal tegmental ascending system change the total amount and turnover rate of ACh in the forebrain and the recovery of cholinergic levels and turnover rate in forebrain regions may be one correlate of the animal's ability to tolerate such lesions. In our model it was shown that 15 days was a sufficient interval to produce tolerance to staged bilateral lesions in the rat. Supported by NIH Grant #NS 12587.

180.17 CORTICAL GRAFTS IMPAIR SPATIAL LEARNING IN ADULT RATS WITH MEDIAL FRONTAL CORTEX LESIONS. Bryan D. Fantie and Bryan E. Kolb. Psychology Department, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

Previous research has suggested that the recovery of behavioural function in animals with brain lesions can be enhanced by transplanting cortical tissue into the damaged areas. In the present study, we wished to investigate whether cortical grafts would improve the performance of rats with medial frontal cortex lesions on a place navigation task which is especially sensitive to the integrity of this brain site.

Nineteen adult male Long-Evans rats had their medial frontal cortex removed by aspiration under sodium pentobarbital anaesthesia. One group (Lesion+Transplant , n=10) received grafts of cortical tissue taken from one-day old pups 8-25 days later. The other group (Lesion Only, n=9) was re-anaesthetized, had their scalp incision opened and then re-sutured. Approximately 30 days later all of the rats were tested for spatial learning in a water maze with a hidden platform.

Both groups of rats with lesions performed more poorly than did unoperated controls and neither operated group ever achieved a comparable level of task mastery. Significantly, Lesion+Transplant rats were worse at the task than were Lesion Only animals. For example, by the fourth day of testing, 7 of the 9 Lesion Only rats were swimming to the platform within a mean latency of 20 s while only 2 of the 10 Lesion+Transplant rats were performing at a similar level (X^2=6.34, p<.02).

Subsequent Histology revealed that the grafts in the two rats that matched the performance exhibited by the Lesion Only group were not viable. In addition, the lesions in the 2 aberrant Lesion Only rats were much larger than those in the other rats and had, in fact, included damage to the cingulate cortex.

These results, therefore, lead us to conclude that viable cortical grafts do not enhance the behavioural recovery of spatial learning in rats that have medial frontal cortex damage. In fact, these transplants cause further impairment.

180.18 FUNCTIONAL AND ANATOMICAL EFFECTS OF STRIATAL NEURAL GRAFTS TO THE EXCITOTOXICALLY LESIONED STRIATUM. O. Isacson*, S.B. Dunnett[1] and A. Björklund. Dept. of Histology, University of Lund, Sweden, [1]Dept. of Exp. Psychology, University of Cambridge, England.

Striatal neural grafts in rats with extensive unilateral excitotoxic ibotenic acid lesions of the caudate-putamen have previously been shown to increase levels of striatal neural marker enzymes GAD and ChAT levels, normalize local cerebral metabolism (2-deoxyglucose-utilization) and reduce behavioral hyperlocomotor activity (Isacson et al., Nature 311:458, 1984). The present experiment extends these findings by determining histological as well as behavioral effects of fetal striatal cell implantation into the striatum and the globus pallidus following selective bilateral striatal lesions.

Rats received bilateral ibotenic-acid lesions (2 x 10 ug) of the AMC 9 days prior to grafting. Grafts were placed either into the antero-medial caudate-putamen (AMC) or directly into the striatal projection area of the globus pallidus (GP). Behavioral testing involved both conditioned (T-maze) and non-conditioned (locomotor activity) tasks.

The groups with AMC-lesions without grafts showed severe deficits in a T-maze delayed alternation task which involved both memory and left/right choice, and they also had a generally increased spontaneous open-field locomotor activity. The grafted animals showed significantly improved performance in delayed alternation. The grafts placed homotopically into the lesioned AMC were more effective in reducing the lesion-induced deficit than those grafts placed directly into the GP. In the grafted groups the performance in the delayed alternation task was positively correlated with graft-size in the lesioned caudate-putamen (r = 0.8, p<0.01). Histological evaluation revealed surviving grafts with high neuronal densities and expression of acetylcholine-esterase in all of the transplanted animals. Striatal grafts growing in the lesioned area of the AMC were larger in volume than those placed in the intact GP (mean: 7.1 vs 3.8 mm^3) and they significantly reduced the lesion-induced striatal atrophy.

This study provides further evidence that fetal striatal grafts can survive in the excitotoxically lesioned striatum and form a neural substrate that ameliorates functional deficits, both in conditioned and non-conditioned behaviors, produced by striatal lesions.

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180.19 Reversal of spontaneous locomotor hyperactivity following fetal transplants of the striatum occurs independent of DA mechanisms. A.W. Deckel[1,3], T.H. Moran[1], and R.G. Robinson[1,2], Dept of Psychiatry and Behavioral Sciences, [1]Dept of Neuroscience, [2]Johns Hopkins Univ Sch of Medicine, Baltimore, MD., 21205., Dept of Psychology, [3]Kessler Institute of Rehabilitation, W. Orange, N.J., 07052

Kainic acid (KA) striatal lesions in female Sprague-Dawley rats cause a hyperactivity in spontaneous locomotor activity, and a relative hyperactivity following injection with amphetamine. Previously, we found that fetal striatal (str) implants grafted directly into the KA lesioned str reverse the spontaneous locomotor activity caused by the lesions. This experiment examined the effects of pre and post synaptic DA stimulation on the activity of rats who recovered in their spontaneous locomotor activity following KA lesions and fetal str transplants.

Thirty-three adult female Sprague- Dawley rats were assigned to 1 of 4 groups, including lesion-only (LO; n=7), lesion and transplant (LT; n=9), transplant-only (TO; n=8), and controls (CN; n=9). LO and LT animals received bilateral KA injections (0.8 ug/0.4 ul per 4.5 min per side) in the anterior str; TO and CN received vehicle only. Seven days later, LT and TO received bilateral implants of day 17 fetal rat str. Beginning at 5 months postsurgery, horizontal, vertical, and stereotypical locomotor activity was tested in a computerized activity monitor (Omnitech). Each animal was run for 2 hr periods in the monitor under 3 drug conditions, each condition separated from the next by 1 week. The trials included saline (1.0 ml/kg, i.p.), amphetamine (1.0 mg/kg, i.p.), and apomorphine (0.2 mg/kg, s.c.); drugs were administerd randomly in order to control for drug order effects.

LO animals showed a persistent hyperactivity compared to both CN and LT in their spontaneous (saline) horizontal activity; transplants in the LT normalized behavior (F(1,27)=7.9, p=<.01), with LT appearing nonsignificantly different from controls. Conversely, apo and amp led to a persistent hyperactivity of horizontal and vertical activity in both LT and LO groups compared to CN, with no transplant effect apparent. Transplants did, however, significantly affect LT stereotypical movements compared to LO under the apomorphine condition. Brains were cut at 25um, and tritiated spiperone autoradiography and H+E staining was done. While the transplants survived robustly in both the lesioned and intact brains, few DA receptors were found in them. In summary, fetal str transplants reversed spontaneous locomotor deficits following KA str lesions, but had little effect on locomotion under contions that stimulated DA. We conclude that the transplants do not reinstate the disturbed nigral-striatal circuity, but lead to recovery by other, as yet unknown, mechanims. ACKNOWLEDGEMENTS: This work was supported by a grant from the Huntington's Disease Foundation.

180.20 **Transplants of glial cells after frontal cortex lesion promotes behavioral recovery in rats.** J.P. Kesslak, P.C. Mazur*, M. Nieto-Sampedro and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

Lesions of the frontal cortex in rats produce a deficit on a reinforced alternation task. Transplants of embryonic frontal cortex have been shown to enhance recovery after the lesion[1]. However, the time course for recovery is so rapid that it is unlikely that neural connections with transplants could have been established, suggesting that biochemical actions may be involved. Damage to the CNS elicits production of endogenous neurotrophic and neurite promoting factors[2]. Glial cells proliferate around the wound and in gelfoam in the wound cavity and may be, inpart, responsible for the production of trophic activity[1]. We examined the behavioral effect of transplanting into a frontal cortex lesion cavity; 1) gelfoam that had remained for 5 days in a wound cavity of another animal and, 2) purified astrocytes grown in culture.

Adult male albino Sprague-Dawley rats were used. Five experimental groups were formed: i) Sham-operations; the scalp was retracted with no further damage. ii) Frontal cortex ablations, where the wound cavity was filled with saline-soaked gelfoam. iii) Frontal cortex ablation which received gelfoam transplants from a wound cavity from a previously damaged rat. iv) Frontal cortex ablation which received transplants of gelfoam containing cell-free extracts derived from wound-gelfoam. v) Frontal cortex lesion with transplants of purified astrocytes grown in culture.

Ten days after surgery animals were run on a reinforced alternation task. Results were analyzed using ANOVA. Groups which received wound-gelfoam and glial transplants did not differ from the sham-operated group, and learned significantly faster than the lesion group which received saline-soaked gelfoam. The group receiving wound-fluid extracts did not differ from the saline-gelfoam group. These results indicate that behavioral recovery after frontal cortex lesion is facilitated by transplantation of non-neuronal cells. Replacement of lost neurons is not necessary, although it may be sufficient, for behavioral recovery on this task.

1. Labbe et al., (1983) Science, 221: 470.
2. Nieto-Sampedro et al., (1983) J. Neurosci., 3: 2219.

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181.1 Mild neonatal stress influences adult analgesia following morphine. H. Schreiber III, B. Zito*, M. Warren*, A. Davis* J. Trambley*, B. Gonzalez*, M. Ortiz*, L. Salazar*, G. Gonzalez*, Div Behav Sci., N.M. Highlands Univ., Las Vegas, N.M. 87701.

A burgeoning literature delineates the complex relationship between stress and analgesia, but most studies involve relatively recent situational stress. We report here that neonatal stress substantially reduces morphine-induced analgesia in adult rats. In Experiment 1, Sprague-Dawley albino litters and dams were left undisturbed or litters received early handling (mild hypothermia produced by removal from nest and dam, 3 min/D, first 10 Ds p partum). At 130 Ds, after 10 Ds of adaptation, rats (split litters) received morphine sulfate (2.5 mg/kg, i p) or saline and daily tail-flick testing (preinj, 30, 60, 90, 120 min p inj) or not for 14 Ds. Then, all rats received morphine and were tested. A significant Time of Testing by Sex by Drug History by Early Treatment interaction was found, $F(4,192)=3.0$, $p<.02$. Early handling reduced analgesia by about 80% in male rats at 30 min p inj, regardless of previous exposure to the apparatus, but not among morphine history rats. In Experiment 2, litters received early handling or not as before, but half of the litters were returned to a dam-absent nest. Dams were returned after 1 hr. This manipulation of the dam can abolish the early handling effect on stereotypy (Schreiber et al, 1978). Nonetheless, at 155 Ds when rats received morphine (2.5 mg/kg) and tail-flick testing (preinj, 30, 90 min p inj.), early handled rats showed little morphine-induced analgesia in comparison with controls, $F(1,16)=9.64$, $p<.01$, regardless of maternal separation. In Experiment 3, Long-Evans hooded litters received early handling or not as before; all dams were removed for 1 hr with half of the dams left undisturbed and half exposed to stressed donor pups. This manipulation of dams is believed to alter maternal behavior in a fashion similar to early handling (Smotherman and Bell, 1980). Control groups for maternal separation and observation were included. At 100 Ds, females received morphine (5 mg/kg, i.p.) and were tested on a hotplate (preinj, 30, 60, 90 min p inj). Analysis of the average latency to paw-lick or footstamp showed a main effect of early handling $F(1,32)=6.44$, $p<.02$ (early handled Ss, less analgesia) and a main effect of dam exposure to donors, $F(1,32)=3.94$, $p=.056$ (offspring of exposed dams, less analgesia). Other experiments involving inhibition of locomotion and catatonia will be discussed. No rat in any experiment was subjected to more than threshold pain. We conclude that stress-related neonatal manipulations are potent influences on the behavioral effects of morphine in adulthood.

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181.2 EFFECTS OF DIPRENORPHINE INJECTED INTO THE VTA ON ICS IN THE RAT. M. Washburn* and E.A. Stein. Department of Biology, Marquette University, Milwaukee, WI 53233.

The neurochemical and neuroanatomic substrates of brain stimulation reward (ICS) have been intensively studied using numerous techniques including lesions, pharmacologic manipulations and stimulation site mapping. It now appears that the catecholamines play a significant role in this behavior. Recently, the endogenous opioid peptides have been implicated as modulators of the so-called "standard neurotransmitters" in a number of neuronal systems and behaviors including ICS. However, while agonists such as morphine and the endorphins have reliably been shown to affect both rate and threshold of responding, considerable controversy exists as to the effects of opiate receptor blockers on this behavior. As such, we have undertaken a mapping study of the effects of diprenorphine (Dpr), a μ/δ opiate receptor blocker, on ICS.

Male, Holtzman rats were implanted with monopolar, 250 μ nichrome electrodes aimed at the medial forebrain bundle (flat skull coordinates: -3.3, 1.75, 8.8). In addition, bilateral 26 g stainless steel guide cannulae were implanted 1 mm dorsal to the nucleus accumbens (+1.7, $\pm$1.5, 6.8), medial prefrontal cortex (+2.7, $\pm$0.5, 3.0) and ventral tegmentum (-4.8, $\pm$1.0, 8.45). Following recovery, rats were trained to press for 300 msec trains of 0.5 msec cathodal pulses. When stimulation rates stabilized, eight current levels, representing 10 to 100% responding, were chosen and randomly presented to rats daily until again rates stabilized. A within subjects design was then employed with rats receiving one of three doses of Dpr (1.5, 3 or 5 μg/inj bilaterally in 0.5 μl saline) or saline vehicle once per week. Prior to recording responding at each current during a five min test, subjects are given an additional 30 second interval to acclimate to the new current.

Administration of Dpr into the VTA was not able to alter ICS rate or threshold responding. There were no significant shifts in the current-response curve. These results are discussed in light of a possible modulatory role for opioids in ICS.

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181.3 IN VIVO ALTERATIONS OF OPIATE RECEPTOR BINDING AFTER AVERSIVE AND REWARDING STIMULATION. M.J. Blake and E.A. Stein. Department of Biology, Marquette University, Milwaukee, WI 53233.

Early studies demonstrated that intracranial brain stimulation (ICS) had analgesic or aversion ameliorative properties in addition to its rewarding qualities. Subsequent work has shown both electrophysiological and pharmacological distinctions between the systems which mediate these effects. Even though the pain-suppressive system from the brain to the spinal cord can be separated from the reward system, activation of the reward system can attenuate the perception of negative environmental stimuli. Observations in our lab have shown that rats actively escaping aversive footshock will attenuate this behavior when presented simultaneously with non-contingent ICS. This provides further evidence that reward systems can override pain perception mechanisms in addition to suggesting that non-contingent ICS has rewarding properties. With regard to numerous studies implicating the endogenous opioid peptides in both pain and reward mechanisms, in vivo autoradiography was employed to determine the changes in opiate receptor occupancy that occur between rats receiving ICS, footshock or both.

Male Holtzman rats were implanted with monopolar stimulating electrodes aimed at the ventral tegmental area. Following a one week recovery, rats were placed in stimulating chambers and primed to press for ICS. Animals showing robust ICS were implanted with chronic jugular catheters and after a three day recovery, the current range supporting ICS was determined for each rat. Animals were then trained to press a lever to escape 300 msec trains of aversive footshock applied to the grid floor every second. One response resulted in turning the footshock off for 5 seconds. Rats were then divided into four groups. The first group received non-contingent ICS, the second group received escapable footshock, the third group received escapable footshock and non-contingent ICS simultaneously, and the fourth group served as a sedentary control. Following their respective treatment, rats were injected with .002 mg/kg H^3-diprenorphine and processed for autoradiography following a previously described technique (Seeger, T.F. et al., Brain Res., 305:303, 1984). After an appropriate exposure, x-ray film (LKB Ultrofilm) was developed and analyzed using an Eyecom II microdensitometer (Spatial Data).

Analysis of results revealed that rats receiving ICS regardless of the presence of footshock had unilateral alterations in opiate receptor binding in several nuclei previously implicated in reward. This suggests that ICS results in a change in opioid release that is unaffected by incoming painful stimuli. The opioid involvement in reward and its relation to central pain mechanisms will be discussed. Supported in part by NIDA 02234 and a fellowship from Marquette University to MJB.

181.4 CHRONIC ADMINISTRATION OF ACTH AND/OR NALTREXONE: EFFECTS ON MORPHINE-INDUCED ANALGESIA. Z.H. Galina and Z. Amit. Center for Studies in Behavioral Neurobiology, Dept. Psych, H-1013, Concordia Univ. Montreal, Quebec, Canada, H3G 1M8.

The substrate for the behavioral effects of ACTH are unknown but it has been suggested that these effects may be mediated by an action at opiate receptors. We thought that a useful way of determining the substrate would be to analyze what effect the chronic administration of ACTH and/or naltrexone would have on morphine analgesia.

Thirty-two male wistar rats which had been part of a previous experiment examining the locomotor effects of ACTH and naltrexone were used. They were given two s.c. injections of either ACTH (20IU/kg), naltrexone (10mg/kg) or saline for 24 consecutive days. The groups were: saline-saline, ACTH-saline, naltrexone-saline, and ACTH-naltrexone. Four days after the final day of drug administration (day28) half the rats in each group received morphine (1mg/kg) or saline five min. before being placed on a hot-plate (54°C). Latency to initial back pawlick and jump/escape were recorded.

Pawlick data indicated (ANOVA) an effect of morphine, and interactions between ACTH x naltrexone, and ACTH x naltrexone x morphine. Post hoc tests revealed that the chronic administration of ACTH or naltrexone alone reduced the latency to pawlick compared to saline controls. The conjoint administration of ACTH and naltrexone prevented this reduction. Morphine given to all groups reduced the latencies independent of group designation.

Jump/escape data indicated an effect of ACTH, and morphine, and interactions between ACTH x naltrexone. Post-hoc analysis revealed that ACTH alone increased the latency to jump but that this could be prevented by simultaneous naltrexone presentation. Morphine increased the latencies to jump in the saline group and this was potentiated in all drug groups.

In summary, we found that the long term administration of ACTH increased latency to jump but decreased latency to pawlick; and potentiated the effects of morphine. Conjoint administration of naltrexone blocked the ACTH effects on both jump and pawlick measures. The effects of morphine were potentiated by chronic administration of either ACTH, naltrexone or ACTH and naltrexone on the jump measure; pawlick was unaffected. Taken together these results suggest that ACTH may operate through opiate receptors to effect analgesia in a complex fashion depending on the measure used.

181.5 DIFFERENTIAL ACTIONS OF INTRACEREBROVENTRICULAR CCK ON AMPHETAMINE-INDUCED LOCOMOTION AND STEREOTYPY IN RATS.
Aaron Ettenberg and Friedbert Weiss Department of Psychology, Univ. of California, Santa Barbara,CA 93106

Cholecystokinin octapeptide sulfate ester (CCK-8) has been reported to have long lasting antipsychotic effects in humans and neuroleptic-like effects in animals. Since CCK has recently been found to coexist, and be co-released, with dopamine (DA) in a subset of mesencephalic (A10 and, to a lesser extent, A9) neurons, it has been suggested that its antipsychotic/neuroleptic effects may stem from a central antagonism of DA neurotransmission. However, in at least one important behavioral assay of DA function (i.e. hyperlocomotion-stereotypy model), we have found that CCK failed to produce effects comparable to those of classical DA-antagonist neuroleptic agents. While both CCK and typical neuroleptics (e.g. haloperidol) attenuate the locomotor increase induced by low doses of amphetamine (AMP), only the neuroleptics antagonize the stereotypies induced by higher doses of AMP. In fact, we report here that CCK produced a marked potentiation in stereotypies while completely reversing the locomotor effects produced by amphetamine administration.

To stimulate hyperlocomotion or stereotypy rats were injected (s.c.) with d-AMP (1 or 3 mg/kg) 15 min before ICV infusion of sulfated CCK-8 (1 or 2 ug/rat). Locomotor activity was measured in cages equipped with automated photocell-activated counters. Stereotyped behaviors were rated by a trained observer who was unaware of the treatment conditions of the animals. Ratings involved an objective assessment of the presence or absence of particular behaviors (e.g. head bobbing, gnawing...) as well as a zero to four point behavioral rating scale similar to those used by others. Ratings were taken at 5 min intervals over a 2 h period immediately following peptide infusion. CCK-8 antagonized AMP-induced (1mg/kg) hyperlocomotion to levels indistinguishable from baseline (at the high dose). The same peptide treatments produced a dose-dependent potentiation in the stereotypy induced by AMP (3mg/kg). These data are comparable to the effects others have observed with the class of "atypical" neuroleptics (e.g. clozapine). We, therefore, hypothesize that a) CCK differentially affects DA function in the mesolimbic and nigrostriatal systems, and b) CCK has a mode of action more comparable to "atypical" rather than classical neuroleptic agents.

181.6 "ATYPICAL" NEUROLEPTIC-LIKE PROPERTIES OF CCK IN A ROTATIONAL-BEHAVIOR MODEL OF DOPAMINE FUNCTION
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A fragment of the gastrointestinal peptide, cholecystokinin (CCK-8), has recently been identified to coexist with the putative transmitter, dopamine (DA), within a subset of mesencephalic DA neurons. Although the functional nature of this CCK-DA coexistence remains uncertain, a growing body of behavioral data suggest that CCK may have antagonist/neuroleptic-like effects on DA neurotransmission. For example, CCK has been reported to have antipsychotic activity in humans and neuroleptic effects in animals (i.e. to delay acquisition, and facilitate extinction of active and passive avoidance behaviors, to suppress exploratory behavior, to reduce responding for rewarding brain stimulation, and to antagonize amphetamine (AMP)-induced hyperlocomotion). However, the neuroleptic-like actions of CCK do not appear to extend to cataleptogenic potency nor to antagonism of AMP-induced stereotypy. In our own work, CCK reversed the hyperlocomotion induced by low doses of AMP but potentiated the stereotypy resulting from higher doses. These results are similar to those observed with the "atypical" neuroleptics (e.g. clozapine) and might suggest that CCK and atypical neuroleptics only weakly influence behaviors mediated by the dorsal striatum (catalepsy, stereotypy), but more strongly inhibit behaviors mediated by the nucleus accumbens (hyperlocomotion). In the present study we compared the DA antagonist actions of CCK, haloperidol (HAL), & clozapine (CLZ) in a rotational model involving unilateral striatal infusions in AMP pretreated rats.

Rats received unilateral intrastriatal infusions of either CCK (2,4,8ug), CLZ (5,20ug), or HAL (5ug) 15 min after s.c. injection of d-AMP (1mg/kg). The animals were then placed into rotational chambers where the number & direction of 360° turns were recorded automatically every 5 min for 1 h. As others have reported, HAL produced strong and almost exclusive ipsilateral circling. In contrast, CLZ & CCK produced relatively weak circling which was highly variable in direction. These data suggest that CLZ & CCK only weakly affect AMP-stimulated DA neurotransmission in the striatum. The reported neuroleptic-like actions of CCK may, therefore, be more comparable to "atypical" rather than typical antipsychotic compounds.

181.7 CHOLECYSTOKININ (CCK) POTENTIATES THE RATE-DECREASING EFFECTS OF MORPHINE ON SCHEDULE-CONTROLLED LICKING IN RATS.
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CCK, a brain-gut peptide, has previously been shown to antagonize the effects of morphine on a variety of measures, such as analgesia (Faris et al., Science, 219:310, 1983) and food intake (Morley, J.E., Life Sci., 30:479, 1982). However, the ability of CCK to antagonize the effects of morphine on schedule-controlled responding has not been demonstrated. The present research examined the interaction of CCK with cumulative doses of morphine on operant licking. As a comparison the prototype opioid antagonist, naltrexone, was also given in combination with morphine. In one component of a two-component multiple schedule, rats (23 hrs. water deprived) were required to respond in the presence of a white stimulus light by licking a dry tube. Responding was reinforced under a fixed-ratio 50 (FR 50) schedule of water presentation, i.e., 2.5 seconds access to water at another tube. In the other component responding had no programmed consequences (timeout). Each session consisted of four 10-min timeout components alternating with four 10-min FR components. Cumulative dose-effect curves for morphine were obtained by giving an injection before each of the four FR components; successive injections increased the cumulative dose in equally spaced logarithmic steps. In general, as the cumulative dose of morphine increased (3.2-18 mg/kg, i.p.), the overall response rate decreased. In two of three subjects CCK alone (10-32 ug/kg, i.p.), produced little or no effect, except small rate-decreasing effects in the first FR component. When CCK was given in combination with morphine, however, the morphine dose-effect curve tended to shift to the left. In contrast, after pretreatment with naltrexone (1 mg/kg, i.p.), these same doses of morphine produced little or no effect on the overall rate of responding. Increasing the cumulative doses of morphine (18-75 mg/kg) indicated that naltrexone shifted the dose-effect curve approximately 3/4 log-unit to the right. Accordingly, these results would indicate that CCK does not interact with morphine in the same way as naltrexone when operant licking is the behavioral measure.

181.8 ATYPICAL NEUROLEPTIC-LIKE EFFECTS OF NEUROTENSIN ON APOMORPHINE INDUCED BEHAVIORS.
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The examination of the effects of drugs on behaviors induced by both low and high doses of apomorphine constitutes a simple and reliable procedure to detect potential neuroleptics and differentiate between typical and so-called atypical antipsychotics (Protais et al., Eur. Pharm., 94: 271, 1983). Whereas behaviors induced with small doses of the dopamine agonist are antagonized by both typical and atypical neuroleptics, stereotypy produced by high doses of the dopamine agonist is markedly decreased only by typical antipsychotics.

Many behavioral and neuropharmacological effects of neurotensin resemble more those of atypical than typical neuroleptics (Jolicoeur et al., Hdbk. Neurochem. 8:93, 1985). Although neurotensin has been shown to be ineffective in reducing stereotypy produced by dopamine agonists, to our knowledge, the effect of this peptide on behaviors induced by low doses of apomorphine has not been examined. In the present study, the effects of neurotensin on yawning and penile erections induced by 100 µg apomorphine were investigated. The following doses of the peptide were administered intracerebroventricularly 30 min prior to apomorphine injections: 0.9, 1.87, 3.75 and 30 µg. Number of yawns and the presence of erections were monitored for 1 hr following apomorphine administration. Results indicate that apomorphine induced yawning was significantly decreased with 1.87 µg neurotensin and totally abolished with 3.75 and 30 µg of the peptide. Also, no penile erections were observed in animals treated with the two higher doses of the peptide. In a parallel experiment, none of the above doses of neurotensin significantly affected stereotyped sniffing induced by 600 µg of apomorphine, confirming previous similar observation.

Together, these results lend support to the suggestion that the profile of neuropharmacological effects of neurotensin is more akin to that of atypical neuroleptics. Furthermore, since yawning and penile erections induced by low doses of apomorphine are generally attributed to stimulation of dopaminergic autoreceptors, whereas stereotypy is though to be mediated by activation of post-synaptic dopaminergic receptors, the results of the present study constitute behavioral evidence that neurotensin may have a selective inhibitory action on dopamine autoreceptors. Supported by the Medical Research Council of Canada.

181.9 NEUROPEPTIDE Y (NPY)-INDUCED FEEDING: COMPARISON WITH RAT PANCREATIC POLYPEPTIDE (rPP), HUMAN NPY, AND PEPTIDE YY (PYY) IN MALE AND FEMALE RATS. J. T. Clark* and S.P. Kalra (SPON. E. Smith), Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL 32610

Porcine Neuropeptide Y (pNPY), a 36 amino acid tyrosine rich peptide which has been localized in various hypothalamic and extrahypothalamic sites and co-exists with adrenergic transmitters, is a potent stimulator of feeding activity in satiated, ovariectomized rats (Clark et al, Endocrinol, 115:427, 1984). In the present study, we have compared the feeding response induced by various members of the pancreatic polypeptide family in intact male and female rats. Intraventricular (Ivt) injections of pNPY during the final hours of the light period induced feeding in a dose-related fashion. While the lowest dose tested (0.1 µg) was without effect, higher doses (0.5, 2 and 10 µg) uniformly elicited feeding with a latency of 12-15 min in male rats. With the most effective dose, 2 µg (0.47 nM), the increased food intake was due to an increased local eating rate, and not due to an increased time spent eating. In contrast, the pattern of feeding behavior seen after rPP was quite different and less impressive. During the first hour, only one dose of rPP (2 µg) evoked an increase in food intake due to an increased time spent eating. Further, the effects of pNPY (0.5 and 2 µg) on food intake were significantly greater during the dark phase than during the light phase. Interestingly, increased food intake in nocturnal tests (4 h) was due solely to augmented intake during the first 60 min after pNPY injection, the increased response gradually dissipated to return to control levels between 60 and 240 min. We found pNPY equally effective in intact cycling female rats (2 µg = 2.33 ± 0.28 g/60 min). As in males, the increased food intake was due to increased local eating rate. On the other hand, hNPY or PYY was about half as effective as pNPY in stimulating feeding in female rats and the increased food intake was due to increased time spent eating. These results show that (1) although all members of the pancreatic polypeptide family so far tested stimulate feeding to a varying degree, pNPY was uniformly most effective in male and female rats and (2) either single, as in hNPY (Met^{17} NPY), or multiple amino acid substitutions, as in rPP and PYY, decreased appetetive effects. Thus, these findings are in line with the proposal that NPY, or an NPY-like peptide, discharged in critical areas of the rat brain may normally induce feeding behavior in rats. (Supported by NIH HD 08634 and a postdoctoral fellowship HD 06660).

181.10 VENTRAL TEGMENTAL AREA INJECTION OF DIFFERENT NEUROPEPTIDES : DIFFERENTIAL EFFECTS ON FEEDING AND OPERANT BEHAVIOR. A.E. Kelley*, M. Cador*, L. Stinus* and M. Le Moal. INSERM U-259 Psychobiologie des Comportements Adaptatifs, rue Camille Saint-Saëns, 33077 Bordeaux, France.

The ventral tegmental area (VTA) contains the dopaminergic A10 (DA-A10) neurons which innervate various forebrain limbic regions. The VTA also has a high distribution of the peptides neurotensin (NT), substance P (SP), and enkephalin. Previous studies have shown that these peptides injected into VTA induce a psychomotor stimulation which is dependent on the activation of the DA-A10 neurons. However, little is known about the effects of such peptide injections on consummatory behavior. The present study is divided into two parts; 1) Effects of VTA-peptide injections on feeding behavior, and 2) Effects of VTA-peptide injections on operant responding for food reward.

1) The behavioral consequences of these injections were studied in both food-deprived and non-deprived rats. During a 30-min test, the following parameters were measured : latency to eat, total food intake, food spillage and duration of eating. Similar measures were taken for drinking. In deprived rats SP(0.5, 3.0 µg) increased latency but did not affect other parameters, and had no effect in satiated rats. NT (0.5, 2.5 µg) increased latency and markedly reduced food consumption in deprived rats, and had no effect in non-deprived rats. DALA (0.1, 1.0 µg) enhanced feeding in both deprived and non-deprived animals.

2) The effects of VTA injections of NT (0.0175, 0.175, 0.5 µg) SP (0.1, 1.0, 3.0 µg), neurokinin α (NKA, 0.1, 1.0, 3.0) and DALA (0.01, 0.1, 1.0 µg) were assessed on fixed-interval (FI) responding for food reward. Stable baseline responding and the typical FI scallop were obtained on an FI-40 sec schedule. The index of quarter life was taken as an indication of the temporal pattern of responding. NT induced a dose-dependent reduction in responding without affecting quarter-life, and reduced the number of reinforcements taken. SP and NKA did not affect the level of responding, although responding on the non-reinforced lever was augmented by both peptides. DALA induced a dose-dependent increase in responding which was rate-dependent, and reduced the quarter-life. DALA effects were similar to the classic pattern of responding observed after amphetamine.

These results indicate that although all these peptides elicit behavioral activation and may affect DA neuronal activity, the bahavioral responses can be differentiated with respect to feeding and operant behavior. It is possible that the endogenous peptides modulate the DA systems differentially. The findings are discussed in terms of both motor and motivational mechanisms.

NEUROPEPTIDES AND BEHAVIOR II

182.1 **CHRONIC DESMETHYLIMIPRAMINE BUT NOT LITHIUM MODIFYS LOCOMOTOR RESPONSE TO CORTICOTROPIN RELEASING FACTOR.** **C.L.Ehlers*,R.I. Chaplin, G.F. Koob (SPON:A.J.Mandell) Div. Neuroscience, Res. Inst. Scripps Clinic, La Jolla, CA 92037**

Recent studies have emphasized a role for Corticotropin Releasing Factor (CRF) in the etiology of affective disorder. Increased levels of CRF in the spinal fluid, blunted ACTH response to IV infusion of CRF, and non-suppression of cortisol to dexamethasone administration have all been reported in certain groups of depressed patients. Since these abnormalities in the hypothalamic-pituitary-adrenal system are frequently normalized following successful drug treatment, we sought to investigate whether chronic lithium (LI) or desmethylimipramine (DMI) would modify locomotor response to intracerebroventricular (ICV) administration of CRF in animals.

Thirty-six male Wistar rats were implanted with stainless steel ICV cannula and two weeks later were randomly assigned to one of four treatment groups: LI diet (n=12),Control diet (n=9),daily DMI injections (n=7) (10mg/kg), or daily Water injections (n=8). After 21 days of Lithium or control diet, and eighteen days of DMI or water injections one half of the rats received ICV injections of 0.15 nMols of CRF(ovine) in 5 microliters of saline or saline alone. Five days later the rats who initially received saline got CRF and visa versa. Locomotion was measured following the injections for 3 hours in computer automated cages equipped with two red photocell beams. Repeated measures ANOVA revealed that ICV CRF as opposed to saline injections, produced a significant increase in locomotion over the three hour period in all 4 groups (SAL VS CRF group effect: LI DIET,$p<.001$; C DIET,$p<.001$;DMI,$p<.01$; WATER,$p<.02$).Additionally the pattern of locomotor response to CRF was significantly modified by chronic DMI treatment but not by the control injections (ANOVA group time $p<.05$) with DMI treated rats displaying a more prolonged locomotor response to CRF. However no differences in amplitude or pattern of response to CRF were seen in chronic lithium treated rats as compared to rats on control diet. These studies suggest that the clinical effectiveness of DMI, but not lithium, may be partially enacted through systems responsive to CRF.**(supported by AA06059 and the Mac Arthur foundation)**

182.2 CENTRAL ADMINISTRATION OF CRF DELAYS THE ONSET OF MATERNAL BEHAVIOR AND INCREASES PUP-KILLING IN OVARIAN STEROID-TREATED RATS. A.J. Prange, Jr., J.D. Caldwell*, P.J. Brooks* and C.A. Pedersen. (SPON: D.L. Evans). Department of Psychiatry, Biological Sciences Research Center and the Neurobiology Curriculum, University of North Carolina at Chapel Hill, NC 27514

Corticotropin-releasing factor (CRF) has been reported to inhibit sexual and feeding behavior. Several investigators have observed that maternal behavior (MB) in rats is suppressed by stress. Since CRF has been shown to be released under certain stressful conditions, we have hypothesized that this neuropeptide may play some role in stress-inhibition of MB.

Virgin Sprague Dawley rats (200-250 gm) from Zivic Miller Laboratories were ovariectomized (OVXed). Eight days after OVX a Silastic capsule one cm in length containing 8.8 mg of 17 β-estradiol (E) was implanted SC in each animal. Ten days after OVX one long (90 mm) Silastic capsule containing 120 mg of progesterone (P) was implanted SC in each animal. Twenty days after OVX and 24 hrs before introduction of pups, P implants were removed from each animal. One hr prior to introduction of pups CRF (0.5, 1.0, 2.0, or 4.0 µg in 10 µl of normal saline) or normal saline (NS) vehicle alone were infused ICV. Immediately after ICV infusions, three 1-5 day old rat pups were placed in the cage of each animal. An observer, ignorant of ICV treatment, then looked for and recorded MB over the first six hrs and the 25th hr of pup contact.

Over the first two hours of pup contact, the incidence of full maternal behavior (FMB) was significantly lower in animals receiving 1 µg (4/10), 2 µg (3/10) or 4 µg (2/12) CRF compared to animals receiving NS (27/32); $p < .01$, 1 µg vs NS; $p < .003$, 2 µg vs NS; $p < .0001$, 4 µg vs NS; (Fisher's exact probability test). The incidence of FMB after 0.5 µg CRF (8/14) was not significantly different compared to animals receiving NS. By the end of the sixth hr of pup contact, animals receiving 1, 2 or 4 µg of CRF still had significantly lower cumulative incidences of FMB than animals receiving NS. At the end of the 25th hr of pup contact, there were no differences between CRF and NS recipients in cumulative incidences of FMB. Animals receiving 1, 2 or 4 µg of CRF displayed a significantly greater incidence of killing in the first two hrs of pup contact compared to control animals receiving NS (10/32 vs 1/32, $p < .003$).

We conclude that CRF inhibits the ovarian steroid-induced onset of MB and promotes infanticide. Our results are consistent with the hypothesis that endogenous CRF may contribute to the suppression of MB observed under stressful conditions.

182.3 CORTICOTROPIN-RELEASING FACTOR (CRF) POTENTIATES ACOUSTIC STARTLE REFLEX[1] (ASR) IN RATS:[2] BLOCKADE BY[3] CHLORDIAZEPOXIDE[4]. N.R. Swerdlow*, M.A. Geyer[2], W.W. Vale[3], and G.F. Koob[4]. [1]Dept Neurosci, M.S.T. Program, School of Medicine, U.C.S.D., La Jolla, CA 92093, [2]Dept Psychiatry, U.C.S.D., La Jolla, CA 92093, [3]The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037 and [4]Div Neurosci and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

CRF is a 41 amino-acid polypeptide distributed throughout the CNS that has been shown to have potent neurotropic properties and to stimulate the release of ACTH and beta-endorphin from the anterior pituitary. Interestingly, CRF acts within the CNS to stimulate numerous behavioral changes in the rat that mimic behaviors normally exhibited during conditions of high stress. The purpose of the present study was to examine the behavioral properties of CRF using a reflex response - the ASR - that is known to be sensitive to states of stress or fear.

The ASR is an easily quantified contraction of skeletal musculature in response to an intense acoustic stimulus. In rats, the ASR has been shown to be sensitive to states of stress or fear, since ASR amplitude is enhanced when the acoustic stimulus is presented during another stimulus (light, for example) that has previously been paired with shock. This 'fear-potentiated' startle is attenuated by drugs, including benzodiazepines, that have anxiolytic properties in humans.

In the first experiment, ASR amplitude was measured in rats (n=32) following icv administration of 0, 0.1, 1.0 or 10.0 ug rCRF. ASR amplitude was significantly potentiated by the 1.0 ug dose. In a second experiment, ASR amplitude was measured in rats (n=36) given 1.0 ug rCRF icv following pretreatment with the anxiolytic chlordiazepoxide (CDP: 0, 2.5, 5.0, 10.0 mg/kg ip). CRF-enhanced ASR was decreased in a dose-dependent manner by CDP; this effect was statistically reliable at the 2.5 mg/kg dose of CDP. In a third experiment, this dose of CDP produced no significant decrease in ASR potentiated by d-amphetamine (2.5 mg/kg sc; n=12) or strychnine (0.75 mg/kg ip; n=12).

Our results indicate that CRF, like stress, potentiates ASR amplitude; this effect of CRF is reversed by the anxiolytic CDP at doses of CDP that do not decrease amphetamine- or strychnine-potentiated ASR. CRF may serve as a critical neural substrate of stress-enhanced acoustic startle.

182.4 EFFECTS OF SYNTHETIC ACTH(4-9)-ANALOGUES ON DIFFERENT LEARNING TASKS IN MICE AND RATS. F.J. Hock, H.J. Kruse* and H.J. Gerhards*. HOECHST AG, D-6230 Frankfurt/M. 80, FRG

Considerable interest has developed over the past several years in the functions of neuropeptides in behavioral processes. There is now a large body of evidence, based largely on the work of deWIED and his colleagues, that ACTH and its related neuropeptides modulate learning behavior. ACTH and several of its analogues have been reported to facilitate one-way active avoidance acquisition and to attenuate ECS-induced amnesia. Hoe 427 and S 82 5972 A are ACTH(4-9) analogues selected from a group of ACTH(4-9) analogues because of their potency in behavioral tests.

(1) Inhibitory (passive) avoidance. Mice were treated 60 min prior to training with Hoe 427, S 82 5972 A, or saline. The animals were trained in a through-shaped straight alley runway with a guillotine door separating a light (L) and dark (D) compartment. Each mouse was placed in the L side facing away from the door. The door was opened and when it stepped through to the D side, a 1 mA, 1 s FS was delivered. Animals were given either electroshock (ECS, 25 mA, 200 ms) immediately after training or they received scopolamine (SCO, 3 mg/kg, i.p.) 5 min prior to training to produce amnesia. The minimal effective dose (MED) for attenuating ECS amnesia was 0.03 µg/kg s.c. for S 82 5972 A and 0.1 µg/kg s.c. for Hoe 427. For SCO amnesia, the MED was 0.3 µg/kg s.c. for S 82 5972 A and 0.03 µg/kg s.c. for Hoe 427.

(2) One-way Shuttle Box. Rats were socially deprived for 5 weeks before training. The animals were trained on 5 consecutive days in an active avoidance paradigm. Each rat was placed on a grid and the onset of shock was signalled by a 10 sec light conditioned stimulus (CS). The rat could escape or avoid the shock by jumping onto the platform. The animals were given 10 trials each day with a 50 sec intertrial interval. No drug was given on the first day; on days 2 - 5, rats were treated with Hoe 427 or saline 60 min prior to training. The number of avoidances was measured for each day. A dose of 10 µg/kg s.c. Hoe 427 was effective in facilitating acquisition of this task.

(3) 8-arm Radial Maze. Rats were trained in an 8-arm radial maze (STÄUBLI et al., Beh. Neur. Biol. 40, 58, 1984) to find a food pellet. After this task was well learned, a delay was introduced between the 4th and 5th choice of the trial. On the 5th day of training with a delay, the animals received Hoe 427 or saline 60 min before the start of the 1st trial. In addition, the animals received an amnestic dose of SCO (0.5 mg/kg, s.c.) 30 min before the delay interval. Two doses of Hoe 427 tested, 3 and 10 µg/kg s.c., antagonized the SCO-induced amnesia: On days 6 - 8 the Hoe 427 treated groups made significantly fewer errors than the control group. By day 8, the performance of the control group had not returned to the pre-SCO treatment level.

182.5 INHIBITORY ACTION OF MSH ON LORDOSIS: POSSIBLE SEROTONERGIC MEDIATION. L.H. Raible* and B.B. Gorzalka*(SPON: J.P.J. Pinel). Dept. Psychol., Univ. British Columbia, Vancouver, B.C., Canada V6T 1W5.

Research in our laboratory has indicated that centrally administered alpha-melanocyte stimulating hormone (MSH) inhibits sexual receptivity in rats (as measured by the lordosis quotient; LQ = lordosis/mount x 100). Work by Leonard et al. (J. Neurosci. Res., 2: 39, 1976) and Root-Bernstein & Westall (Brain Res. Bull., 12: 425, 1984) suggests that MSH and serotonin (5-HT) may interact physiologically. In addition, recent evidence indicates that serotonin type II receptor (5-HT_2) activity facilitates receptivity (Mendelson & Gorzalka, Pharmacol. Biochem. Behav., in press, 1985). The current experiments were designed to determine if the inhibitory action of MSH is due, in part, to an MSH-induced decrease in 5-HT_2 receptor activity.

In Experiment 1, the ability of quipazine (3 mg/kg, ip), a 5-HT_2 agonist, to attenuate the inhibitory action of MSH (200 ng/4 µl, icv) was examined. Ovariectomized estrogen and progesterone primed Sprague-Dawley rats were assigned to one of four groups: saline + saline; saline + quipazine; MSH + saline; MSH + quipazine. Results indicated that mean receptivity in the MSH group was significantly lower than mean receptivity in the remaining three groups, which did not differ from each other. In Experiment 2, subthreshold doses of MSH (20 ng/4 µl, icv) and of pirenperone (10 µg/kg, ip), a 5-HT_2 antagonist, were administered alone or in combination to determine if the two would summate to produce an inhibitory effect. The four groups were as follows: saline + saline; saline + pirenperone; MSH + saline; MSH + pirenperone. Results indicated that the mean LQ for the MSH + pirenperone group was significantly lower than the mean LQs for the remaining three groups, which did not differ from each other. Experiment 3 was designed to determine if the inhibition observed in Experiment 2 could be reversed by quipazine. The four groups were as follows: saline + saline + saline; saline + saline + quipazine; MSH + pirenperone + saline; MSH + pirenperone + quipazine. The doses of MSH and pirenperone used were those employed in Experiment 2 while the dose of quipazine used was that employed in Experiment 1. Results indicated that the mean LQ for the MSH + pirenperone + saline group was significantly lower than the mean LQ for the MSH + pirenperone + quipazine group, which did not differ from the mean LQ for the saline only group.

These results suggest that the inhibitory action of MSH on receptivity is mediated, at least in part, by an MSH-induced decrease in 5-HT_2 activity.

182.6 CENTRALLY ADMINISTERED RAT HYPOTHALAMIC GROWTH HORMONE-RELEASING FACTOR STIMULATES FOOD INTAKE IN FREE-FEEDING RATS F.J. Vaccarino, D. Feifel*, J. Rivier[1], W. Vale[1], and G.F. Koob[2]. Dept. of Psychology, Univ. of Toronto, 100 St. George Street, Toronto, Ont., Canada M5S 1A1; [1]Peptide Bio. Lab., Salk Inst. for Biol. Stud., La Jolla, CA; [2]Scripps Clinic and Res. Fdn., La Jolla, CA.

Recent findings from our laboratory (*Nature*, 314:6007, 1985) suggest that rat hypothalamic growth hormone-releasing factor (rhGRF) can stimulate food intake in hungry rats. We found that intracerebroventricular (icv) microinjections of rhGRF in pmole doses increased food intake in food-deprived rats. Since peripheral injections of rhGRF or growth hormone did not influence food intake, these findings were taken to suggest that rhGRF may be acting centrally to stimulate food intake. While these results demonstrate that central injections of rhGRF can stimulate food intake in food-deprived rats, the extent to which rhGRF stimulates food intake in non-deprived rats is unknown. Thus, the present study examined the effects of central rhGRF microinjections on food intake in rats with food and water made available ad libitum.

The food (Purina Rat Chow) and water of adult male Wistar rats with unilateral cannula implants aimed at the left or right lateral ventricle was measured for a 90 minute period (9:00-10:30 AM) on five consecutive days. On the sixth day rats were tested for food and water intake following icv microinjections of one of the following rhGRF doses: 0.0, 0.4, 4.0 or 40.0 pmoles. RhGRF was administered in a 2 µl volume of 0.01% ascorbic acid vehicle with a 30 gauge injector using gravitational force.

The results demonstrate that icv microinjections of rhGRF produce increases in food intake. The mean percent baseline food intake ± SEM following icv microinjections of 0.0 pmoles (vehicle), 0.4 pmoles, 4.0 pmoles and 40.0 pmoles rhGRF was 86 ± 7, 130 ± 17, 169 ± 37 and 133 ± 16, respectively. No significant effect of rhGRF on water intake was observed. There were no significant differences in baseline food intake across the four groups tested. The present results demonstrate that in addition to stimulating food intake in food-deprived rats, rhGRF can stimulate food intake in free-feeding rats. Based on the finding that neither growth hormone nor rhGRF influence food intake following peripheral administration, the present rhGRF-induced increase in food intake may reflect a direct central action of rhGRF. This further suggests that central and peripheral functions of rhGRF may collectively underly growth processes in an integrated manner. This research was supported by a grant from the Life Sciences Division at the University of Toronto.

182.7 **INTRAHIPPOCAMPAL INJECTION OF CYSTEAMINE DEPLETES SOMATOSTATIN AND PRODUCES COGNITIVE IMPAIRMENTS IN THE RAT.** T. J. Walsh*, D. F. Emerich*, A. Winocur, C. Banki*, G. Bissette and C. B. Nemeroff (SPON: A. L. Riley). Univ. North Carolina School of Medicine, Chapel Hill, NC, 27514, Univ. Pennsylvania Sch. Medicine and Duke University Medical School.

Somatostatin (SRIF) is a tetradecapeptide which is widely distributed in the mammalian CNS. While the role of SRIF in the inhibition of growth hormone release is well established its' potential non-endocrine functions remain uncertain. Recently, several lines of evidence indicate that SRIF modulates learning and memory processes. For example, SRIF attenuates the amnestic effects of ECS (Vecsei et al., Peptides 4, 293, 1983). Furthermore, the concentrations of SRIF are reduced in the hippocampus (HPC), and other brain regions, in neuropsychiatric disorders which are characterized by cognitive impairments (Davies and Terry, Neurobiol. Aging, 2, 9,1983). The studies presented here sought to further examine the functional role of SRIF in the HPC.

Preliminary studies revealed that 30 ug of cysteamine bilaterally injected into the HPC produced a significant decrease of SRIF content (55 %) in this structure 72 hrs following surgery. The content of met-enkephalin in the HPC was not affected by this regimen. The concentrations of SRIF in the olfactory tubercles, striatum, frontal cortex and hypothalamus (HYP) were not affected.

A second series of studies examined the behavioral effects produced by intrahippocampal injections of cysteamine. Male Fischer rats bilaterally injected with cysteamine (30 ug site) 72 hrs prior to training exhibited impaired retention of a passive avoidance response. Initial step-through latencies during the training trial and sensitivity to footshock were not affected by cysteamine. Thus, the retention deficit probably represents an impairment in the acquisition or retention of the task. In contrast, systemic administration of cysteamine (200 mg/kg, i.p.) reduced SRIF content only in the HYP and produced no cognitive deficits. Intrahippocampal cysteamine also produced a persistent decrease in locomotor activity. This finding is in contrast to numerous studies showing that manipulation of the HPC proper, its subfields, and its cholinergic afferents reliably increases motor activity. Another unexpected observation was that intrahippocampal cysteamine produced a significant increase (72 %) in the concentration of TRH in the HPC.

The data presented here suggest that (1) cysteamine produces a regionally specific decrease of SRIF following intracerebral injection, (2) decreases of SRIF in the HPC are associated with cognitive deficits, (3) the decreases of SRIF observed in Alzheimer's Disease probably have clinical significance, (4) there might be important interactions between SRIF and TRH in the HPC, and (5) cysteamine is a useful tool for exploring the neurobiology of SRIF.

182.8 BEHAVIORAL CHANGES FOLLOWING CENTRAL INJECTION OF CYSTEAMINE IN RATS. C. Bakhit and N. R. Swerdlow, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Recent studies of the peptide somatostatin (SS) in the CNS have documented its presence, calcium-dependent release, high-affinity binding, excitatory or inhibitory effects on cortical and hippocampal neural activity, and behavioral potency when administered intracerebroventricularly (icv). Altered SS activity has been implicated in the pathophysiology of several CNS disorders, including Alzheimer's disease. In the present study, we utilized the SS depleting agent cysteamine (CYS) to examine the effects of CNS SS depletion on locomotor activity and passive avoidance behavior in rats.

Male Wistar rats (n=51) were surgically implanted with icv cannulae. Each rat was injected daily for four days with either 0, 250 or 350 ug CYS icv. One day later, one group of rats was decapitated, and cortex (CX), hippocampus (HC) and hypothalamus (HT) were removed for analysis of tissue SS-14 levels. A second group of rats was tested for their levels of locomotor activity three days following the final CYS injection using automated photocell cages. Beginning one day later, these rats were trained on two consecutive days in a simple runway passive avoidance paradigm; testing took place one day after training. Following completion of testing, these animals were decapitated, and regional SS-14 levels were measured as above.

SS-14 levels in CX, HC and HT showed a significant dose-dependent decrease one day following four daily icv CYS injections. Following completion of behavioral testing, however, SS-14 levels were recovered in CX and HT in animals treated with 250 ug CYS; SS-14 levels in HC were actually elevated in these animals. Levels in CX and HC in animals treated with 350 ug CYS recovered to control levels in CX and HC by this time, but remained depressed in HT.

Photocell activity was significantly elevated in rats treated with 250 ug CYS, but not 350 ug CYS, compared to controls (0 ug). In contrast, passive avoidance performance was significantly impaired in rats treated with 350 ug CYS, but not 250 ug CYS, compared to controls. Our results suggest that changes in CNS SS-14 activity may disrupt central substrates of complicated behaviors such as locomotor activity and passive avoidance. Supported by NIAAA grants AA07273 and AA03504 and NIH NRSA PHSGM 07198-10 to NRS.

182.9 SUBSTANCE P ANALOGUE ANTAGONISTS ACT AS SUBSTANCE P AGONISTS IN THE RAT SEPTUM. J.R. Zucker*, H. Lai* and A. Horita* (SPON: R. Haschke). Depts. of Anesthesiology, Pharmacology and Psychiatry, Univ. of Washington, Seattle, WA 98195

Intraseptal microinjection of Substance P (SP) has been shown to reduce the hippocampal turnover rate of acetylcholine (Wood et al, Neuroscience 4:1479, 1979). In the present study, we micro-injected saline, SP, and two analogues of SP ([D-Arg^{1}, D-Pro^{2},D-Trp^{7,9}, Leu^{11}]-SP and [D-Pro^{2}, D-Trp^{7,9},]-SP) into the septum of rats pretreated with pentobarbital and measured the duration of loss of righting reflex and change in choline uptake into hippocampal synaptosomes. Synaptosomal choline uptake from a medium containing 0.3 µM choline chloride and 0.4 µCi of [^{3}H] choline was performed for 4 minutes at 38°C. Nonspecific uptake was from an identical medium containing 2 µM hemicholinium-3 and was approximately 55% of total uptake. Uptake into hippocampal synaptosomes from unanesthetized rats was 31.1 (SEM±1.0) pM choline/4 min/mg protein.

Compared with microinjection of saline, all three drugs potentiated pentobarbital narcosis. Additionally the pentobarbital-induced reduction (27%) of hippocampal synaptosomal choline uptake was potentiated (44-56%) by all three drugs. There was a negative correlation ($r=-0.96$, $p<0.02$) between the durations of loss of righting reflex and hippocampal synaptosomal choline uptake. These data suggest that pentobarbital narcosis may be inversely related to septal-hippocampal cholinergic activity. Significantly, the two analogues of SP that were tested in the septum appear to be acting as SP agonists at that site, in their ability both to reduce septal-hippocampal cholinergic activity and to potentiate pentobarbital narcosis. This is in contrast to their reported action as SP antagonists in various peripheral tissues.

182.10 **PROLACTIN INDUCES YAWNING IN YOUNG ADULT MALE RATS.** N.J.Laping* and V.D.Ramirez (SPON:J.Chan). Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Previously we reported that prolactin (PRL) infused directly into the caudate nucleus of freely moving rats was capable of activating dopaminergic terminals and in some cases yawning behavior was observed (Chen, J.C. et al., In International Congress on Prolactin, 1984, p. 161). In this report we examined the effect of systemic PRL on yawning behavior. First, we confirmed that in our laboratory and under our conditions, apomorphine (APO) induces yawning in young adult male rats at doses where it is considered to be a dopamine antagonist (binding to dopamine autoreceptors). Also, we have found that these animals yawn more in the afternoon at 16:00h (28 yawns/60 min) than at 10:00h (16/60 min) in response to APO (100 µg/kg body weight).

Herein we report that a subcutaneous injection of low doses of ovine prolactin (oPRL) can induce yawning in young adult male rats (n=8 per group). The most effective doses were 0.25 and 2.5 µg/kg body weight (5 and 4.5 yawns/60 min at 10:00h vs 0.3 in control animals). Doses of 0.025, 0.05, 25 and 250 µg/kg were less effective. The number of yawns per hour were 0.6, 3.0, 2.6 and 0.6 respectively. In addition, grooming behavior was observed with the higher doses of oPRL. Interestingly, yawning in response to oPRL changes over the course of one circadian cycle with the highest number of yawns occurring at 16:00h (11 yawns/80 min). The other times tested, 6:00h, 10:00h and 24:00h showed a mean response of 4, 4.5 and 8 yawns per 80 min respectively. A control group received 0.25 µg/kg boiled oPRL at 16:00h and showed a mean of 2 yawns per 80 min. The average onset of yawning began 37 minutes after the oPRL injection whereas with APO the latency of the response was 12 min at 10:00h. Also, the latency for both oPRL and and APO treatments shift to a shorter time; 23 min and 6 min respectively in the afternoon at 16:00h. This shift was only significant for the oPRL treated animals.

These results indicate that oPRL in addition to other hypophysial peptides such as ACTH and MSH can stimulate yawning. It is proposed that PRL after initial activation of the nigrostriatal dopamine system secondarily induces yawning by inhibiting this system via a negative feedback mechanism using autoreceptors. This may explain the longer latency of ovine prolactin to induce yawning as compared to apomorphine.

182.11 NEUROTENSIN AND 3,3'-DIMETHYLPROLINE-TRH (D-TRH): EFFECTS ON SPONTANEOUS BEHAVIOR AND REINFORCEMENT PROCESSES IN RATS. G. Meisenberg, W.H. Simmons* and Y. Sayeed*. Dep. of Biochem., Ross Univ. Med. Sch., P.O. Box 266, Roseau, Dominica (West Indies) and Dep. of Biochem., Loyola Univ. Med. Sch., Maywood, IL 60153.

After intracerebroventricular (i.c.v.) injection in rats, neurotensin (NT), tested at doses of 0.46, 1.86, 7.5 and 30 μg, significantly reduced locomotor activity (7.5 and 30 μg) and grooming behavior (0.46 and 30 μg) in the open field when determined 10-15 minutes after the injection. D-TRH, tested at doses of 0.063, 0.25, 1.0 and 4.0 μg, reduced locomotion (1.0 μg) and rearing (0.25, 1.0 and 4.0 μg) and induced wet-dog shakes (0.25, 1.0, 4.0 μg). NT (0.46-30 μg) did not significantly change responding for electrical self-stimulation of the lateral hypothalamus (square wave, bidirectional pulse-pairs, 100 Hz, train duration 0.2 seconds) when determined 10-20 minutes after i.c.v. administration. D-TRH significantly suppressed responding at 0.25, 1.0 and 4.0 μg. After administration of NT (1.86 and 7.5 μg) and D-TRH (0.063 and 0.25 μg) in the ipsi- or contralateral nucleus accumbens, self-stimulation was increased after ipsilateral injection of 0.063 μg D-TRH and decreased after ipsilateral injection of 0.25 μg D-TRH. All other treatments were ineffective. In the conditioned place-preference paradigm, both 4.0 μg of D-TRH and 30 μg of NT did not induce significant preference or aversion for the treatment-compartment, suggesting a lack of pronounced rewarding or aversive properties or ambivalent motivational effects at these doses. These data suggest that NT does not specifically attenuate reinforcement processes at the level of the nucleus accumbens while low doses of intra-accumbens D-TRH cause enhancement of self-stimulation and high doses of D-TRH reduce self-stimulation. Both peptides did not show significant aversive properties, suggesting that peptide-induced reductions of motor activity and the induction of wet-dog shakes, respectively, are not secondary to peptide-induced aversion.

182.12 THYROTROPIN RELEASING HORMONE (TRH) REMAINS ELEVATED IN LIMBIC REGIONS FOR 1-2 WEEKS FOLLOWING ELECTROCONVULSIVE SHOCK (ECS). A. Sattin, T.G. Hill*, J.L. Meyerhoff and M.J. Kubek, Depts. of Psychiat. and Anat., Indiana U. Sch. Med. and VAMC, Indianapolis, IN 46223; Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, DC 20012.

We have previously reported large increases in TRH content of specific limbic and cortical regions of rat brain 2 days following 3-5 alternate-day ECS (Life Sci. 34, 1149-52, 1984; 36; 315-20, 1985). This phenomenon is not elicited by subconvulsive shock and a single ECS elicits a smaller effect only in pyriform cortex (Pyr). This effect was seen only in Pyr, hippocampus (HC), amygdala (Ay) and posterior cortex (P.Ctx). Several other limbic regions and frontal Ctx were unaffected.

Tonic-clonic seizures were induced in male S-D rats (150-180g) with 35-60 millicoulombs (60 Hz a.c.). Sham ECS rats received identical handling but no current. Sham rats (n=12) and ECS rats (n=8) received either 1 or 3 treatments on alternate days, and were sacrificed 2, 6 and 12 days later. Brains were removed immediately, dissected, weighed and frozen on solid CO_2. TRH was assayed by specific RIA following HAc extraction and results expressed as pg/mg tissue (mean ± SEM). Student t-tests (2-tailed) were performed following log transformation of original data.

Significant increases in TRH were seen two days after 1 ECS in HC (6.03+0.34 vs. 10.51+0.91, $P < .001$) and Pyr (3.83+0.34 vs. 10.30+1.18, $P < .001$) where it remained elevated for 6 days (9.29 +0.64, $P < .001$ and 7.30+0.67, $P < .001$, respectively). Two and 6 days after 3 alternate-day ECS, TRH was increased in HC (16.63 +1.30, $P < .001$; 8.58+0.66, $P < .005$), Pyr (30.09+2.17, $P < .001$; 14.59+1.87, $P < .001$) and Ay (42.77+1.65, $P < .001$; 29.19+1.86, $P < .02$). By 12 days TRH remained significantly elevated only in Pyr (5.60+0.29, $P < .005$). In P.Ctx the ECS effect on TRH lasted only 2 days after 3 seizures (2.36+0.32 vs. 3.14+0.29, $P < .01$) and no significant effects were seen in striatum, anterior Ctx + olfactory tubercle or hypothalamus with either 1 or 3 ECS.

These data confirm our previous results showing a greater increase in TRH after 3 ECS than after 1 ECS. Those differences now appear to be reflected in the greater magnitude and duration of the effect in Pyr than in HC, Ay and Ctx. The results suggest a possible long-term neuromodulatory role for TRH neurons and/or their processes in HC, Ay and Pyr, especially in Pyr where both TRH receptors and TRH metabolism are high. These data also support a role for endogenous TRH as antiepileptic and/or antidepressant, although the mechanism(s) responsible for such changes in TRH in specific CNS regions remain unknown. (VA Res. Serv. and AM 28260).

NEUROPEPTIDES AND BEHAVIOR: VASOPRESSIN

183.1 **MICROINJECTION OF KAINIC ACID INTO THE MEDIAL PREOPTIC AREA OF GOLDEN HAMSTERS INHIBITS VASOPRESSIN-DEPENDENT FLANK-MARKING BEHAVIOR** C.F. Ferris* D. Meenan,* J. Pollock,* H.E. Albers (SPON: P. Grigg). Department of Physiology, University of Massachusetts Medical Center, Worcester MA. 01605.

Scent-marking, a form of olfactory communication, is a complex behavior exhibited by many animals in which urine, feces, sweat or glandular secretion is disseminated in the environment. Golden hamsters scent-mark by rubbing specialized glands, flank glands, against vertical objects. This behavior, called flank-marking, can normally be elicited by odors or aggressive encounters from other hamsters. We have previously reported that microinjection of arginine vasopressin (AVP) into the medial preoptic area (MPOA) of hamsters triggers flank-marking (Ferris et al., Science 224:521, 1984) and that microinjection of a specific AVP antagonist into the same site blocks both the effect of injected AVP as well as flank-marking elicited by odors from other hamsters (Ferris et al., Neurosci. Lett., in press). The present studies investigated whether the neurotoxin, kainic acid, microinjected into the MPOA could inhibit flank-marking behavior. Male hamsters were anesthetized and implanted with guide cannulae stereotaxically aimed at the MPOA. The correct placement of the guide cannulae was tested by microinjecting animals with 0.1 ng of AVP in a volume of 10 nanoliters. Animals responding to AVP were then tested on the following night for flank-marking behavior elicited by placing the hamster into the recently vacated home cage of another hamster. On a subsequent day animals were microinjected with 0.2 μg of kainic acid in a volume of 20 nanoliters or a vehicle control of 1 M NaOH. Four days later animals were again tested for odor-elicited flank-marking behavior. The odor-elicited flank marks observed over a 10 min period prior to these injections was 7.0±.83 and 9.0±1.14 for the kainic acid (n=5) and control (n=5) groups, respectively. Following the microinjections there was a significant ($p<.01$) decrease in the number of flank-marks observed in the kainic acid group (1.8±1.1) while flank-marking remained unchanged in the control group (9.0±2.24). When tested with AVP, animals treated with kainic acid flank-marked significanly fewer times ($p<.01$) than animals treated with the vehicle control (6.0±5.0 and 41.2±13.8, respectively). Microinjection of kainic acid (0.2ug/20nl) into the 3rd ventricle (n=3) and other sites in the hypothalamus (n=4) did not inhibit the odor-elicited flank-marking. These data indicate that the discrete microinjection of kainic acid into the area of the MPOA, responsible for the expression of flank-marking behavior in golden hamsters, can inhibit flank-marking behavior elicited by placing a hamster into the recently vacated home cage of another hamster. (Supported by HD-18022 and GM-34798).

183.2 DIFFERENTIAL PAVLOVIAN CARDIAC CONDITIONING FOLLOWING ANALOGS OF VASOPRESSIN AND ACTH. James D. Valentine* and Linda L. Hernandez. WJB Dorn VA Hospital, and University of S.C., Columbia, SC 29201

Previous work has shown that both des-Gly-Arg^9-vasopressin (DGAVP) and ACTH4-10 enhance bradycardiac orienting responses in rabbits. Furthermore, both ACTH4-10 and Asu-Arg-vasopressin delay disappearance of heart-rate conditioned responses (HR CRs) when administered before extinction of simple conditioning. Since DGAVP and ACTH4-10 have little, if any, peripheral endocrine activity, these effects probably occur via central mechanisms. The present experiment examined the effects of DGAVP and ACTH4-10 on discriminative HR CRs in rabbits, when administered both before initial acquisition and before extinction testing.

Rabbits were subjected to differential Pavlovian conditioning using 2-sec tones as conditional stimuli (CSs) and a 500 msec paraorbital shock as the unconditioned stimulus (US); one tone (CS+) was followed by shock and one tone (CS-) was not. Four consecutive daily sessions were conducted. Session 1 consisted of 60 acquisition (paired CS-US) trials. Sessions 2 and 3 consisted of 30 acquisition, followed by 30 extinction (CS-only) trials. Session 4 included 10 acquisition and then 50 extinction trials. Either saline, DGAVP (5 or 25 μg/kg, s.c.) or ACTH4-10 (50 or 250 μg/kg, s.c.) were administered 1 hour before both sessions 1 and 3.

The results showed that DGAVP had few effects during initial training compared to saline. However, the 5 μg/kg dose decreased bradycardiac HR CR magnitude during sessions 2 and 3, and both doses delayed the rate of deceleration during these sessions. During session 4, following no differential treatment of the groups, 25 μg/kg DGAVP increased the magnitude of the HR deceleration. In contrast, 50 μg/kg ACTH4-10 increased HR CR magnitude throughout initial training. The 250 μg/kg dose had a similar effect, but only early in session 1. Early in session 2, when no differential treatment had occurred, both doses of ACTH decreased the magnitude of the HR CR. In session 3, 50 μg/kg ACTH increased HR CR magnitude during early trials, whereas 250 μg/kg decreased HR CR magnitude throughout this session. Additionally, in sessions 3 and 4, 50 μg/kg ACTH increased the magnitude of the HR deceleration to the CS-.

Thus, DGAVP appears to delay extinction of discriminative HR CRs; this compound had few effects on initial acquisition but altered the topography of the HR CR during subsequent sessions. In contrast, ACTH4-10 enhanced HR CRs during initial training and thus may affect stimulus processing or attention functions. During later sessions the lower dose also appeared to disrupt the discrimination since it enhanced decelerations to the CS-.

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183.3 VASOPRESSIN REACTIVATES MEMORY AFTER HYPOTHERMIA INDUCED AMNESIA T. P. Tinius*, B. E. Beckwith, K. A. Tinius*, P. Traynor*, and N. Wagner* Psychol. Dept. Univ of North Dakota, Box 7187, Grand Forks, ND 58202.

Studies testing the effect of vasopressin on memory processes have often failed to use an established model of animal memory, such as the contextual cues theory (Spear, The processing of memories: Forgetting and retention, 1978), to explain how vasopressin influences memory. The present experiment was designed to assess whether vasopressin would reactivate memory at a 24 hr. retention interval when an amnestic treatment was administered after the learning trial. Sixty-two Holtzman albino rats were either pretrained or nonpretrained prior to the learning trial of a one trial step through passive avoidance task. Pretrained animals were placed in the dark chamber of the passive avoidance box for 2 minutes immediately followed by 1 trial to enter the dark chamber on day 1, and 2 additional traits to enter the dark chamber prior to the learning trial on day 2. The nonpretrained animals were placed in a clear plexiglass box for 2 min., taken out, and placed in the box for another 10 sec. on day 1, and then placed in the clear plexiglass box for two 10 sec. periods prior to the learning trial given in the passive avoidance chamber on day 2. Immediately after the learning trial all animals were immersed in ice water until their body temperature was reduced to 10 C. Twenty-three and one-half hours after the learning trial half of the pretrained animals and half of the nonpretrained animals were injected with Arginine Vasopressin (1 ug/.1 ml i.p.), while the remaining animals were injected with an equal volume of placebo. One-half hour later, all animals were given a single retention trial.

The results revealed that the pretrained animals treated with vasopressin had a marginally longer initial latency to enter the dark chamber than did the pretrained animals treated with placebo, a significantly longer latency than the nonpretrained animals treated with placebo, but not a significantly longer latency than the nonpretrained animals treated with vasopressin. The pretrained animals treated with vasopressin spent significantly more time on the safe side than all other groups. Thus, vasopressin reactivated memory only when the animals were pretrained to the apparatus.

Pretraining may establish inactive contextual cues which are different from contextual cues established at the learning trial and disrupted by the amnestic treatment. Vasopressin administered prior to the test trial appears to reactivate the cues established during pretraining which in turn reactivates memory at the test trial. The results also suggest that the peptide hormones adrenocorticotropin (ACTH) and vasopressin reactivate memory after an amnestic treatment through different mechanisms of memory retrieval.

183.4 EFFECTS OF VASOPRESSIN ON CRF-INDUCED BEHAVIOR. D.R. Britton, M. Varela*, A. Garcia*, M. Rosenthal*. Dept. of Physiology, Univ. of New Mexico Sch. of Medicine, Albuquerque, N.M. 87131

Arginine vasopressin (AVP) has been known for some time to have common effects with CRF_{1-41}. Both peptides release ACTH from pituitary cells in vitro and in vivo. More recently it has been shown that intracerebral ventricular (icv) administration of AVP decreases the release of endogenous CRF into the hypophyseal portal circulatoin (Plotsky et al., 1985). Both CRF and AVP are most highly concentrated not only in the same brain regions, but are co-localized in some of the same neurons (Roth et al., 1982; Swanson et al., 1984). In addition, both CRF and AVP have been shown to prolong extinction of a conditioned avoidance response. On the other hand, they appear to have some effects which are antagonistic to one another. CRF, when administered to animals in their home cages appears to have arrousing or stress-like properties (Britton et al., 1982, 1984; Sutton et al., 1982). On the other hand, AVP has been reported to have sedative like properties (Krejci et al., 1979).

Given the relationship between these two peptides, we tested animals which had been given a standard behaviorally active dose of 0.5 ug of CRF icv and co-treated with AVP either icv or sc. Animals given CRF alone displayed typical CRF home cage behavior. They showed a decreased food consumption following a 24 hr. fast. They had increased grooming and increased locomotion. Animals treated with AVP alone demonstated a dose related (up to 10 ug/Kg, s.c.) primary effect of sedation. There were no significant changes in amount of grooming. There was a slight decrease in the amount of time spent eating and an increase in the number of observations of inactivity. The combination of CRF and AVP resulted in an increase in the number of inactive periods compared to either CRF alone or AVP alone. Given the previous demonstrations of hypertension produced independently by both of these treatments, it is possible that the observed decrease in activity with combined treatment reflects additive effects on blood pressure.

183.5 INDUCTION OF GROOMING BY NEUROHYPOPHYSEAL NONAPEPTIDES AND ANALOGUES: BLOCKADE WITH PROSTAGLANDIN SYNTHESIS INHIBITORS J.D. Caldwell*, C.A. Pedersen, F. Drago#**, V.J. Hruby+**, and A.J. Prange, Jr. Biological Sciences Research Center and the Neurobiology Curriculum, Department of Psychiatry, University of North Carolina, Chapel Hill, NC; #Institute of Pharmacology, University of Catania, Catania, Italy; +University of Arizona, Department of Biochemistry, Tucson, AR

We have demonstrated that oxytocin (OXY) injected intracerebroventricularly (ICV) increased grooming behavior in both male and female rats. We now report the comparative grooming behavioral efficacy and potency of OXY, arginine vasopressin (AVP), arginine-vasotocin (AVT) and various analogues of OXY after ICV administration. AVP and AVT both demonstrated inverted U dose-response relationships across a dose range of 0.05 - 5 µg while OXY followed a linear dose-response curve. Behavior was measured as the number of 15 second intervals in which body or paw licking or scratching occurred over a 30 min observation period beginning 25 mins after ICV infusion of peptides. Maximum grooming scores following AVP and AVT injections, while not significantly greater than for OXY, were seen at doses of 0.5 and 0.1 µg respectively; whereas maximum grooming resulted after injection of 10 µg OXY. At the 0.1 and 0.5 µg doses AVT and AVP induced significantly ($p < .05$) more grooming than equal doses of OXY (Bonferroni's comparisons). We concluded from these data that the neural substrate mediating grooming is more sensitive to AVT and AVP than OXY. We then compared the grooming effectiveness of 1 µg OXY to equimolar doses of several analogues of OXY with various uterotonic potencies. Deamino-oxytocin, which is almost twice as potent as OXY in inducing uterine contraction, was slightly but not significantly less potent than OXY in inducing grooming. [deamino-4-homoglutamine]-OXY, which has 20% of the uterotonic potency of OXY, displayed 70% of the potency of OXY at this dose. Analogues oxypressin, [9-decarboxyamido]-OXY, [deamino-7-alanine]-OXY, and [3-cyclohexylalanine]-OXY did not significantly enhance grooming. It appears from these data that for some OXY analogues their uterotonic potency does not parallel their grooming-inducing potency. Injections of either of the prostaglandin synthesis inhibitors indomethacin (5 mg/kg) or acetylsalicyclic acid (50 mg/kg) ip thirty minutes before ICV injections significantly ($p < .05$) blocked the excessive grooming resulting from 1 µg OXY (Tukey's comparisons). From these data we suggest that OXY may induce grooming behaviors by increasing prostaglandin activity.

183.6 OSMOTIC STRESS MIMICS EFFECTS OF VASOPRESSIN ON INHIBITORY AVOIDANCE TASK. C.J. Lebrun, T.L. Wall, M. Le Moal* and G.F. Koob. (SPON: B. Will). Div. Preclin. Neurosci. Endocrin., Scripps Clin. and Res. Fdn., La Jolla, CA; *INSERM, Unite 259, Bordeaux, France.

Arginine vasopressin (AVP) or antidiuretic hormone (ADH), has been hypothesized to have a role in the retention of learned responses in addition to its clinical physiological functions of water retention and modulation of blood pressure. However the relationship of the behavioral effects produced by exogenously administered AVP and the behavioral function, if any, of endogenously released AVP have never been evaluated. The purpose of the present study was to determine if a potent peripheral osmotic stimulus, the intraperitoneal injection (IP) of hypertonic saline, at doses known to release AVP both centrally and peripherally produce behavioral effects similar to those produced by exogenously administered AVP. To test this hypothesis, we examined the effects of peripheral injections of AVP and hypertonic saline (NACL) on the retention of an inhibitory avoidance task (Martinez et al., Physiol Behav. 19: 139-149 (1977). On Day 1, the rat was placed in the shock compartment for 2 min, and then given a pretraining trial in which the rat is placed in the start area, and allowed to step through into the shock compartment without receiving shock. On Day 2, two more pretraining trials were given followed by an acquisition trial, during which the rat received a shock. Immediately following the learning trial, the rats were injected either subcutaneously with AVP (2 µg/rat; 4 µg/rat) or intraperitoneally with NACL (0.25M; 0.5M; 1M). A retention test was given 24 hours later. The results showed (see Table) that an interoceptive osmotic stimulus (hypertonic saline) known to release AVP can mimic the effects of exogenous AVP on learned behavior. We also report that the facilitation of retention of the inhibitory task induced by this osmotic stimulus (NACL 1M) is reversed by pretreatment with the AVP pressor antagonist [1 deaminopenicillamine 2- (O-methyl) tyrosine arginine vasopressin] (dPtyr(Me)AVP) suggesting that endogeneously released AVP may also produce behavioral effects. Table mean (± SEM) latency to enter shock compartment 24 hours after training.

SAL	AVP 2 ug*	AVP 4 ug*	NACL 0.25M	0.50M*	1.0M*
79(15,05)	188(35,85)	160(40,28)	143(37,31)	231(32,91)	237(33,93)
n=44	n=10	n=10	n=11	n=11	n=11

*significantly different from saline $p < 0.05$

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183.7 KINDLING IN SPONTANEOUS HYPERTENSIVE RATS AND WISTAR KYOTO NORMAL RATS. R.S. Greenwood, H.C. Sullivan*, and K. Winstead*. Dept. of Neurol. and Neurobiology Prg., UNC Sch. of Med., Chapel Hill, NC 27514.

Recent studies have suggested that vasopressin may have a role in the generation of some types of seizures. Intraventricularly administered vasopressin has been reported to cause seizures that can be blocked by a vasopressin receptor blocker. Amygdala but not pyriform cortex kindling rates are slower in rats deficient in vasopressin, homozygous Brattleboro rats, compared to Long-Evans controls. Amygdala kindling rates in animals with elevated levels of vasopressin have not been studied. The spontaneous hypertensive (SH) rat has higher plasma and urinary excretion of vasopressin than the genetically related Wistar Kyoto normal (WKYN) rat. In vitro hypothalamic slices from SH rats show greater release of vasopressin than slices from WKYN rats. In the present study we compared the kindling rates and characteristics of SH rats and WKYN rats.

Bipolar stimulating electrodes were implanted in the right amygdala of SH and WKYN rats. After one week, daily kindling stimulation (1 sec train, 60 cps, 400 uA) was begun. Kindling was continued until the animal had reached 3 consecutive stage 5 seizures.

Kindled seizures in SH rats and WKYN rats were not behaviorally different at any kindling stage . Afterdischarge (AD) duration was significantly shorter in SH rats at the start of kindling but not when stage 5 seizures had been achieved. SH rats required significantly fewer stimulations and fewer AD than WKYN rats to reach stage 2, stage 3, stage 4, and stage 5 seizures.

The more rapid rate of kindling in SH rats and the shorter duration of AD in SH rats at the start of kindling is consistent with the hypothesis that vasopressin may play an important role in amygdala kindling.

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STRUCTURE AND FUNCTION OF IDENTIFIED CELLS II

184.1 APPARENT MEMBRANE PROPERTIES OF THE MAUTHNER CELL'S LATERAL DENDRITE DEPEND STRONGLY UPON ELECTRICALLY COUPLED AXONS: A HYPOTHESIS BASED ON CABLE THEORY. W.D.Crank* (Spon: S.B.Udin), Div. Neurobiology, Dept. Physiology, SUNYAB, Buffalo, NY 14214.

The goldfish Mauthner (M-) cell has a low input resistance (<200 k-ohm) and a fast time constant (T≅0.4msec) when measured at the soma. These properties have been used to estimate membrane specific resistance, R_M=200ohm-cm^2, and specific capacitance, C_M=2.0uF-cm^{-2} (Crank & Faber, Neurosci. Abstr. 1983). On the lateral dendrite (LD), about 250-500um from the soma, numerous eighth nerve axons are electrotonically coupled to the M-cell by gap junctions. Does the coupling of axons to the LD influence its apparent membrane properties? I have used cable theory to investigate this possibility. Axons are treated as membrane cylinders resistively coupled to an R-C representation of LD membrane, which is assumed isopotential.

This cable model shows that subsequent to instantaneous charging of LD membrane, and after rapid redistribution of charge to coupled axons, voltage decay is ultimately governed by an effective membrane time constant (T_E) having a magnitude between the limits set by the membrane time constant of the M-cell (T_M) and that of the axons coupled to it (T_A). Assuming T_M=1.0msec and T_A=0.20msec (R_M=10^{3}ohm-cm^2; C_M=1.0uF-cm^{-2}; and input resistance of a single axon, R_A=10^{7}ohm), the weighting of M-cell and axonal influences on T_E depends on gap junctional resistance, R_J, of a single axon and the density, n, of axons coupled to LD membrane surface. When R_J=10^7 ohm and n=one axon per 667 um^2, computations yield T_E=0.230 msec and effective membrane resistivity, R_E=118ohm-cm^2. Effective specific capacitance is inferred as: C_E=T_E/R_E=1.95uF-cm^{-2}. When the influence of axons is reduced by increasing R_J to 3x10^{7}ohm, results are: T_E=0.261msec, R_E=210 ohm-cm^2, and C_E=1.24uF-cm^{-2}. These examples reveal that while a membrane may have one set of intrinsic properties (e.g. R_M=10^{3}ohm-cm^2, C_M=1.0uF-cm^{-2}), coupling axons to it can lead to quite different apparent properties--decreased specific resistance, increased specific capacitance, and more rapid time constant--which resemble those estimated for the M-cell. Furthermore, the balance of influences depends on strength of electrotonic coupling.

In summary, the apparent electrical properties of LD membrane are predicted to be influenced in a major way by coupled eighth nerve axons. Because the region of eighth nerve innervation of the M-cell is displaced from the soma, axonal coupling may only partially account for properties measured there.

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184.2 COMPUTER SIMULATIONS OF CABLE PARAMETERS PREDICT SIMPLE STRUCTURE-FUNCTION RELATIONSHIPS FOR FROG MYELINATED AXONS. P.G. Funch and A. Belanger*, Div. Neurobiology, Dept. Physiology, SUNY at Buffalo and Biomedical Computing Unit, Yale University School of Medicine, New Haven, CT.

Detailed biophysical data have been obtained using voltage clamp techniques on either nodes of Ranvier or internodes of frog peripheral myelinated fibers (Stämpfli, R.F. Adv. Neurol. 31:11, 1981). However, to date little data exist on the cable parameters of these axons (space constant, λ; time constant, T_m; input resistance, R_{in}) which are required for understanding current flow during action potential propagation. Thus, a distributed electrical model of a myelinated axon was analyzed using NEUROS (P. Guthrie, NIH), a modification of the electrical circuit analysis computer program SPICE, which was suggested by Shepherd and Brayton (Brain Res. 175:377, 1979) for application to neural modelling of arbitrary systems of compartments and physiological properties. Each of 8 internodes was divided into 5 identical parallel RC compartments; each node was a single compartment. The two ends of the "axon" were terminated by resistances ($2R_{in}$) and capacitances ($T_m/2R_{in}$) which simulated semi-infinite cable extensions. Using the electrical and morphological parameters for a typical 14 µm diameter fiber (D) (Stämpfli, ibid.), the model yielded R_{in} = 22 Mohm, λ = 0.320 cm, and T_m = 160 µsec.

Cable theory predicts the following size dependencies for unmyelinated axons: $\lambda \propto d^{1/2}$; $R_{in} \propto d^{-3/2}$; and T_m = constant. There are no corresponding theoretical predictions for myelinated axons. Thus, in order to assess them, the 10 µm diameter axon (d) was scaled to 2 and 4 µm using the following observed structural relationships: 1) internodal length (L) $\propto$ d; 2) Nodal length = 1.27 µm (i.e., nodal area = 4d); and 3) Number of myelin lamellae $\propto$ d (i.e., d/D=constant). This scaling procedure maintained constant ratios of: (total internodal)/(nodal) membrane resistance = 2.55; and (total internodal)/(nodal) capacitance = 2.62. With these structural constraints, the model demonstrated that the following simple predictions hold: $\lambda \propto d$; $R_{in} \propto d^{-1}$; and T_m = constant (e.g., for d = 4 µm, R_{in} = 55.2 Mohm, λ = 0.128 cm, and T_m = 160 µsec). Interestingly, these results confirm Tasaki's observation (Amer. J. Physiol. 127:211, 1939) that an action potential (120 mV) can passively decrement across two successive inactivated nodes (by a factor of $e^{-(3L/\lambda)}$ = 0.153), and yet still be sufficiently large (18mV) to bring the third node to threshold. More generally, it appears that the cable properties of some myelinated axons may have simple dependencies on size which, if experimentally verified, should facilitate our understanding of their functioning.

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184.3 **ACTIVE AND PASSIVE PROPAGATION IN INHOMOGENEOUS AXONS: THEORETICAL AND SERIAL EM STUDIES OF VARICOSE UNMYELINATED NERVES.** S. A. Ellias*, M.Greenberg*, and J. K. Stevens. Neurology Service, Mass. Gen. Hosp., Boston Mass., 02114, and Playfair Neurosci. Unit, Toronto Western Hospital, Ontario, Canada.

Complete computer assisted serial E.M. reconstructions of over 70 small unmyelinated rat sciatic nerves reveal that most are actually non-uniform and highly varicose, with diameters ranging between 0.2 microns and 2.0 microns within a single axon. This study analyzes the effects of these inhomogeneities on the "average space constant" and the average conduction velocity in varicose axons. The passive properties are obtained by integrating a generalized ricatti differential equation for a "unidirectional local space constant", l(x). The "average space constant" is defined as the reciprocal of the spatial average of the reciprocal of l(x). The average velocity of propagation of action potentials is calculated separately by numerically integrating Hodgkin Huxley equations using a predictor-corrector method. We find that the average velocity is proportional to the average space constant in varicose axons. This linearity is consistent with the finding that local velocity was proportional to a local space constant in axons with a special class of flare or taper (S. S. Goldstein and W. Rall, Biophys. J., 14:731 1974). Thus, the linear relation between velocity and space constant for axon cylinders may be generalized to inhomogeneous structures under certain assumptions. The dependence of this conclusion upon channel density and the average spatial period of the inhomogeneities will be discussed. In simulations using sinusoidal modulation about a mean radius, the average space constant and velocity were found to be 80% of the velocity of the mean cylinder and 12.5% higher than that of the smallest inside cylinder. In simulations using radii obtained directly from reconstructed unmyelinated rat sciatic nerve, the average space constant and velocity were always close to those expected for the smallest neck (not the larger varicosity) for all but the closest spacings of varicosities. This suggests that simple measurement of axonal diameter can not be used as a predictor of conduction velocity. Moreover, we believe that these findings may have important implications to small fiber neuropathies.

184.4 **HIGHLY IRREGULAR SHAPES OF NORMAL TYPE C AXONS: SERIAL EM STUDY**
Michael Greenberg[1], John Stevens[2], and Samuel Ellias*[3], 1&2) Playfair Neuroscience Unit, Toronto Western Hospital, 1) Department of Anatomy, and Division of Neurosurgery, 2) Department of Physiology, University of Toronto, Toronto, Canada, 3) Department of Neurology, Massachusettes General Hospital, Boston

We have performed serial EM reconstruction of 5-micron length segments of 79 mouse sciatic axons and 20 retinal ganglion cell axons to determine if axons follow rules of shape-generation similar to those described in dendrites (Sasaki, et al, J Cell Biol 98:1279, 1984). Morphometry of axonal cross-sections throughout the length of each reconstruction shows that most axons are highly varicose structures. As a first-order index to variability, the ratio of each axon's maximum cross-sectional area to its minimum is provided below (note: max/min=1 describes a cylinder).

(n=99)	Max/Min	Percentage of Axons
	1	10%
greater than or equal to	2	90%
greater than or equal to	4	25%
greater than or equal to	8	10%

This tabulation indicates that the majority of axons are non-uniform cylinders, with a substantial percentage varying in cross-section areas by factors greater than four. Serial EM reconstruction of the contents of the axons revealed that the regions of axonal expansions, or varicosities, contained organelles, especially mitochondria.

We conclude that (1) at least the type C axons are highly varicose, and consequently one cannot be certain of the dimensions of an axon from single-section measurements. (2) Since mitochondria, as well as other organelles, have been observed to be moving (Allen, et al, Science, 218:1127, 1982), axonal varicosities associated with mitochondria move also; this suggests that axons at any given point along their lengths may vary their cross-sectional area in time. (3) Ellias, Greenberg, Stevens (this session) report that varicosities have important functional consequences upon electrical properties of axons. (4) The presence of organelle-associated varicosities could provide mechanisms to explain certain neuropathies.

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184.5 A COMPUTER-ASSISTED VIDEO TECHNIQUE FOR PREPARING PICTURES OF INTRACELLULARLY FILLED, WHOLE-MOUNTED NEURONS IN THE CRICKET.
D.G. Tieman* and R.K. Murphey (SPON: S.B. Tieman). Neurobiology Research Center, State University of New York, Albany, NY 12222.

We have developed a computer-assisted video technique for preparing images of cobalt-chloride impregnated, whole-mounted neurons in the cricket. The primary advantages of the technique are its rapidity and accuracy as a method for gathering, storing and depicting anatomical data. The technique employs optical sections of the whole mount and can produce high resolution photographs with essentially infinite depth-of-field. The depth information available in the optical sections makes the creation of stereo pairs relatively simple.

The technique employs a real-time 512 x 512 digitizing frame store (INTELLECT 100), an LSI-11/23 computer, a 32M byte hard disk and software that we have developed. A TV camera is attached to a microscope and successive "optical sections" are stored digitally as the focussing depth is systematically changed. For the cercal ganglion of the cricket, we routinely store an area of about 500um x 500um with 10-20 optical sections sampled at 16um intervals. After storage, each optical section is feature-enhanced to emphasize elements that are sharply focussed. The final two-dimensional image is generated from the "stack" of optical sections. For each of the 256K points in the final image, a pass is made through the corresponding points in the "stack" of optical sections and the blackest value is saved for use in the final image. For the normal image, the stack is traversed perpendicular to the plane of optical section; for the stereo pairs, the stack is traversed twice, once slanting left of perpendicular and once slanting right of perpendicular. Our procedure requires about 30 minutes of experimenter time to store the optical sections and then 2 to 3 hrs of computer time for enhancing and combining the optical sections. Thus, we have developed an easy and reliable method for demonstrating the 3-D organization of individual neurons in whole-mount preparations. The method is of further interest since it can be easily modified for use with sectioned material.

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184.6 NEUROANATOMICAL MODELING WITH CADCAM
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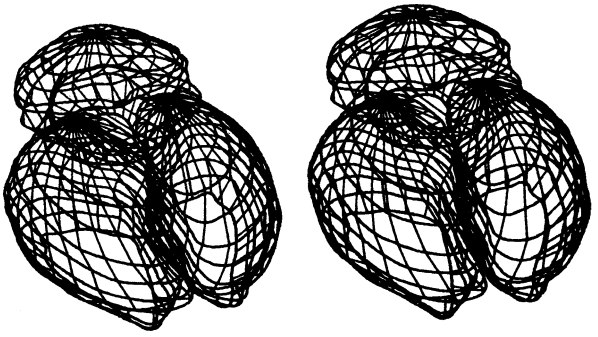
The increasing availability of programs for computer assisted design and modeling CAD-CAM has prompted an examination of the potential of these programs for making neuroanatomical models. Although originally designed for use with combinations of readily defined geometrical objects or primatives, more advanced versions have facilities for rendering models composed of elements with less regular shapes, such as those associated with biological forms. To demonstrate how programs of this type can be used for neuroanatomical modeling, solid 3-D models of a rat brain, and one of its major components, the hippocampal formation, have been made with the general surface (gsu) feature of Control Data's ICEM solid modeler. In addition to discussing the nature of the data required by the modeling program and describing the process by which this data is obtained from an neuroanatomical specimen, some of the features of the models that promise to be of value in neuroanatomical studies, such as stereo imaging of objects within objects and "slicing" in variable planes, will be illustrated.



184.7 MORPHOLOGICAL AND BIOCHEMICAL EVIDENCE FOR CATECHOLAMINERGIC ACTIVITY IN TISSUE CULTURE OF BRAIN STEM EXPLANTS. B.A. Bennett, M. Morris* and D.K. Sundberg*. Dept. of Physiology and Pharmacology, Bowman Gray Sch. Med., Winston-Salem, NC 27103.

Brain stem ascending and descending catecholaminergic systems are important in controlling endocrine and autonomic function. Areas A1 and A2 of the brain stem were used for tissue culture experiments because of the abundance of noradrenergic (NE) neurons which send input to hypothalamic regions. Both immunocytochemical localization and chromatographic/electrochemical measurement were used to determine the presence of the catecholamines and their synthetic enzymes. Microdissected explants were obtained from neonate rats (3-5 days) and maintained in tissue culture for 1 to 3 wks. For histology, the explants were fixed with acrolein and stained with rabbit anti-tyrosine hydroxylase (TH) or dopamine B hydroxylase (DBH). All tissues were processed using the Vectastain method and the material analyzed by light microscopy. After either 1 or 2 wks in culture, both A1 and A2 regions showed evidence of TH and DBH staining. The TH stain was much more intense with numerous cell bodies with processes present in the explant. The DBH stain was more diffuse, localized mainly in terminals in the explant.

Media and tissues were analyzed for catecholamine content using electrochemical detection after HPLC separation of alumina extracts. The results were similar for the 2 regions. Both media and tissue contained an amine which co-eluted on 2 chromatographic systems with dyhydroxyphenylethyl glycol (DHPG), a monoamine oxidase metabolite. Small amounts of dopamine (DA) were measureable, but NE was not detected. There was also an increase in the DHPG content with tissue culture (a 30 fold change from 1 to 3 wks).

Catecholamine biosynthesis was evaluated by determining the incorporation of ^{3}H-tyrosine (1 hr incubation) into the amines. There was significant incorporation of label into DA.

A model is described for the culture of specific brain stem regions. It is viable on the basis of several criteria: identification of catecholamine synthesizing cells, the presence of catecholamines in both tissue and media, and the ability to incorporate an amino acid precursor into DA. It may prove useful in the investigation of mechanisms controlling autonomic function.

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184.8 GABA AND 5HT IMMUNOREACTIVE NEURONS IN ASCARIS. Antony O.W. Stretton and Carl D. Johnson (SPON: N. Tumosa) Department of Zoology, University of Wisconsin, Madison, WI 53706.

We have previously shown using biochemical assays that acetylcholine synthesis is present in identified branches (commissures) of three classes of excitatory mototneurons (DE1,DE2 and DE3), but not in two classes of inhibitory motorneurons (DI and VI)(J. Neurosci.,in press). ACh is a likely transmitter of the excitatory motorneurons. There have been numerous suggestions that 4-aminobutyric acid (GABA), a compound that hyperpolarizes and relaxes Ascaris muscle cells, may be an inhibitory transmitter at nematode neuromuscular synapses. Consistent with this possibility, we have found that the VI and DI cells are stained with GABA specific antisera. Cell bodies and processes in the ventral nerve cord, commissures and processes in the dorsal nerve cord are all stained. One interesting feature, revealed by staining, is the presence on the inhibitory motorneuron commissures of a short longitudinal branch in the sublateral nerve cord. These branches are prominent in the first three segments, but are not present on VI or DI commissures posterior to the first VI in the fourth segment. Branches off the VI commissure are always in the dorsal sublateral nerve cord, whereas DI commissure branches are in the ventral sublateral nerve cord. Preliminary electrophysiological experiments (by J. Angstadt) suggest that the inhibitory motorneurons recieve excitatory synaptic input from sublateral neurons. In addition to these cord motorneurons, there are at least 10 cells in the ganglia surrounding the nerve ring, one cell in the tail and two cells in the pharynx which stain with the GABA antisera.

There are a bilaterally symmetrical pair of cells in the posterior pharynx which stain intensely with the antisera to serotonin (5HT). Each cell has short posterior branches and two long anterior processes which project forward to the level of the nerve ring where they end in a profusion of small branches. In the female no other cells stain. In males there are, in addition, 5 intensely staining cells in the posterior ventral nerve cord and a number of weakly staining (3 or 4 bilaterally symmetrical pairs) cells in the posterior lateral lines.

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184.9 EVOLUTIONARY PROGRESSION IN AN IDENTIFIED SYNAPTIC CONTACT. S.R. Shaw and I.A. Meinertzhagen. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

In principle, the evolution of a neural network could proceed either by the loss or gain of component neurons inherited from a common ancestor, or by neuronal respecification affecting synaptic connectivity. We have explored the latter possibility in homologous neurons of Diptera (flies), by tracing the connections within the synaptic modules, the cartridges, of the first optic neuropil, the lamina. The compound eye is claimed to have a monophyletic origin (Paulus 1979), suggesting that the lamina cartridges also should be homologous structures; their cellular composition is remarkably constant across different groups, adding further weight to this proposed homology.

The lamina cartridges have been analysed from electron microscopy of short (40-100) series of consecutive sections from 3 representative families of the phylogenetically older Nematocera, and 9 from the more advanced monophyletic suborder Brachycera. In the older families up to the Tabanidae (Brachycera), the photoreceptor terminals form equivalent populations of synaptic contacts, at each of which they are presynaptic to a pair of processes, one each from two axial monopolar cells (homologues of L1 and L2 in Musca). The Mecoptera, which share a common ancestor with Diptera, have the same configuration. The archetype of the synapse is thus dyadic. In the advanced brachyceran families above the Bombyliidae, in Hennig's (1973) taxonomic scheme, however, one extra neuronal process is added at each pole of the postsynaptic pair L1, L2. This tetradic configuration, hitherto the only one described, is thus a later, derived form of the synapse. The two polar elements stem from profiles which resemble, and may thus also be homologous with, α-processes of amacrine cells known in Musca. The transition from dyad to tetrad is not simply a flexibly derived advanced functional trait, for it is no more readily reversed than it was rapidly developed in evolution: flightless, parasitic advanced Brachycera retain the tetradic configuration. Evidence corroborating synaptic evolution comes from fine structure. Both the platform which surmounts the presynaptic ribbon at the tetrad, and the postsynaptic cisternae in L1 and L2 are also derived, advanced structures. Neither is present in lower Diptera, and both are poorly developed in families of intermediate position.

These trends suggest a gradual evolution of some features of this particular synapse, on a time scale which fossil evidence indicates to be about 200 MYr. Within the same period, a single quantum step in postsynaptic configuration has occurred once within the Brachycera, from dyad to tetrad. We believe that this is the first clearly documented case of the evolution of synaptic contacts.

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184.10 AXON SIZE DISTRIBUTION: EVOLUTIONARY VARIATION AMONG FLIES. A. J. Benson* and D. G. King (SPON: W. Yau). Dept. of Zoology and Dept. of Anatomy, Southern Illinois University, Carbondale, IL 62901.

Axon diameter is closely related to several functional properties including conduction velocity (or electrotonic decrement in passively conducting axons), synaptic divergence ratio, and current available for electrical transmission. Larger axons should be faster and better able to activate numerous postsynaptic neurons. But smaller axons may be more economical, with lower metabolic costs to support extensive axoplasm. Thus large axons might be expected in pathways where speed, individual reliability, or extensive synaptic output have selective advantage, while small axons might be favored where such factors have less adaptive significance. The most extreme examples of evolutionary specialization affecting individual axonal size are the phylogenetically diverse occurrences of giant fibers in startle-response pathways.

The cervical connective of flies encompasses several thousand axons whose cross-sectional areas can differ by more than three orders of magnitude. A common pattern for axon organization is revealed by the spacial arrangement of identifiable axons and tracts. These display a fair constancy of size and position which is apparent through bilateral symmetry within individuals, close similarity among individuals within a species, and less precise but still recognizable similarities among different species.

Most larger axons are concentrated in a dorsomedial tract, while smaller axons are located ventrally and laterally. The relative size of dorsal-tract axons varies widely among species, so that the proportion of the connective devoted to the 10 largest axons ranges from less than 5% to more than 30%. This proportion appears to be greatest among flies with exceptional flying skill such as asilids, bombyliids and syrphids. One pair of "giant" dorsomedial axons is found in most but not all cyclorrhaphans. One or two pairs of very large axons also occur ventrally in many species; occasionally these large ventral axons assume "giant" proportions. Many flies possess a coherent ventromedial bundle of very small (less than 0.5μm) axons. The number of axons in this bundle is quite variable; no such tract is apparent in several species of diverse taxonomic affinities.

These observations suggest that diversity in the dipteran ventral nervous system may provide an excellent opportunity to explore the genetic and developmental constraints which influence evolutionary differentiation of neuronal form, particularly axon diameter.

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184.11 BEHAVIORAL MUTATION *pas* CAUSES CYTOLOGICAL ALTERATIONS IN THE GIANT FIBER PATHWAY AND IN THE GUT OF *DROSOPHILA MELANOGASTER*. D. G. King, Department of Anatomy and Department of Zoology, Southern Illinois University, Carbondale, IL 62901.

The *Drosophila* giant fiber pathway includes cervical giant fibers (CGF), peripherally synapsing interneurons (PSI), and motor axons to the tergotrochanteral muscle (TTM) and the dorsal longitudinal muscle (DLM). In normal flies the CGF synapses with both PSI and TTM axons, while the PSI contacts both TTM and DLM axons. Synaptic transmission from CGF to TTM axon appears to be electrical while CGF transmission via PSI to DLM axons requires one chemical synapse (King & Wyman, *J. Neurocytol.* 9:753, 1980; Tanouye & Wyman, *J. Neurophysiol.* 44:405, 1980). In flies with the mutant genotype *pas*, the CGF is unable to drive action potentials in the DLM, while the latency for CGF activation of the TTM is increased. Motor axons to the TTM and DLM function normally when stimulated directly (Thomas & Wyman, *J. Neurosci.* 4:530, 1984).

Several cytological differences between wild-type and *pas* flies have now been observed. In the nervous system, the PSI can be identified by its chemical synapses onto DLM axons in both *pas* and wild-type flies, although its axon is much less conspicuous in *pas*. Membrane apposition between PSI and CGF and between PSI and TTM is reduced or absent in *pas*, while contact between CGF and TTM is at least as prominent in *pas* as in wild-type, with well-developed chemical synapses. In the cardia (an organ formed around the invagination of the esophagus into the anterior midgut), *pas* flies show enlargement of both short and tall secretory cells (which contribute to formation of the peritrophic membrane) and reduction of the clear zone near the distal limit of the foregut.

These observations confirm that *pas* does alter the connectivity of identifiable neurons. However, they also suggest that the action of this gene is not specific to these neurons, nor even to the nervous system, but involves some general cellular change that effects other organ systems as well. If one assumes that electrical transmission in the giant fiber pathway is mediated by gap junctions interconnecting the CGF, PSI and TTM axons, the neural alterations in *pas* could result from reduction or loss of these gap junctions. Certainly failure of effective short-latency transmission between CGF and DLM is consistent with this hypothesis. Increased latency of CGF to TTM transmission in *pas*, in spite of direct contact between the CGF and TTM axons, suggests that although electrical coupling between these axons might be eliminated in *pas*, the direct CGF-TTM synapse may still allow slower chemical transmission. Extensive gap junctions form a normal feature of gut epithelium in flies; whether alterations in these junctions may also provide a sufficient explanation for the observed modifications of the cardia in *pas* remains unknown.

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184.12 SEARCH FOR THE METACEREBRAL GIANT CELL IN DIVERSE GASTROPODS. R.P. Croll. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

A serotonergic, metacerebral giant cell (MCG) has previously been identified in diverse species of two sublasses of gastropods, the opisthobranchs and pulmonates, and it thus represents one of the best existing examples of putative cellular homologies. However, arguments for a common phylogenetic origin for these cells in different species cannot be based solely on similarities between cells in a few extant species. These similarities could be the result of convergent evolution rather than homology. Additional evidence for homology could come from a broader survey of gastropod species. In this way, one can examine whether the MCG is present in all species of opisthobranchs and pulmonates, whether species differences in feeding behaviour (in which the MCG is involved) are correlated with changes in the cell's form and function, and whether a possible phylogenetic origin for the cell can be identified.

In order to address these questions, I have started such a survey. The MCG, in addition to being serotonergic and bilaterally symmetrical, and having a very large soma, is charaterized by its axonal projection out of the cerebral ganglia down the cerebrobuccal connective (CBC). Based upon these characteristics, MCG's in different species can be tentatively identified on the basis of immunohistochemical staining for serotonin and colbalt "backfilling" of the CBC. Once identified, additional information about secondary axonal branching patterns is possible with silver intensification of intracellular cobalt injections. These techniques have now been applied to numerous species of opisthobranchs (including Aplysia californica and Hermissenda crassicornia), pulmonates (including Achatina fulica and Helix aspersa) and prosobranchs (including Littorina litorea and Crepidula fornicata).

Preliminary results confirm earlier studies suggesting that the occurrence of MCG's is generalized throughout the opisthobranchs and pulmonates, despite differences in feeding behaviour. Differences in secondary branching patterns have been noted. Attempts to locate the MCG in the third gastropod subclass, the prosobranchs, which have been hypothesized to represent the evolutionary origin of the opisthobranchs and the pulmonates, have not been successful. It appears that the serotonergic axons in the buccal ganglia and CBC of the prosobranchs arise from one or more clusters of cells in the cerebral ganglia rather than from a single large cell. The evolutionary significance of these findings will be discussed.

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184.13 MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF LAMINA GANGLION CELLS IN CRAYFISH. L.T. Wang-Bennett and R.M. Glantz. Department of Biology, Rice University, Houston, Texas 77005.

Intracellular labeling techniques employing either horseradish peroxidase or lucifer yellow were used to identify lamina ganglion cells physiologically and morphologically. The physiological properties of the penetrated cells: response waveform to light stimuli; receptive field; intensity-response function; and effect of current on light response, etc. were examined. The cell types were revealed following histochemical processing of the eye preparation. This study comprises the first account of the electrophysiological phenomena at the lamina ganglion in Crustacea.

Of the 50 cells studies, we have obtained data on several large to medium field cells (defined by the extent of dendritic spread on the lamina) such as: Tangential$_1$, (Tan$_1$), Tangential$_2$ (Tan$_2$), T-cell, multipolar cell and monopolar type 5 (M5), etc. (see Strausfeld and Nässel 1981 for terminology). Small field monopolar cells (M1-M4) exhibit similar physiological properties; thus, distinguishing among these cells relies upon morphological data.

A major physiological distinction can be made among monopolar cells based on the light response waveforms: nonspiking-hyperpolarization vs. spiking cells. The monopolar cells' hyperpolarizing light response has a transient and a plateau phase (sometimes accompanied by a depolarizing off response). Responses show 30-60% reduction due to lateral inhibition. In some cases the response reversed in polarity when the inhibitory fields were stimulated. The range of intensity-response function is 3.0 log units (s=+ 0.7, n=7). Response onset leads medullary sustaining fiber by 7.9 msec (n=9). Spiking monopolar cells have been recorded from the lamina ganglion. These cells exhibit depolarizing light response with an initial spike rate of 100 Hz.

Light responses of Tan$_1$ and Tan$_2$ are similar in waveform; the hyperpolarizing responses have no separate transient and plateau. Tan$_1$ has dendritic coverage of 151 µm (s=+ 68; n=4) which is accompanied by a 40° receptive field and 5 log units of intensity-response range. The dendrites of Tan$_2$ intersect the entire retinotopic projection; these cells exhibit a diversity of synaptic unitary events. Tan$_1$ and Tan$_2$ show strong spatial summation. This is in contrast to monopolar cells which show no sign of spatial summation. The cell body of Tan$_1$ is located in the medulla. Its axon runs centrifugally into the lamina where its terminals innervate the laminar cells. Tan$_2$ also has a cell body in the medulla and has very broad medullary and lamina lateral branches. The two branches are seen at two different focal planes. Laminar T-cell soma originates in the external chiasma. Spikes are recorded from these cells and its I-R function covers 3 log units.

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185.14 IDENTIFICATION OF PEPTIDERGIC NEURONS WITH MONOCLONAL ANTIBODIES RAISED AGAINST HOMOGENATES OF WHOLE CENTRAL NERVOUS SYSTEMS OF THE SNAIL LYMNAEA STAGNALIS. J. van Minnen*, H.H. Boer* and T.A. de Vlieger* (SPON: European Neuroscience Association). Dept. of Biology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

Monoclonal antibodies were raised against homogenates of whole central nervous systems (CNS) of the pond snail Lymnaea stagnalis. Selection of antibody producing hybridomas to neural antigens was carried out by staining sections of the CNS with supernatants of the cultures. About seventy percent of the hybridomas produced antibodies against antigens present in the CNS or in the perineurium. Approximately ten percent of the hybridomas were directed against neurons or groups of neurons. Certain antibodies stained selectively known neuroendocrine centres of the snail such as the Caudo-Dorsal Cells (ovulation hormone) and Light Green Cells (growth hormone) and the endocrine Dorsal Bodies, which produce a female gonadotropic hormone. A number of hybridoma cell lines produced antibodies against hitherto unidentified neurons.

It is argued that the antibodies were raised against biologically active peptides and/or their precursors and can be used for the purification of these peptides.

Some of the antibodies also stained neurons in other species (insects and vertebrates).

184.15 MORPHOMETRIC ANALYSIS OF HAMSTER FACIAL AND HYPOGLOSSAL NEURONS FOLLOWING CRUSH INJURY. T.E. Durica and S.K. Jacob. Rush Medical College, Department of Anatomy, Chicago, IL 60612.

Both the short and long term neuronal responses to a crush injury were examined for facial and hypoglossal neurons of the hamster. The right facial and hypoglossal nerves were crushed and the neurons were examined at 5, 30 and 60 days postoperative (dpo). The left side served as control and there were 5 animals for each postoperative time. Point count stereology was used to measure a variety of neuronal cellular components: these included cytoplasmic, nuclear, nucleolar, and total cell area. At 5 dpo, for both the facial and hypoglossal neurons, there was a significant increase in all the measured components which typifies the classical chromatolytic response in motoneurons. At 30 dpo, in both facial and hypoglossal neurons, all measured components were still larger than that for control neurons. However, the size of these components was decreased when compared to those of 5 dpo neurons. Observations on the animals' behavior indicated a functional return for both the facial and hypoglossal musculature at this time. At 60 dpo, for both facial and hypoglossal neurons, all neuronal components were still enlarged being similar in size to that seen at 30 dpo. The only noted difference was in the nucleus of the hypoglossal neurons which was increased in size and was similar to the size of the neuronal nucleus of the 5 dpo hypoglossal neuron. There was no significant loss of neurons at any postoperative time examined in either the facial or hypoglossal motoneuron population. These results show major changes in measured components that may indicate possible alterations in neuronal metabolism during recovery from crush injury. These observations show that crush injury not only produces a significant short term response but also elicits a significant long term response. The long term response suggests that the changes in neuronal architecture may be of a permanent nature even though a functional return has been seen after a relatively short time following crush injury.

184.16 **GENERATION OF ENRICHED POPULATIONS OF CULTURED PHOTORECEPTOR CELLS BY SELECTIVE NEURONAL DESTRUCTION.** L.E. Politi* and R. Adler (SPON: E. Adler-Graschinski). Wynn Center, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205, and INIBIBB, Bahia Blanca, Argentina.

A monolayer culture system for photoreceptor cells has been recently developed (Adler et al., J. Cell Biol. 99:1173-1178, 1984). Although glial, endothelial, fibroblastic and pigmented epithelial cells are not present in the cultures, biochemical analysis of these photoreceptors is complicated by the presence of other retinal neurons. We have now attempted to eliminate these neurons using kainic acid (KAc) and/or β-bungarotoxin (BBT), which do not affect photoreceptor cells but destroy specific retinal neuronal types (Morgan, Pr. Ret. Res. 2:249, 1982; Hirokawa, Br. Res. 250:309, 1982).

Initially, 8-day chick embryo neural retina cultures were treated for 24 hrs after 6 days in vitro. Both KAc and BBT were capable of destroying many process-bearing neurons in a concentration-dependent manner, and without causing any obvious changes in photoreceptors. Maximal KAc effects (neuronal loss=55-60%) were seen at ca 1.5 mM. BBT destroyed as many as 75% of the neurons at concentrations of 1 nM. This maximal neuronal loss could not be increased by combining KAc+BBT treatments. However, ongoing experiments indicate that combined KAc+BBT treatment results in maximal responses already after 2 hr, when neither KAc nor BBT alone approach the 24 hr level. Neuronal sensitivity to KAc and BBT appeared to be developmentally regulated. Thus, neuronal destruction by KAc was negligible after 2 days in vitro (div), detectable at 4 div, and maximal at 6-8 div.

In summary, then, treatments with KAc and/or BBT offer an important first step for the generation of enriched populations of cultured photoreceptor cells. Their integrity in treated cultures is now being further evaluated by EM, autoradiography and immunocytochemistry. Moreover, procedures are being sought to eliminate a population of small, non process-bearing round cells present in the cultures which are resistent to KAc and BBT.

Supported by Grant EY04859 and Core Grant EY07047. Dr. Politi is a fellow of the CONICET from Argentina.

EFFECTS OF CHRONIC DRUG ADMINISTRATION I

185.1 DETECTION OF DRUG INDUCED NON-STATIONARITIES OF THE ELECTRICAL RHYTHMS OF THE NUCLEUS ACCUMBENS AND AMYGDALA. C.C. Turbes, G.T. Schneider*, R.J. Morgan and T.N. Solie.* Dept. of Anatomy, Creighton University School of Medicine, Omaha, NE 68178.

These are studies of the electrical activity of the extracellular space of the nucleus accumbens and the amygdala. The extracellular microenvironment is a complex medium capable of transmitting information between neurons. The extracellular electrical fields are recorded with microelectrodes. These experiments involve twelve cats. Monopolar and bipolar electrodes with 100 to 200 micron tip exposures are used. Only electrodes with the resistance of 20 megohms or less are used. The electrodes are capable of recording both slow wave potentials and multiple unit spike potentials concurrently. The analog signals are recorded on FM tape and analog to digitally converted and processed with a Varian V-72 minicomputer. In these studies we are interested in electrical activity between 1 Hz to 1 KHz. The d and l isomers of amphetamine are used to enhance the coherence of frequencies from 50 Hz to 1 KHz. To study the interrelationships between these spatially distant neuronal populations, the following signal processing methods are used: coherence, partial coherence and phase spectra. We have utilized certain parametric methods such as autoregressive and cross regressive time stationary spectral filters. The Fast Fourier Transform (FFT) algorithms are used for spectral estimates of continuous analog signals. A large number of epochs of the predrug, during the action of the drug, and postdrug data are used to develop the spectral filter models. The positive values above the average for each frequency are collected and plotted. These methods are used to further characterize the amphetamine induced changes in coherence spectra, autospectra and cross spectra of the nucleus accumbens and certain amygdala nuclei.

There are increases in coherence of certain frequencies above 50 Hz to 1 KHz induced by the amphetamine isomers. Partial coherence estimates showed the interaction of the amygdala and nucleus accumbens. Coherence is influenced by other brain areas synaptically connected. The parametric methods showed synchronous changes at the same frequencies involved in the coherence spectra induced by the amphetamine isomers.

185.2 CHANGES IN SPONTANEOUS FIRING RATE OF NIGRAL NEURONS AFTER CHRONIC HALOPERIDOL. R.F.Gariano*, S.J.Young* and P.M.Groves. Department Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

Chronic treatment with neuroleptic drugs often leads to disabling and sometimes irreversible abnormal movements termed dyskinesias in humans and experimental animals. We are currently investigating the effects of chronic treatment with haloperidol on electrophysiologically identified dopaminergic and non-dopaminergic substantia nigra (SN) neurons in urethane-anaesthetized rats. Animals in the experimental group were administered haloperidol in tap water (0.02mg/ml) ad lib., equivalent to 2mg/kg/day, for four weeks, followed by an 8 day drug free washout period prior to recording. Immunoassay performed at the end of the 8 day drug free period showed that plasma levels of haloperidol were below the limits of detection (1.0ng/ml), corresponding to brain tissue levels of less than 2% of treatment levels (Ohman,R., et al. N.S. Arch. Pharmacol. 299:105, 1977). Approximately 30% of the CHAL rats were cataleptic during the first week of treatment; tolerance developed to this behavioral effect thereafter. A matched control group (CTRL) was maintained on haloperidol-free tap water, available ad lib. All experiments were performed blind with respect to treatment group.

Spontaneous firing rates of pars compacta dopaminergic neurons in the CHAL group were increased 22% over corresponding values for the CTRL group (5.2 vs 4.3 Hz, t=3.03, df=192, $p<.005$). The subset of dopaminergic neurons which were antidromically activated from the striatum showed a similar alteration in firing rate (4.7 vs 3.7 Hz, t=2.10, df=60, $p<.05$). Using the method of terminal excitability testing (Groves,P.M., et al. Brain Res. 221:425, 1981), we are presently investigating the possibility that these effects on firing rate are related to changes in the sensitivity of cell body and/or terminal dopamine autoreceptors. Conversely, spontaneous firing rates of non-dopaminergic pars reticulata neurons in the CHAL group were decreased 35% compared to CTRL pars reticulata neurons (25 vs 38 Hz, t=5.89, df=137, $p<.0001$). These results are of interest in view of the highly stable firing rate of SN dopamine cells across the sleep-waking cycle, the growing recognition of the non-dopaminergic nigral cells in basal ganglia output, an apparent reciprocal relationship between the dopaminergic and non-dopaminergic elements of the SN, and the proposal that GABAergic dysfunction in the basal ganglia may be causally related to the appearance of tardive dyskinesia in humans and primates following long-term neuroleptic therapy (Fibiger,H.C., K.G. Lloyd. TINS 7(12):462, 1984).

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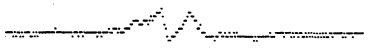
185.3 A COMPUTERIZED TECHNIQUE FOR THE STUDY OF MOUTH MOVEMENTS: EFFECTS OF AGE AND CHRONIC NEUROLEPTICS. Ronald See*, Gaylord Ellison, and Jack Kinney*. Dept. of Psychology and Computer Sciences, UCLA, Los Angeles, CA 90024

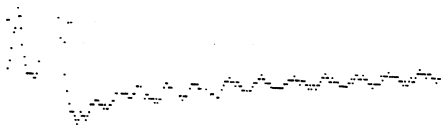
Long-term neuroleptic administration has been shown to increase vacuous chewing in rodents, suggesting the possibility of a rodent model of tardive dyskinesia. We have devised a novel method for precisely quantifying oral movements using direct input of video images into a computer. With this technique, subtle alterations in orofacial movements which would be hidden to a human observer can be precisely quantified.

Rats are habituated to a plexiglass tube with an open hole at one end. For testing, 2 small spots of UV-sensitive ink are placed on the upper and lower jaw, and the animal is placed in the tube inside a soundproofed chamber. The only illumination present is from a UV lamp beneath the animal's jaw. The output from a closed-circuit TV camera focused on the animal's mouth region is fed into a specially constructed computer board. This measures the distance (number of TV rasters) between the two spots and places this information into memory 60 times/sec. Oral movements are recorded as the distance between the two dots changes. The resulting data is analyzed for frequency, duration, and train length of oral movements. A very high correlation between oral movements as determined by computer and those reported by the human observer is obtained, particularly for large amplitude oral movements.

In an initial study to establish the validity of this scoring technique, 24 female Sprague-Dawley rats were divided into three equal groups (one control, one low-dose haloperidol, and one high dose haloperidol). Haloperidol was administered using silastic implants containing haloperidol base. These animals were observed once each month for 8 months of continuous haloperidol administration and once each week for 4 weeks following explant. The types of changes in oral movements observed will be discussed.



(Computer record of a typical double open-close oral movement)



(Computer record of an atypical oral movement train following a head movement.)

185.4 HALOPERIDOL TREATMENT OF RATS ALTERS STRIATAL NA,K-ATPASE LEVELS. S.P. Mahadik, P. Aceto*, F. Vilim, A. Korenovsky*, S.E. Karpiak. Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, Biochemistry & Molecular Biophysics, Coll. Physicians & Surgeons, Columbia U., 722 W 168th St., NY, NY. 10032.

Neuroleptic treatment of schizophrenic patients often induces extrapyramidal side effects, namely tardive dyskinesia (TD). Since neuroleptics are still a major method of treating schizophrenia, determination of the molecular mechanisms underlying TD may establish methods by which this motor disorder can be eliminated. In the study of such mechanisms, several animal models are available.

One of the neuroleptics, haloperidol, causes an imbalance in dopaminergic-cholinergic transmission, probably due to depolarization blockade of dopaminergic neurons in the nigro-striatal pathway [1]. We are studying the effects of chronic haloperidol treatment on Na,K-ATPase in rat striatum, where the enzyme is present in high concentration in synaptic terminals and plays a role in synaptic transmission, as well as acting as the Na/K pump. Male Sprague-Dawley rats (250gr) were injected daily with haloperidol (2mg/kg i.m.) for various periods of up to 28 weeks. Striatal membranes were prepared from treated rats and saline controls at 1,2,3 days and 2,5,24 & 28 weeks. Na,K-ATPase (determined as total ATPase minus Mg-ATPase with 4mM ouabain, 40mM Tris-HCl, 10mM MgCl2, 150mM NaCl, 40mM KCl, 2mM EDTA, 2mM EGTA, 4mM Tris-ATP, pH 7.5) showed no changes after 1,2 or 3 days of haloperidol treatment. After 2 weeks the level of Na,KATPase increased by 28% and remained elevated (28-40%) for up to 28 weeks. The kinetic properties of Na,K-ATPase as a function of K conc. showed a change in Vmax (3.57 mM vs 2.46 mM for control) but no change in Km (0.45 mM vs 0.46 mM for control) The change in Vmax suggests an increase in the level of Na,K-ATPase. Since we have previously found that chronic haloperidol treatment caused an increase in acetycholinesterase [2], we conclude that chronic haloperidol treatment results in changes of several synaptic membrane enzymes, and these changes may be responsible, or at least contribute, to the dopaminergic-cholinergic imbalance seen in the striatum.

1. Chiodo & Bunney. J. Neurosci. 3:1607 (1983).
2. Mahadik et al., Soc. Neurosci. Abstr.10:1200 (1984).

185.5 BEHAVIORAL EFFECTS OF LONG-TERM HALOPERIDOL: INTERACTIONS WITH AMPHETAMINE. A.D. Levy & G. Ellison. Dept. Psychology, UCLA, Los Angeles, CA 90024.

Long-term administration of antipsychotic drugs in humans can produce tardive dyskinesia, as well as other behavioral alterations. In this laboratory we have attempted to model these disorders in rats given continuous long-term administration of haloperidol (HAL), via subcutaneously implanted silicone reservoirs containing 100 mg of HAL. These HAL pillows are designed to release approximately 0.7 mg/kg/day over 8 months of administration. In this experiment we attempted to determine whether amphetamine (AMPH) administration, either concurrent with, or following HAL treatment would alter the syndrome of increased oral movements, or other behavioral effects induced by HAL.

Two groups of 10 rats were implanted with HAL pillows, while two other groups received control pillows. One month later, one HAL and one control group were implanted with slow release AMPH pellets, designed to release approximately 4 mg/kg/day over the 30 days of treatment. During the course of AMPH treatment, locomotor activity and body weights were recorded daily. After 30 days of AMPH administration, animals were monitored monthly for oral movement behavior on a newly developed computerized scoring system. At other times rats were scored for oral movements and activity measurements in a home cage environment. Animals were also observed for limb tremor or changes in gait.

The animals demonstrated some interesting behavioral trends during concurrent AMPH and HAL administration. Animals receiving HAL only showed decreased locomotor activity counts, while rats administered only AMPH showed elevated activity counts for about 5-10 days compared to controls. HAL blocked the increased activity induced by AMPH; rats showed virtually no change from the depressed baseline activity counts. AMPH reduced body weights over the first week of administration, gradually returning to control values. HAL, however did not alter the reduction of body weight observed with continous AMPH administration.

185.6 BEHAVIORAL AND RECEPTOR CHANGES IN NIGROSTRIATAL LESIONED RATS AFTER CHRONIC DOPAMINE AGONISTS. D.R. Liskowsky, A.T. Salvatierra, W.J. Weiner, and L.T. Potter*. Depts. of Pharmacology and Neurology, Univ. of Miami School of Medicine.

Each of five groups of Sprague Dawley rats received unilateral 6-hydroxydopamine lesions of the substantia nigra. Two weeks following lesioning, rats were challenged with apomorphine (2mg/kg) and the resultant rotational behavior quantified. Animals were then given either: bromocriptine (10 mg/kg), ciladopa (20 mg/kg), levodopa/carbidopa (200-20 mg/kg), placebo, or no drug. After 3 weeks of drug administration rotational behavior was again quantified. Twenty-four hours later, D2 receptor binding in the lesioned and intact striata was measured using 1.25 nM ^{3}H-spiroperidol, ± d-butaclamol. The D2 receptor binding of the animals in the no drug group was determined 24 hours after the 'pre'-treatment apomorphine challenge.

Results demonstrated that the placebo group had a significantly higher level of D2 receptors than the no drug group. Analysis of the D2 binding between the lesioned and intact sides indicated increased receptors on the lesioned side in the placebo group (+34%) compared to the no drug group (+19%). The placebo group also demonstrated an increase in apomorphine-induced rotations from 'pre' and 'post' treatment trials. Bromocriptine and ciladopa groups each demonstrated D2 receptor binding levels similar to that seen in the no drug group. 'Pre' to 'post'-drug treatment rotational behavior increased in the bromocriptine group, but not in the ciladopa group. Levodopa/carbidopa produced D2 receptor levels intermediate to those in in the other treatment groups. Differences between lesioned and intact striata (+ 34%) were the same as in the placebo group. No increase in apomorphine-induced rotations were found between 'pre' and 'post' treatment trials.

These results demonstrate the ability of certain agonists to prevent behavioral and receptor changes which usually occur following nigrostriatal lesion. They also demonstrate the differences between direct acting agonists and the dopamine precursor, levodopa. These differences may relate to the varying efficacies of these agents in the treatment of Parkinson's disease.

185.7 [^{3}H]-SULPIRIDE BINDING SITE CHANGES CORRELATED WITH CHRONIC COCAINE ADMINISTRATION IN RATS. S.B. Goehring*, M.J. Kuhar and N.E. Goeders (SPON: A. Goldberg). Department of Neuroscience, Johns Hopkins University School of Medicine and Laboratory of Neuroscience, NIDA Addiction Research Center, Baltimore, MD 21205.

A variety of clinical and animal data suggest that the repeated intermittant administration of cocaine and related psychomotor stimulants may be associated with a behavioral sensitization whereby the same dose of the drug results in increasing behavioral effects. This investigation was initiated to determine the effects of chronic cocaine administration on the binding of sulpiride, a relatively specific ligand for D2 dopaminergic receptors, in the rat brain.

Ten adult male Sprague Dawley rats (230 to 250 g) were divided into two groups. The rats in the first group received single daily intraperitoneal injections of saline while the rats in the second group received daily injections of cocaine (10 mg/kg,i.p.) for 15 days. Twenty minutes following the last injection, each rat was sacrificed by decapitation, the brains removed and the striatum and nucleus accumbens dissected over dry-ice. Saturation studies were carried out with [^{3}H]-sulpiride to characterize changes in dopamine receptors (i.e., K_d and B_{max} values) resulting from the treatment conditions using a previously reported procedure (Zahniser and Dubocovich, J. Pharm. Exp. Ther., 227, 592, 1983). The B_{max} values (mean fmol/mg protein $\pm$ SEM) obtained for sulpiride binding in the striatum and nucleus accumbens of the saline treated rats (441 $\pm$ 34 and 316 $\pm$ 28, respectively) were in agreement with previous reports. Chronic daily injections of cocaine resulted in a significant decrease in the concentration of sulpiride binding sites in the striatum (291 $\pm$ 22; $p < 0.005$) and a significant increase in the concentration of sulpiride binding sites in the nucleus accumbens (407 $\pm$ 27; $p < 0.025$). No significant differences in K_d values were seen in either brain region.

These data suggest that chronic cocaine administration may result in differential effects on D2 receptors in the nigro-striatal and mesolimbic dopaminergic systems. These results are in general agreement with those of K. Akiyama et. al. (Biol. Psyc., 17, 223, 1982) who reported similar changes in [^{3}H]-spiperone binding sites following chronic amphetamine administration. Autoradiographic experiments are currently in progress to further characterize these effects. (Supported by USPHS Grants DA 00266, MH 00053, MH 25951, NS 15080 and MH 09111).

185.8 DISCRIMINATIVE STIMULUS PROPERTIES OF ANORECTIC DRUGS COMPARED TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. D. M. Wood* and M. W. Emmett-Oglesby. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107.

Rats were trained to discriminate the stimulus properties of cocaine using a two-lever choice paradigm in which food reinforcement was delivered for responses on the correct lever: one lever was correct after cocaine (10 mg/kg, i.p.) injection, and the other lever was correct after saline injection. Following training, cocaine (2.5-10 mg/kg) was dose-dependently generalized to the cocaine training stimulus, and methylphenidate (1.25-5.0 mg/kg), phenmetrazine (0.64-10.0 mg/kg), phentermine (0.64-10.0 mg/kg), and diethylpropion (0.32-2.5 mg/kg) substituted for the cocaine stimulus; however, fenfluramine (1.25-5.0) and phenylpropanolamine (5.0-20.0) only partially substituted for the cocaine stimulus. Subsequently, training was halted and cocaine, 20.0 mg/kg, was injected every 8 hours for 7 days. As compared to acute generalization and substitution testing, chronic administration of cocaine shifted the entire generalization curve for cocaine two-fold to the right. Similarly, the substitution curves for methylphenidate, phenmetrazine, and phentermine were also shifted two-fold to the right. However, the substitution curve for diethylpropion was shifted in excess of four-fold to the right, and the substitution curves for fenfluramine and phenylpropanolamine did not change following chronic cocaine administration. All drugs that substituted fully for the cocaine training stimulus and that showed an approximate 2-fold shift of the dose-effect curve during tolerance to cocaine are CNS stimulants with well-established abuse potential. In contrast, diethylpropion is reported to have less dependence liability than these other agents, and it shows a more profound degree of cross-tolerance. In addition, fenfluramine and phenylpropanolamine are also considered to have lesser abuse potential than the preceding agents; acutely, they only poorly substitute for cocaine, and they also show a markedly different cross-tolerance profile than these other drugs. These data suggest that cross tolerance to the discriminative stimulus properties of cocaine may be used as a bioassay in determining dependence liability of anorectic drugs.

Supported in part by AOA Grant 82-11-045 and by Burroughs Wellcome Grant F-85-15 and a Thompson Pharmaceutical Grant to DMW.

185.9 THE DEVELOPMENT OF BEHAVIORAL RESPONSES TO d-AMPHETAMINE IN RATS EXPOSED PRENATALLY TO ETHANOL. J.H. Hannigan and B.A. Blanchard*. Department of Psychology, State University of New York at Albany, Albany, NY 12222.

Rats exposed to ethanol in utero demonstrate a variety of behavioral changes in early postnatal life, including overactivity in an open field. To determine dopaminergic involvement in this overactivity we studied the development of behavioral responses to amphetamine by rats subjected to fetal alcohol exposure (FAE).

Pregnant Long Evans hooded rats were fed liquid diets containing 35% ethanol-derived calories (35%EDC) from gestation day 6 through 19. This dose of ethanol has produced reliable behavioral effects without gross morphological abnormalities. Other animals were either pair-fed the liquid diet with sucrose-substituted calories (0%EDC), or were given water and standard lab chow (LC). At all other times all groups were given water and chow. Pups were weaned on postnatal day 21 and housed in pairs with same-sex littermates. On the day before testing all animals were allowed to explore the open field (38 cm X 38 cm) for 20 min. At 28 or 42 days of age, animals were weighed and injected s.c. with either freshly-prepared d-amphetamine sulfate (1.0 mg/kg) or an equivalent volume of saline/ascorbate vehicle, and placed into the open field for 120 min. Automated monitors recorded horizontal activity and stereotypy.

Rats in the 35%EDC control group showed a small but reliable increase in horizontal activity relative to the 0%EDC and LC groups at both 28 and 42 days. Overall, the 42-day-old animals were more active than the 28-day-old animals. All groups injected with amphetamine had elevated activity relative to vehicle-treated controls. The 28-day-old rats demonstrated a longer latency to onset and a shorter duration of the activating effects of amphetamine than the 42-day-old rats. There were no significant differences between prenatal treatment groups on any of the behavioral effects of the amphetamine treatment.

These results, to date, suggest that amphetamine treatment may eliminate overactivity in rats prenatally exposed to ethanol. However, the absence of FAE-induced overactivity after amphetamine may be due to 'ceiling' effects on activity. Significant increases in stereotypy in all groups at this moderate dose support this possibility. Experiments at other doses of amphetamine are directed currently at the question of FAE-induced differential sensitivity to amphetamine.

(Supported in part by grants AA03249 and AA00077 from NIAAA to E.P. Riley.)

185.10 INVOLVEMENT OF BRAIN TRH RECEPTORS IN BEHAVIORAL SUPERSENSITIVITY INDUCED BY CHRONIC METHAMPHETAMINE. M. Sato, S. Kajita*, K. Akiyama*, N. Ogawa† and S. Otsuki*. Depts. of Neuropsychiatry and †Neurochemistry (Institute for Neurobiology), Okayama University Medical School, Okayama 700, JAPAN.

Thyrotropin releasing hormone (TRH), a tripeptide, is distributed throughout central nervous system and involved in a variety of functions some of which can be attributed to dopaminergic mechanism. Chronic administration of methamphetamine (MAP) results in lasting augmentation of behavioral response to dopamine agonists which has been extensively utilized as an experimental model of acute excerbation of paranoid psychotic state (Sato et al, Biol. Psychiatry, 18:429-440. 1983). The present study was aimed at investigating brain TRH mechanism in such behavioral supersensitivity. Male Sprague Dawley rats were treated with either MAP (4 mg/kg/day) or saline for 14 days. After one week of drug free period, a single challenge of either MAP (4 mg/kg) or saline was made, giving rise to 4 treatment groups: chronic saline + saline challenge (S-S), chronic saline + MAP challenge (S-M), chronic MAP + saline challenge (M-S) and chronic MAP + MAP challenge (M-M). The animals were killed 1 hour after the challenge injections, and the brains were dissected out into 7 regions for measurement of TRH-like immunoreactivity (TRH-LI) and specific [3H]TRH binding (at 12 nM). There was no significant difference in TRH-LI in any brain region among the four groups. A challenge of MAP did not affect specific [3H]TRH binding in any brain regions. In the striatum, the specific binding decreased significantly ($P<0.01$) in M-S (11.0$\pm$ 0.7) and M-M (10.5 $\pm$ 0.3) as compared to S-S (18.1 $\pm$ 1.4). In the nucleus accumbens, it decreased significantly ($P<0.05$) in M-M (39.9 $\pm$ 2.2) as compared to S-S (50.6 $\pm$ 3.8), where values are expressed as mean $\pm$ S.E.M. (fmol/mg protein). It did not differ among the treatment groups in other brain regions. The lasting decrease in brain TRH receptors in the rats treated with chronic MAP was shown in this study. Since there is a report by Spindel et al (Brain Res. 216:323-331, 1981) that haloperidol reverses amphetamine-induced decrease in TRH concentrations in the striatum, it might be feasible that such change in brain TRH mechanism occurs secondarily to MAP-induced change in brain dopaminergic system and contributes to a lasting behavioral supersensitivity by chronic methamphetamine.

185.11 THE NEUROTOXIC EFFECT OF MPTP ON THE DOPAMINERGIC CELLS OF THE SUBSTANTIA NIGRA IN MICE IS AGE-RELATED. G.A. Ricaurte*, J.W. Langston, I. Irwin*, L.E. DeLanney* and L.S. Forno*. Depts. of Neurology, Stanford Univ. School of Med., Stanford, CA; Santa Clara Valley Med. Ctr. and the Institute for Medical Research, San Jose, CA 95128.

There are currently conflicting reports as to whether or not MPTP destroys nigrostriatal dopaminergic neurons in mice (Heikkila et al, Science 224: 1451, 1984; Hallman et al, J. Neurochem. 44(1): 117, 1985), as it has been shown to do in monkeys (Langston et al, Br. Res. 292: 390, 1984). Because the validity of using the MPTP-treated mouse as an animal model of Parkinson's disease depends critically on whether dopamine (DA) cell loss occurs in this experimental animal as a result of MPTP administration, this study re-evaluated the effect of MPTP on mouse nigrostriatal dopaminergic neurons using combined chemical and anatomical methods. Male C57BL/6 mice of various ages were used. MPTP was injected intraperitoneally according to either of two dosing regimens: 30 mg/kg/day for 10 days or 20 mg/kg/hour for four hours. After various survival periods, some mice were killed for chemical assay of dopaminergic nerve terminal markers (DA levels, metabolites and uptake); others were used for morphological assessment of dopaminergic neuronal integrity. In young mature (6-8 week old) mice, MPTP destroyed a large fraction of striatal dopaminergic nerve terminals, but left the majority of cell bodies in the pars compacta of the substantia nigra (SN) unaffected. In contrast, in older (8-12 months old) mice, MPTP induced widespread degenerative changes throughout the zona compacta of the SN. This suggests that older mice are more susceptible to the neurotoxic effects of MPTP and indicate that, in older mice, MPTP does indeed destroy nigrostriatal dopaminergic neurons. In this regard, the older MPTP-treated mouse would appear to be a valid animal model of Parkinson's disease. How aging leads to increased sensitivity to the neurotoxic effects of MPTP is not yet clear, but is under investigation. However, it is of interest that the neurotoxic effects of MPTP on the dopaminergic cells of the substanitia nigra should prove to be age-related since this neurotoxin is being used to study Parkinson's disease, which has long been linked to the aging process.

185.12 HEPATIC AND RENAL CHANGES IN MONKEYS TREATED WITH 1-METHYL-4 TETRA-HYDROXYPYRIDINE (MPTP) L.Fagel*, R.J.Schwartzman, P.F.Reyes*, G.Alexander* (Sponsor: John Bertoni, M.D.) Thomas Jefferson University, Department of Neurology, Philadelphia, Pa. 19107.

Although the neurotoxic effects of MPTP on primate and rodent CNS have recently been described, the visceral changes that occur in MPTP treated animals have not been fully investigated. We examined the livers and kidneys of 5 adult Macacca Fascicularis monkeys, 3 of which developed Parkinsonian manifestations after intravenous MPTP administration. Two animals were sacrificied 7 days after the initial injection of MPTP and one, a month later. Two animals were used as controls. All animals were perfused in vivo through the left ventricle with 4% formalin and 1.0% glutaraldehyde solutions. Multiple sections of grossly normal looking livers and kidneys were obtained from each animal and processed for histochemistry and electron microscopy. From these samples, 10 U thick sections were paraffin embedded and stained with hematoxylin-eosin, periodic acid Schiff with and without diastase and sudan black. Renal sections were also stained for urate, phosphate, and calcium crystals. At present, only sections of the liver have been processed for ultrastructural studies. Fatty changes and focal lymphocytic infiltrates in the liver were observed in both the control and treated animals that were sacrificed 7 days after treatment. However, these abnormalities were more severe in the treated group. The distribution and type of fatty change in the treated animals were different from those of the control. Furthermore, hepatic necrosis and eosinophilic infiltrates were noted only in experimental animals. The liver of the animal sacrificed a month after treatment showed focal parenchymal necrosis and chronic inflammatory infiltrates without fatty change. Histopathological examination of the kidney of the same animal revealed massive intratubular deposition of crystalline material, proliferative glomerular changes and chronic inflammatory infiltrates.

We believe the visceral alterations we have observed could result from the direct toxic effects of MPTP and/or its metabolites on the parenchyma, hypersensitivity reaction, fluid and electrolyte imbalance and starvation. Even if these lesions are non-specific, hepatic and renal damage should be considered in assessing the neurotoxic complications of MPTP.

185.13 NEUROTOXIC DAMAGE TO NIGROSTRIATAL SYSTEM FOLLOWING INTRANIGRAL ADMINISTRATION OF THE OXIDATIVE METABOLITES (MPDP AND MPP) OF MPTP. C. J. Sun*, D. J. Richman*, W. Gessner*, A. Brossi*, J. N. Johannessen, S. P. Markey* and C. C. Chiueh, (SPON: J. G. Kenimer), Food and Drug Administration, Rockville, Maryland, 20857, NIADDK and NIMH, Bethesda, Maryland, 20205.

Previous experiments indicated that systemic or intranigral administration of MPTP failed to produce a permanent lesion in the nigrostriatal system of rodents (Chiueh et al., Europ. J. Pharmacol., 100: 189, 1984; Chiueh et al., Fed. Proc., 44: 893, 1985; Hallman et al., J. Neurochem., 44: 117, 1985). Markey et al (Nature, 311: 464, 1984) reported that MPTP is trapped in monkey brain but not in rodent brain after it is oxidized to MPP by MAO-B and/or dehydrogenases. Furthermore, the oxidation process of MPTP appears to be responsible for its neurotoxic effects since pargyline and deprenyl inhibit the formation of MPDP and MPP (Chiba et al., B.B.R.C., 120: 574, 1984; Johannessen et al., Life Sci., 36: 219, 1985) and also prevent the neurotoxic effects of MPTP (Heikkila et al, Nature, 311: 471, 1984; Cohen et al., Europ. J. Pharmacol., 106: 209, 1984).

In C57BL6 mice, MPTP produces a reversible depletion of striatal dopamine but no permanent lesion in the substantia nigra compacta. Its oxidative metabolites cause a decrease in striatal dopamine following intrastriatal administrations (Chiueh et al., Fed. Proc., 44: 893, 1985). In the present study, we synthesized oxidative metabolites of MPTP, i.e. MPDP and MPP, and investigated their neurochemical, behavioral and pathological effects after intranigral administration to rats.

Unilateral intranigral administration (A 3.2, L 2.2, H 2.0) of 1 to 5 ug of MPDP and MPP in 1 ul saline produced a dose dependent depletion of ipsilateral striatal dopamine in rats assayed by HPLC-EC two to three weeks following the treatment. The dopamine levels in the nucleus accumbens were slightly decreased. d-Amphetamine (3 mg/kg s.c.) and apomorphine (1 mg/kg, s.c.) induced a circling locomotion towards the lesioned side in these unilaterally treated animals. The ipsilateral circling scores correlated well with the degree of depletion of striatal dopamine in the individual animal. No receptor supersensitivity was observed. MPP increased the ^{45}Ca influx into cells at the site of injection and produced nonspecific cytotoxic damage seen by histological procedures.

In conclusion, MPDP and MPP, but not MPTP, produced localized cytotoxic damage in nigrostriatal neurons and disrupted their functions following intranigral administration to rodents. The cause of their cytotoxic lesion may be related to the cationic surfactant properties of MPDP and MPP.

185.14 NEUROCHEMICAL EFFECTS OF MPTP IN THE DOG: EFFECTS OF PARGYLINE PRETREATMENT. J.N.Johannessen,C.C.Chiueh,J.P.Bacon*,N.A.Garrick, D.L.Murphy*,R.S.Burns,V.K.Weise*,I.J.Kopin, and S.P.Markey* Lab. Clin. Sci. NIMH and NINCDS, Bethesda, MD 20205

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produces an irreversible parkinsonism in primates and dogs. Monoamine oxidase (MAO) inhibitors block the toxicity of MPTP in primates and rodents, however effects of this pretreatment on the neurochemical sequelae of MPTP injection in the dog have not been examined.

Beagle dogs were injected with MPTP (2.5 mg/kg i.v.) with or without pargyline pretreatment (5 mg/kg s.c. 16 and 2 hr prior to MPTP). Controls were untreated and a fourth group received pargyline only. Animals were killed at various times after injection and their brains assayed for dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), tyrosine hydroxylase activity (TH), and MAO activity using 5-HT, DA, or phenethylamine (PEA) as substrates.

Pargyline blocked the toxicity of MPTP as defined by three criteria. First, the akinesia and muscular ridgidity seen after MPTP were completely absent. Second, the metabolism of MPTP to MPP+, which has been shown to be critical for MPTP toxicity, was largely prevented. Finally, striatal dopamine, which is depleted following MPTP injection, was normal in dogs pretreated with pargyline. Treatment with pargyline alone had no effect on striatal DA. MPTP also produced marked increases in striatal 5-HT (209% of control), while 5-HIAA was decreased to 76% of control levels. In contrast to dopamine, increases in striatal 5-HT were not blocked by pargyline, but were in fact augmented significantly. This appeared to be an additive effect, since pargyline alone also increased striatal 5-HT.

Curiously, while pargyline pretreatment prevented the loss of DA, it did not prevent the loss of DOPAC or HVA caused by MPTP. This finding cannot be explained by the presence of pargyline because animals treated with pargyline alone exhibited normal striatal DOPAC and HVA levels. The effect was reversible, DOPAC and HVA levels returning to normal by 3 months after injection.

To examine whether the lack of DA metabolites seen in pargyline/MPTP treated animals indicates a role for MPTP as a long lasting MAO inhibitor, MAO activities were measured in cortex and striatum. MPTP alone had no effect on MAO activities in cortex, however MAO B activity in caudate was reduced 25%, suggesting that 25% of MAO B activity in the caudate is contained in DA terminals. TH activity in the caudates of pargyline/MPTP treated dogs was not different from control values, precluding the possibility that damage to DA terminals was responsible for the decreased DA metabolites seen in pargyline/MPTP treated dogs.

185.15 MPTP MODEL OF PARKINSON'S DISEASE. B.R. Ransom and D.M. Kunis*. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

In June of 1982, a group of California drug users suddenly developed symptoms of Parkinson's disease after using a synthetic meperidine-like drug contaminated with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (Langston et al., Science 219:979, 1983), and this, ironically has led to the development of a very useful model of Parkinson's disease. MPTP has been shown to selectively destroy dopamine-containing substantia nigra (SN) neurons, probably after being converted by oxidation to 1-methyl-4-phenyl-pyridinium ion (MPP^+), and causes primates to develop parkinsonian symptoms which are benfited by anti-parkinsonian medications such as Sinemet. It has been possible to block both the symptoms and lesions of MPTP treatment with MAO inhibitors. Initial studies suggested that the toxicity of this drug was limited to the brains of men and primates, but recently toxicity has been demonstrated with explant cultures of rat SN (Mytilineou and Cohen, Science 225:529, 1984). Encouraged by this observation we have established dissociated cell cultures of mouse SN and tested this system for MPTP toxicity. The advantages of a culture model of MPTP toxicity would include greatly diminished expense and enhanced experimental control.

Dissociated cell cultures of SN were prepared using 13 day mouse embryos. The mesencephalon was removed, minced and triturated to produce a cell suspension and then plated onto previously prepared monolayers of mouse astrocytes. By two weeks of age these cultures consisted of monolayers of well differentiated neurons whose somal diameters varied between 10 and 20μ. Using the glyoxylic acid method of catecholamine histofluorescence, discreet neuronal staining was observed after preincubation with norepinephrine (10^{-6} M).

MPTP applied to two week old SN cultures at concentrations ranging from 10 to 50 μg/ml produced a dose-dependent destruction of neurons. At 50 μg/ml almost all neurons were destroyed after one week of application. Spinal cord neurons in culture (Ransom et al., J. Neurophys. 40:1132, 1977) were also destroyed by similar applications of MPTP. The MAO inhibitor Pargyline, at a concentration of 100 μM, completely blocked MPTP induced neuronal death in SN cultures. A similar protective effect of Pargyline has been noted in the primate model of drug-induced parkinsonism and is believed to result from the blockade of MPTP oxidation to the charged metabolite MPP^+. Dissociated cell cultures of SN and spinal cord appear highly sensitive to the toxic effects of MPTP and should prove useful in determining its mechanism of action.

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EFFECTS OF CHRONIC DRUG ADMINISTRATION II

186.1 ACUTE AND CHRONIC EFFECTS OF PYRETHROID INSECTICIDES ON OPERANT RESPONDING FOR FOOD. E.A. Stein, M. Washburn*, C. Wolczak*, and A.S. Bloom†. Dept. of Biology, Marquette University, Milwaukee, WI 53233, and †Dept. of Pharmacology, Medical College of Wisconsin, Milwaukee, WI 53226.

The synthetic pyrethroids are potent insecticides with very high topical lethality on insects and concomitant low oral lethality in mammals. They are becoming important agriculturally and therefore the exposure of non-target species should increase. At high doses, rats receiving these compounds will display marked hyperactivity, tremors, convulsions and finally, death. However, low doses, those more likely to be present in the environment, have not been as well studied. The mechanism and sites of action of these agents in the central nervous system of mammals is not known. It appears that operant responding may be a sensitive method to detect changes in CNS function that may not be evident using other procedures (e.g., neurochemical). As such, male Holtzman rats were trained to respond for 45 mg food pellets on a variable ratio schedule (VR25). Rats were placed on a 23-hr food deprivation schedule with water available ad lib. Testing was performed for 30 min daily between 0900 and 1200 hrs. No water was present during testing. To maintain adequate food and water balance, subjects were given free access to both food and water for an additional 30-min period following daily testing. Responses and reinforcements during each session were recorded. Pyrethroids were administered IP once per week and prepared in a 1:1 vehicle of Emulphor and 95% ethanol and diluted with saline. Chemicals tested were technical permethrin, deltamethrin and fenvalerate. All agents were submitted to a dose-response and time course study. In addition, chronic, low doses of each agent were delivered daily for 3 months to examine possible cumulative effects. A within subjects design was used in all studies, with each rat receiving each treatment; however, only one agent per rat was employed. All agents were effective in reducing operant responding for food in a dose-dependent manner. Effects were seen within the first 15 min post injection with duration of effect lasting approximately 30 min to one hr. Operant responding returned to baseline on subsequent test days. No cumulative effects of these agents were seen, as chronic administration of a dose previously shown effective in reducing responding by approximately 20%, failed to produce any permanent alterations in responding when administered daily for periods up to three months. Results of these studies indicate that the synthetic pyrethroid insecticides can disrupt a well learned behavior response at doses significantly lower than doses producing lethality. However, effects were of short duration and appear not to be cumulative. Supported by NIEHS grant ES03122 to EAS and ASB.

186.2 EFFECT OF PYRETHROID INSECTICIDES ON THE BINDING OF ^{3}H-GLUTAMATE TO RAT BRAIN MEMBRANES. A.S. Bloom, L.A. Sutherland*, C.J. Hillard and E.A. Stein. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226 and Department of Biology, Marquette University, Milwaukee, WI 53233.

The pyrethroids are synthetic derivatives of the natural pyrethrins found in the Chrysanthemum. We have previously shown that this class of insecticides partially blocked kainic acid binding to mouse brain membranes (Toxicol. Appl. Pharmacol. 64: 566, 1982). We have now examined the effects of several pyrethroids on ^{3}H-glutamate binding sites in the rat brain. Both in vitro and in vivo drug treatments were studied. In the in vivo studies, groups of male Sprague-Dawley rats were injected ip with either a pyrethroid or its dimethyl sulfoxide (DMSO) vehicle, daily for seven days. DMSO vehicle was found to have no effect on ^{3}H-glutamate binding, either in vitro or in vivo. Seven days of treatment with the potent cyano-containing pyrethroid, deltamethrin (10 mg/kg), produced a decrease in the binding of ^{3}H-glutamate. This dose produced obvious symptomology such as sinuous writhing which is typical of this type of pyrethroid. The decrease in binding was the result of a 36% decrease in the Bmax parameter. A slight increase in the binding constant (K_d) was also observed after treatment with deltamethrin. Slight (12%), but statistically significant decreases in binding were also produced by the cyano-containing pyrethroid, fenvalerate (20 mg/kg) and by permethrin (100 mg/kg), a pyrethroid without a cyano group. However, symptoms were not usually obvious upon gross observation with the latter two treatments. Higher doses of these compounds need to be examined. In other studies, pyrethroids were added to brain membranes, in vitro, using DMSO as a vehicle. Inhibition of ^{3}H-glutamate binding was produced by permethrin (10 μM), fenvalerate (2 μM) and cypermethrin (2 μM). The decrease after cypermethrin was 30%. The data from these studies offer further support for the hypothesis that an action at receptors for excitatory amino acid neurotransmitters is involved in the neurotoxic effects of the pyrethroid insecticides. (Supported by USPHS Grant No. ES03102).

186.3 TRIETHYL LEAD ATTENUATES FEEDING AND DRINKING, AND INDUCES A CONDITIONED TASTE AVERSION, IN ADULT RATS. P. Faubert* and D.A. Czech. Dept. of Psychology, Marquette Univ, Milwaukee,WI 53233

Our laboratory previously reported that adult rats acutely exposed to tetraethyl lead (TTEL) show a significant reduction in both food and water intake, beginning 2-3 days post-exposure and lasting for about a week. Although substrate affected is not known, it is suspected that the toxic agent is triethyl lead (TEL); TTEL is rapidly metabolized to the triethyl form. To further investigate this issue at the behavioral level, we exposed male adult Sprague-Dawley rats to TEL at the same dosage levels as previously used in TTEL studies. Food and water intake and body weight were monitored daily throughout the experiment. Rats were provided with food and tap water ad lib in the home cage. When intakes were stable, rats were injected SC with 3 doses of TEL (1,4 & 7 mg/kg as lead base) or normal saline vehicle. Intakes and body weight were partitioned into blocks of 3 days, both pre- and post-exposure, and evaluated with analyses of variance (ANOVA) and Dunnett's procedures. Significance levels were set at $p < .05$. Food and water intakes were significantly lower in both 4 & 7 mg/kg groups, or 7 mg/kg group only, depending on post-exposure period. The effect again lasted about a week. Body weights were significantly lower than controls at 7 mg/kg only. Weight reduction lagged food/water shifts, as expected, by 1-2 days, and also recovered in about a week.

In a separate experiment, rats were exposed to the same dosage levels of TEL in a conditioned taste aversion paradigm (CTA). Adult male rats were placed on a 23.5 hour water deprivation schedule with food available ad lib for 16 days prior to TEL exposure. Two bottles, both containing tap water, were attached to the home cage during the drinking period. After water intake was reasonably stable, a single bottle containing a 0.1% sodium saccharin solution was offered to the animal during one drinking period (conditioning trial). Approximately 30 min after the drinking period ended, rats were injected SC with TEL or vehicle, as in the first experiment. On the following two days, tap water (2 bottles) was again available. On the third (test) day, rats were offered a choice between tap water and saccharin. Saccharin preferences (proportion of saccharin relative to total intake) were evaluated with ANOVA and Dunnett's procedures. Again, $p < .05$ was considered significant. Saccharin preferences of lead-exposed groups was significantly lower than the control group at all dosage levels (1,4 & 7 mg/kg), in a dose-related manner. Results are discussed.

186.4 **IMPAIRED ASSOCIATIVE LEARNING AND SPARED RULE-LEARNING IN A SERIAL LEARNING TASK FOLLOWING TRIMETHYLTIN EXPOSURE IN RATS.** **S.B. Fountain*, D.E. Schenk*, and Z. Annau.** Department of Environmental Health Sciences, The Johns Hopkins University, Baltimore, MD 21205.

Trimethyltin (TMT) is a neurotoxic organometal which produces a variety of learning and memory impairments in laboratory animals and humans. We investigated the effects of TMT exposure on serial pattern learning in rats. Rats were intubated once with either 0 or 7.0 mg/kg TMT one week prior to the pattern learning procedure. Rats then learned patterns composed of various quantities of BSR pulses; they received BSR quantitites in a predetermined order for lever presses in a discrete-trial operant task. All rats received two serial patterns (18-10-6-3-1-0 versus 18-1-3-6-10-0 pulses of BSR) that alternated within each daily session of 100 patterns. The 18-10-6-3-1-0 pattern is formally simple because it can be described by a single "less than" rule relating successive BSR quantities. The 18-1-3-6-10-0 pattern is formally more complex because no single rule describes the pattern. These patterns were chosen because rats have previously been shown to be sensitive to the rule-based structure of patterns. In the present study, TMT-exposed rats learned their formally simple 18-10-6-3-1-0 pattern of BSR quantities faster than Controls, but were significantly slower than Controls in learning their formally more complex 18-1-3-6-10-0 pattern. However, TMT exposure did not affect the reinforcing properties of BSR or rats' response to nonreward. In addition, TMT did not affect rats' level of asymptotic performance; TMT-exposed rats responded at least as well to their patterns at asymptote as Control rats. Histology showed that TMT produced thinning of hippocampal CA1, CA3b, and CA3c pyramidal cell fields and virtual destruction of CA4 in the intrahilar region. Hippocampal CA2 and CA3a (the Sommer sector) and dentate were largely spared. Our results support the notion that TMT exposure impaired some aspects of the rote processes involved in serial pattern learning in rats, yet spared the rats' ability to encode some representation of the formal rule-based structure of their simple pattern. Because TMT is a relatively selective limbic system neurotoxicant, these results suggest that the processes involved in learning simple versus complex serial patterns may be mediated by different systems in the brain.

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186.5 EFFECT OF SOMAN EXPOSURE ON ACQUISITION OF A TWO LEVER OPERANT ALTERNATION. H.E. Modrow*, N.K. Jaax*, B.L. Harding*, and J.H. McDonough (SPON: B. Hackley). U.S. Army Medical Research Institute of Chemical Defense, APG-EA, MD 21010-5425.

Rats surviving convulsion-producing doses of the toxic organophosphate, soman, exhibit neural damage in a number of limbic sites, including the hippocampus. As experimental hippocampal lesions in rats produce deficits in acquisition of alternation tasks, this study examined whether rats exposed to soman would demonstrate perseveration and deficits in learning a cued alternation task.

Following preliminary training to lever press on a fixed ratio 1 FR1) schedule, 70 rats were divided into 4 groups and injected sc with either saline, 75, 85 or 95 ug/kg soman. After recovery, surviving rats were retrained to lever press on the FR1 schedule. When an animal obtained 100 reinforcements within a 30 min period, alternation training began. Initially, rats were required to simply alternate between the two levers using a FR1 schedule and a 2 sec intertrial interval (ITI) with the correct lever indicated by a cue light. As animals became proficient, the schedule was gradually incremented to the terminal level of FR20 with a 20 sec ITI. Training sessions, 40 min per day, 5 days per week, continued until rats attained criterion ($\leq$ 25% incorrect responses for three consecutive days) or 100 training days had elapsed without criterion performance.

A significant dose-dependent gradation in symptom scores was apparent within one hour after injection ($F(2,67)=23.4, p<.01$). Rats injected with 75 ug/kg had significantly lower symptom scores than rats receiving 85 or 95 ug/kg. There was a significant difference between groups in the number of days required to reach FR 20/20 ITI ($F(3,28)=6.68, p<.01$), with the saline group requiring significantly fewer days than all soman groups. Total days to reach criterion produced similar results ($F(3,28)=6.34, p<.01$). No differences were seen between the three soman groups in the number of sessions to reach criterion. All three groups required nearly twice as long as the saline group. These results confirm previous reports of long-term deficits in learning and performance of soman exposed animals and extend these findings to a cued alternation task. The performance decrements observed in soman exposed animals are best attributed to the irreversible damage to limbic structures such as the hippocampus.

186.6 PYRIDOSTIGMINE-INDUCED FATIGUE IN RAT SKELETAL MUSCLE TWITCH. R. J. Anderson, W.L. Chamberlain*, M. Roesner*, C. Dacko* and D.G. Robertson*. Warner-Lambert/Parke-Davis Pharmaceutical Research and University of Michigan, Ann Arbor, MI.

Pyridostigmine, which does not cross the blood brain barrier, has been suggested as prophylactic treatment for organophosphorus intoxication. Because this cholinesterase inhibitor causes myopathies following chronic administration, the purpose of this study was to determine the relationship between cholinesterase inhibition, changes in responsiveness of skeletal muscle and onset of myopathy. Three dose schedules were used: single daily ip injections (2 mg/kg); constant sc infusion (5 mg/kg/day) and constant sc infusion (25 mg/kg/day). Groups of 5 male rats were withdrawn from the study after 1,4,10 and 20 days of treatment. Under urethane anesthesia (1.5 g/kg) the hindlimb was dissected. Maximal twitch contractions were evoked via sciatic nerve stimulation. Contracture and fatigue were measured during 10 second trains of 20, 50 and 100 Hz stimuli. Drug infusion (25 mg/kg/day) produced significant reductions in muscle contracture during 50 and 100 Hz stimulation. Peak effect was at 4 days and there was partial recovery by 20 days. These animals lost weight during the first 3 days and then gained normally. Throughout the study blood cholinesterase activity was inhibited 40-70%. Drug infusion (5 mg/kg/day) produced a 20% reduction in muscle contracture during 20-100 Hz stimulation, an effect which remained constant during the 20 day study. These rats exhibited decreased blood cholinesterase activity on day 1 and normal enzyme activity thereafter. Weight gain was normal. Daily ip injections (2 mg/kg) produced no significant change in muscle contracture or weight gain. These rats exhibited elevated blood cholinesterase activity on day 4 and normal activity thereafter. These rats showed cholinergic signs immediately after each ip dose but no overt signs 24 hours later, the time of muscle recording. None of the treatments produced significant changes in muscle mass, nor in single twitch contractions. These results show that pyridostigmine produces muscle fatigue at lower doses than those which inhibit enzyme activity, decrease body weight or change muscle mass. The data therefore, indicate that the effects of this drug on muscle tension are not related to cholinesterase inhibition or a result of body weight loss. The data are consistent with the view that fatigue of skeletal muscle is an early sign of the subsequently developing myopathy. (This work supported in part by USAMRDC Contract DAMD-17-C-3187).

186.7 EFFECTS OF ALUMINUM IN VIVO ON NEUROTRANSMITTER-SYNTHESIZING ENZYMES IN RABBITS. I. Vincent*, J.R. Hofstetter*, B. Ghetti, J. Richter, and P. Shea. Departments of Psychiatry, Biochemistry, and Pharmacology, Indiana University Medical Center, Indianapolis, IN 46223.

Aluminum (Al) is neurotoxic in man and other species. Bugiani and Ghetti (Neurobiol. Aging. 3: 209, 1982) have shown that intracisternal administration of a suspension of metallic Al-powder caused neurological signs and the development of neurofibrillary degeneration (NFD) to different extents in different central nervous system regions of the rabbit. In order to investigate the mechanism(s) of the in vivo neurotoxic effects, we administered metallic Al powder (0.15 ml of a 1% suspension in lactated Ringer's solution) into the intracisternal space of New Zealand-White rabbits. The animals were sacrificed when they showed symptoms of neurotoxicity. Control rabbits were injected intracisternally with the vehicle and sacrificed at the same time. The brains were divided midsagittally; one half was fixed for morphological evaluation and the other half was used for biochemical analyses. The following enzyme systems were measured in twelve brain regions: choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), glutamate decarboxylase (GAD), and serine hydroxymethyl transferase (SHMT). The treated rabbits had decreases in ChAT activity in the hippocampus, GAD activity in the cerebellum, and (with somewhat less confidence) TH activity in the striatum. Furthermore, they had increases in TH activity in the cervical spinal cord. Additional rabbits will be treated not only to confirm these effects, but also to determine if the changes in enzyme activity are the result of changes in V_{max} or K_m. These effects may be related to the NFD observed in these regions or in the nuclei which project to these regions. This will be determined by morphological examination of the fixed material. (Supported by PHS Training Grant MH-17107-02, Indiana Department of Mental Health, and PHS Grant R03 AG 04208).

186.8 LOW DOSES OF DELTA-9-TETRAHYDROCANNABINOL SUPPRESS HIPPOCAMPAL AUDITORY EVOKED POTENTIALS AND PERFORMANCE DURING AUDITORY DISCRIMINATION. T. C. Foster*, K.A. Campbell, R.E. Hampson* and S.A. Deadwyler. Dept. of Physiology & Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Marijuana's psychoactive ingredient, delta-9-tetrahydrocannabinol (THC), has been consistently reported to alter memory function in human subjects as manifested by impairment of immediate recall and disruption of temporal coding of events (Miller & Branconnier, Psyc. Bull., 1983). Because THC is also known to affect hippocampal synaptic processes (Foy et al, Brain Res. Bull., 1982), we have investigated the effects of THC on auditory evoked potentials (AEPs) recorded from the outer molecular layer (OM) of the dentate gyrus (OM AEPs) of the rat hippocampus during auditory discrimination learning. These potentials have been shown to reflect information processing in the hippocampus, in that a short latency component (N1) of the OM AEP varies systematically in amplitude as a function of the temporal sequence of the preceding CS+/CS- trials (Deadwyler et al., Behav. Neural Biol., 1985).

OM AEPs were recorded from water-deprived rats (n=9) trained to criterion in a 2-tone discrimination task. CS+/CS- tones were presented randomly for 100 trials prior to THC injection, and 100 or 200 trials following injection. I.P. injections were made with THC dispersed in a suspension at doses 0.5, 1.0, 1.5, and 2.0 mg/kg, or with vehicle (pluronic detergent). These doses of THC did not distort the OM AEP waveform, but did produce a significant 50% decrease in the amplitude of the N1 component ($p<.01$) relative to the pre-injection level. This suppression was observed across all 4 dose levels, but the degree of decrease was not dose-dependent. Partial recovery of pre-injection N1 amplitude was observed after 2-4 hrs. There were no systematic effects of THC on the amplitude of the later N2 component, indicating that the decrease in N1 amplitude was not due to nonspecific THC effects.

Performance on the discrimination task decreased in a dose-dependent fashion after THC injection: There was no effect of 0.5 mg/kg THC on behavior, while at 1.0, 1.5, and 2.0 mg/kg respectively, number of responses to the CS+ decreased by 13%, 30% ($p<.01$), and 22%; and latency to respond (on trials in which responses occurred) increased by 28% ($p<.02$), 20% ($p<.05$), and 13%. Behavioral recovery also began in the 2nd 100 trials following THC injection in most animals. Such effects on behavior have previously been reported only with higher doses of THC.

These results indicate that processing of behaviorally-relevant sensory information in the dentate gyrus is selectively altered by low doses of delta-9-THC.

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186.9 DEVELOPMENTAL EFFECTS OF PERINATAL TREATMENT WITH VASOPRESSIN, ITS ANTAGONIST AND HETEROLOGUES IN WISTAR AND BRATTLEBORO RAT. G.J. Boer*, F.G.M. Snijdewint*, J. Kruisbrink*, D.F. Swaab* and +M. Manning (SPON: European Neuroscience Association).*Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands and +Dept. of Biochemistry, Medical College of Ohio, Toledo Ohio 43699, U.S.A.

Based upon the peculiar brain growth anomalies of the vasopressin (VP)-deficient Brattleboro rat and the early ontogenic appearance of this peptide in normal brain, a role for VP in brain development has been postulated. The more so since VP acts as a neurotransmitter and transmitters are likely to be involved as trophic factors at times functional nervous circuitries are established.

At present perinatal treatment with VP, its antagonists and heterologues is applied to explore the possible effects on body and brain development and on body water regulation, both measured at one month of age. A new controlled drug-delivery technique is introduced, allowing continuous treatment of small peptides in both rat newborns and adults. It makes use of microporous Accurel polypropylene tubing (15 mm length, 1.6 mm OD), lumen-loaded with peptide, heat-sealed at the ends and enfilmed with nitrocellulose. Subcutaneously implanted, peptides are released for periods of over one month, with dosage rates of 1-2% of its content each day (Kruisbrink and Boer, J. Pharm. Sc. 73, 1713, 1984). Control preparations contain water medium only.

VP/Accurel devices (15 µg) implanted in Brattleboro neonates failed to restore stunted brain growth, but devices (100 µg) placed during pregnancy in Brattleboro mothers had a slight growth promoting effect for the offspring when LVP was used instead. Daily injections of Pitressin tannate (0.5 U/100 g b.w.) in both cases revealed the same except that after termination of the postnatal injections the already existing polyurea of the offspring had aggreviated. Accurel devices containing VP or the VP-antagonists $d(CH_2)_5[D\text{-}Arg^8]VP$, $d(CH_2)_5[D\text{-}Ile^2,Ala^4]VP$ and $d(CH_2)_5Tyr(Me)VP$ (all 50 µg loads), implanted neonatally, had no effects on body and brain development in Wistar pups. However, repeated injections twice a day for the first 3 weeks of life (2.5 µg/50 µl saline) revealed a temporarily reduced body growth for VP, LVP, VT and OX, not for the antagonists. A total catch-up was seen at one month of age and brain weight was not affected. However, for VP and its heterologues, a persistent polyurea had been introduced.

The results are only consistent with the postulate that VP stimulates growth when a prenatal role of VP in development is assumed. Relatively high doses, moreover given peak-wise by repeated injections, revealed only transient growth disturbances, but alters body water metabolism dramatically. This might point to a role of antidiuretic activity on the maturation of kidney responsiveness.

186.10 CHRONIC ADMINISTRATION OF ANTIDEPRESSANT DRUGS TO RATS REDUCES THE HEAD SHAKE RESPONSE TO L-5-HYDROXYTRYPTOPHAN. I. Lucki, H.R. Ward*, L.S.Y. Tyau* and A. Frazer. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, VA Medical Center, Philadelphia, PA 19104.

The head shake response is produced in rats by the administration of serotonin precursors or the agonist quipazine, and has been associated with the activation of $5\text{-}HT_2$ receptors (J. Pharmacol. Exp. Ther. 228:133, 1984). The repeated administration of many types of antidepressant drugs reduce the number of $5\text{-}HT_2$ receptors measured in the frontal cortex of rats (Science, 210:88, 1980). The present study examined the head shake response produced by the serotonin precursor L-5-hydroxytryptophan (L-5-HTP) in rats treated chronically with antidepressant drugs.

The head shake response was defined for rats as a rapid radial twisting of the head about the rostral-caudal axis. Head shake behavior was induced by the administration of carbidopa (25 mg/kg i.p.) followed 30 min later by the injection of L-5-HTP (150 mg/kg s.c.). Head shakes were counted during 5-min intervals starting at 30, 60, 90, 120, 150, and 180 min after the injection of L-5-HTP. Separate groups of rats were treated with the tricyclic antidepressants desipramine or amitriptyline, or the atypical antidepressant iprindole, at 10 mg/kg twice daily for seven days. Rats were examined for the L-5-HTP-induced head shake response either 24 h or 72 h after the final antidepressant drug injection. Other rats were examined for the L-5-HTP-induced head shake response after a single administration of the antidepressant drugs.

Chronic treatment with each of the antidepressant drugs for seven days produced a significant reduction of the head shake response produced by L-5-HTP when measured 24 h after the final injection. This agrees with the ability of each of these antidepressant drugs to reduce the number of $5\text{-}HT_2$ receptors following their chronic administration. Chronic treatment with these antidepressant drugs was shown previously to attenuate the head shake response in rats produced by the serotonin agonist quipazine (Lucki & Frazer, 1985). At 72 h after cessation of drug treatment, the head shake response was still significantly reduced in rats treated chronically with amitriptyline or iprindole, but not in rats treated chronically with desipramine.

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186.11 A NICOTINE WITHDRAWAL SYMPTOM IS INCREASED BY A GABA SYNTHESIS INHIBITOR. H. Lal, C.M. Harris and M.W. Emmett-Oglesby. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107

Withdrawal from drugs of dependence is characterized by both overt signs and subjective symptoms. Until recently, only the physical signs have been studied in laboratory animals. Now, however, a novel approach to investigating subjective events of withdrawal has been developed, utilizing the pentylenetetrazol (PTZ) discrimination paradigm (Lal and Emmett-Oglesby, Neuropharmacology 22:1423, 1983). Food deprived rats are trained in a 2-lever food-reinforced operant task to select one lever after PTZ, 20 mg/kg, ip, and the other lever after saline, 1 ml/kg, ip. Specific treatments are then tested for their ability to substitute for PTZ or to block PTZ-lever selection. This procedure provides a rate-independent measure of an interoceptive stimulus (ie., subjective effect) in that the proportion of rats selecting the PTZ-lever reflects the magnitude of a PTZ-like stimulus. Previously, withdrawal from benzodiazepines (Emmett-Oglesby et al, Eur. J. Pharmacol. 92:127,1983; Lal et al., Fed. Proc. 43:931, 1984), morphine (Emmett-Oglesby et al., Neuropeptides 5:37, 1984), and nicotine (Emmett-Oglesby et al., Soc. Neurosci. Abstr. 10:1210, 1984) have been studied, and in each case, withdrawal substituted for PTZ and the substitution was blocked by diazepam. Thus, 1) there is a subjective effect of withdrawal common to 3 classes of dependence-producing drugs, and 2) the withdrawal stimulus can be blocked by a drug which blocks the PTZ stimulus and blocks anxiety in humans. The present experiment was undertaken to determine the neural basis for the subjective effects associated with nicotine withdrawal. Rats were trained to discriminate PTZ, They were then given a course of subcutaneous nicotine injections, at 8 hour intervals, 0.64 mg/kg/inj on the 1st day and 1.25 mg/kg/inj thereafter, for 15 days. Nicotine treatment was then suspended and on the 5th day after the last dose of nicotine, PTZ-lever selection was increased by isoniazid (200 mg/kg), an inhibitor of the GABA-synthesizing enzyme, glutamic acid decarboxylase. This increase in PTZ-lever selection was reversed by diazepam, 5 mg/kg (% PTZ-lever selection: 35% after saline, 75% after isoniazid, 20% after isoniazid plus diazepam). These results demonstrate that nicotine withdrawal can be modulated by treatments affecting the GABA/benzodiazepine complex, suggesting that this neural system may be involved in mediating subjective aspects of nicotine withdrawal.

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186.12 SERUM CHOLINESTERASE ISOZYMES AFTER HALOPERIDOL TREATMENT. A. Korenovsky*, H. Laev* & S.P. Mahadik (Spon: W.C. Clark). Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, College of Physicians & Surgeons, Columbia U., 722 W 168th St., NY, NY. 10032.

A variety of pathophysiological conditions alter levels of serum cholinesterase isozymes (ChE). Some changes are due to release of enzymes from muscle and/or neural tissue. Chronic haloperidol treatment, which causes extrapyramidal side-effects in schizophrenic patients, induces cholinergic disturbances. The extrapyramidal effects, collectively described as tardive dyskinesia (TD), can be suppressed by drugs that enhance acetylcholine activity. We have therefore studied the effect of chronic haloperidol treatment of rats on ChE isozymes in serum. Sera were obtained from 19 rats treated with haloperidol (2mg/kg i.m. daily for 28 weeks) and 21 controls injected with saline. Eight ChE isozymes were separated on slab gels (7.5% acrylamide). Although the two classes of ChEs, acetylcholinesterases (AChE) and pseudocholinesterases (ChE) can be distinguished by their ability to hydrolyze acetylthiocholine and naphthylacetate respectively, five isozymes showed considerable activity with both substrates. AChE isozymes are visualized as dark green bands of copper-thio-oxamide and pseudo-ChE as brown bands of naphthol-fast blue-RR. In sera from haloperidol treated rats, isozyme 1 (slowest migration, AChE only) was absent, isozyme 7 (ChE only) was reduced, isozyme 8 (ChE only) was increased, and isozyme 2 (AChE plus ChE) was increased several fold. Isozyme 3 (predominantly AChE) was unchanged. Isozymes 4,5, and 6 (minor components) did not show significant changes. These results clearly show that haloperidol treatment alters the levels of both AChE and ChE isozymes. Since the source of some of these isozymes is muscle and neural tissue, haloperidol may be having a direct effect on the membranes of these tissues. Alternatively, the action of haloperidol may be indirect, since disturbance of cholinergic transmission is known to cause the release of AChE from muscle and neural tissues. Measurement of serum AChE and ChE isozymes may be useful in determining the differential effects of available neuroleptics.

186.13 CHRONIC AND INTERMITTENT NICOTINE-INDUCED CHANGES IN BODY WEIGHT AND FOOD AND WATER CONSUMPTION. E.D.Levin, M.M.Morgan*, C.Galvez* and G.D.Ellison. Department of Psychology, University of California at Los Angeles, Los Angeles, CA 90024.

A previous study in this lab found that in rats chronic nicotine adminstration via a subcutaneously implanted pellet caused a progressive weight loss for the next nine days. Groups given daily injections of nicotine did not show this effect. The weight loss was followed by a progressive weight gain from day nine until day seventeen when the pellets were removed. After explant, the treated rats showed a dramatic weight gain of sixteen grams in two days, which brought them to a level above the control mean. All nicotine-treated groups in this experiment showed hypoactivity in the home cage.

A second experiment was designed to increase the effect of postnicotine weight gain by keeping the rats on the nicotine pellet longer (70 days) and by including a group exposed to nicotine on an intermittent basis (rounds of two weeks exposed and two weeks unexposed). Consumption of food and water was measured to determine the involvement of variation of intake in the nicotine-induced weight changes. The results have replicated the previous findings of a progressive weight loss in the nicotine-exposed groups followed by a weight gain after day nine. Accompanying the weight loss was a decline in food and water consumption. The food and water consumption in the exposed groups increased starting seven days after implantation. Consumption was back to control levels by eleven days postimplantation. After removal of the pellets on day fourteen, the intermittent group increased food and water consumption to levels higher than controls and rapidly gained weight passed the control mean. A more robust post-nicotine weight gain would greatly facilitate subsequent studies of the mechanisms behind the effect.

186.14 ALTERATION OF OVARIAN FUNCTION IN FETAL ETHANOL EXPOSED IMMATURE RATS: POSSIBLE HYPOTHALAMO-HYPOPHYSEAL DEFECT. P.K. Rudeen, J. Hagaman and C.A. Kappel Department of Anatomy, The University of Missouri School of Medicine, Columbia, Missouri 65212.

Exposure to ethanol during pregnancy has been demonstrated to result in decreased body weight, increased mortality, smaller litter size and alteration of central nervous system structure and function. The sexually dimorphic nucleus of the preoptic area, an area involved in the regulation of reproduction in the rat, is altered by fetal ethanol exposure. Little is known about the effects of fetal ethanol exposure on reproductive organ function and regulation. Two experiments were performed to determine the effects of in utero ethanol exposure on ovarian function and its neuroendocrine regulation in the offspring rat. Pregnant female rats were given liquid diets containing either ethanol (5% w/v), a liquid diet containing isocaloric amounts of maltose dextrin, or standard laboratory chow and water throughout pregnancy and continuing until weaning. In the first experiment, 30 day-old female rats from each group were unilaterally ovariectomized. The oviduct was removed from the ovary and the ovary weighed to the nearest 0.1 mg. Ten days later, the animal was autopsied, blood collected and the remaining ovary removed and weighed. In the second experiment, when the female pups reached 30 days of age, each animal received 20 I.U. of Pregnant Mare's Serum Gonadotropin (PMS) alone or PMS followed by 10 I.U. of Human Chorionic Gonadotropin (hCG) to induce ovulation. At autopsy, each oviduct was removed from the ovary, the ova were counted and the ovary and uterus were weighed to the nearest 0.1 mg. Blood was collected and the serum harvested. Serum progesterone levels were measured by radioimmunoassay. In the first experiment, the female rats whose mothers were on a control diet during gestation showed greater relative compensatory ovarian hypertrophy (COH) than did the female offspring from fetal alcohol exposed rats ($p<0.01$). In the second experiment, animals in both the alcohol and control groups which were given PMS/hCG had greater ovarian weights ($p<0.01$) than those animals given PMS alone indicating the LH analog (hCG) enhanced ovarian function. The ethanol exposed animals showed an enhancement of ovarian response due to increased ovarian weights ($p<0.01$), greater number of ova shed, and increased blood progesterone levels when compared with the other control groups given the PMS/hCG treatment. The results indicate the reduction of COH by fetal ethanol exposed rats possibly due to the reduced hypothalamo-hypophyseal response to the loss of steroids following the removal of one ovary. In addition, the intensified response of the ovary to exogenous hormones (PMS/hCG) in the fetal alcohol exposed immature rat may also indicate a loss of the sensitivity of the hypothalamus to steroid feedback. (Supported by NIAAA Grant No. AA05893)

186.15 TOLERANCE DEVELOPS ONLY TO THE RATE DECREASING EFFECTS OF CHLORDIAZEPOXIDE AND ATROPINE UNDER A DIFFERENTIAL REINFORCEMENT OF LOW RATE (DRL) SCHEDULE IN RATS. J. M. Carney*, M. Nakamura*, S. B. McMaster* and H. D. Christensen. Department of Pharmacology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Male Sprague Dawley rats were trained to respond under a DRL 10 sec schedule of food reward. Six rats were assigned to the chronic atropine group and six rats were assigned to the chronic chlordiazepoxide group. The 80 minute session was divided into 8 components. The session began with a 10 min extinction component, which alternated with a 10 minute DRL 10 sec component during which correct responding resulted in delivery of a 45 mg food pellet. The session ended after completion of the fourth DRL component (4 extinction and 4 DRL components). Control sessions demonstrated a typical pattern of responding for which the total number of DRL responses was 75 ± 6 (SEM) responses/10 minutes. Of these 75 total responses/10 min an average of 43 responses resulted in food reward. The remaining 32 responses resulted in a resetting of the DRL clock and did not result in food delivery. Cumulative doses effect curves for chlordiazepoxide (1.0, 3.2, 10, 32 mg/kg) and atropine (1.0, 3.2, 5.6 and 10 mg/kg) were determined by injecting the appropriate dose at the start of the extinction component. Chlordiazepoxide doses of 3.2 and 10 mg/kg produced increases in the total number of non-reinforced DRL responses. At the 10 mg/kg dose chlordiazepoxide also produced a decrease in the number of reinforced resopnses. Atropine increased non-reinforced DRL responses at relatively low doses 1.0 and 3.2 mg/kg. Higher doses (5.6 and 10 mg/kg) produced decreases in both reinforced and non-reinforced responses. Daily administration of 32 mg/kg chlordiazepoxide (Group I) or 10 mg/kg atropine (Group II) demonstrated the development of tolerance to the rate-decreasing effects of these drugs on reinforced DRL responding. Redetermination of the dose-effect curves failed to demonstrate the development of tolerance to the stimulant effects atropine or chlordiazepoxide on non-reinforced DRL responding. These data demonstrate that the CNS systems involved in controlling reinforced and non-reinforced DRL responding are different.

186.16 BENZODIAZEPINE HYPOTHERMIC TOLERANCE: ANALYSIS OF THE ROLE OF DRUG-PREDICTIVE ENVIRONMENTAL STIMULI AND CROSS-TOLERANCE TO ETHANOL. A.J. Goudie and J.W. Griffiths*. Dept. of Psychology, P.O.Box 147, Liverpool University L69 3BX, England.

Tolerance to the hypothermic effect of midazolam, a short acting benzodiazepine, was assessed in rats with a counterbalanced discrimination conditioning procedure (Cunningham, C.L., et al., Pharmacol. Therap., 23: 365, 1984) in which specific environmental cues were always associated with drug or saline administration. Marked (almost complete) tolerance developed over 16 days of treatment (given every other day) to the acute hypothermic effect (loss of 2.3°C core body temperature) of midazolam (4 mg/kg i.p.). However, the observed tolerance was not environmentally specific since it was present in subsequent tests in the absence of drug-predictive environmental cues. Similar findings were obtained in a subsequent study with a lower dose of midazolam (1.6 mg/kg i.p.) in which the drug was given every fifth day rather than on alternate days. With the lower dose of midazolam tolerance developed more rapidly (over 5 exposures), as expected. However, the observed tolerance was again not environmentally specific, since it was also present in tests in the absence of drug-predictive cues. Since there is some evidence (Baker, T.B., Tiffany, S.T. Psych. Review, In Press) that environmentally specific tolerance is more likely to develop with low drug doses which are given infrequently, such data provide convincing evidence for the absence of environmentally specific tolerance with benzodiazepines. Thus, these data, which were obtained with conditioning procedures designed to maximize the role of environmental cues in tolerance acquisition, indicate that tolerance to benzodiazepine-induced hypothermia differs from that seen with other C.N.S. depressants (ethanol, barbiturates) which have been reliably reported to produce conditioned environmentally specific tolerance (Siegel, S., MacRae, J. Trends Neurosci., 7: 140, 1984).

Cross-tolerance to ethanol-induced hypothermia (1.2 or 1.6 g/kg i.p) was absent in subjects which were tolerant to midazolam-induced hypothermia, regardless of whether or not drug-predictive environmental cues were present in cross-tolerance tests. These findings contrast with the hypothesis of Khanna et al. (Eur. J. Pharmac., 59: 145, 1979), based on studies of drug-induced hypothermia, that tolerance develops to drug effects rather than to drugs themselves, since two drugs (ethanol and midazolam) with apparently common effects did not show cross-tolerance.

186.17 DECREASED SEIZURE RESPONSE TO ELECTROSHOCK AND BICUCULLINE IN RATS AFTER ONE WEEK OF DAILY MAXIMAL ELECTROSHOCK TREATMENT. J. Boschulte* and K. Gale (SPON: J.R. Moffet). Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

To determine whether repeated exposure to maximal electroshock (MES) alters the seizure sensitivity of rats, we treated rats with MES daily and recorded the duration of tonic hindlimb extension (THE). MES was applied via corneal electrodes (60 Hz, 200 msec, 150 mA) using a Wahlquist apparatus. THE duration was significantly lower (as compared to first exposure) after 4 days of repeated MES, and continued to decline thereafter. After one week of daily MES, the duration of THE decreased to approximately 50% of that obtained in response to the first MES exposure.

Twenty-four hours following the last MES treatment, rats were tested for responses to intravenous bicuculline (.36 mg/kg). Controls consisted of rats that had been exposed daily to the electroshock apparatus (in contact with corneal electrodes) but received no shock. In rats with prior history of 7 MES exposures there was a significantly reduced incidence of major clonic or tonic seizures in response to i.v. bicuculline when compared with controls not exposed to MES or exposed to only one MES treatment.

These results indicate that a type of "tolerance" develops in response to repeated daily exposure to electroconvulsive shock and that a "cross-tolerance" develops to bicuculline induced seizures. This suggests that repeated seizures may alter the sensitivity of neural substrates involved in seizure development.

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186.18 AVOIDANCE BEHAVIOR FOLLOWING ELECTROLYTIC, AF64A AND KAINIC ACID LESIONS OF THE SEPTUM. D.A. Johnson, B.E. Polenchar, A.L. Beggs, P.R. Sanberg and M.M. Patterson, Department of Psychology and College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

In this study we sought to determine if the effects of electrolytic septal lesions on avoidance behavior are due to damage to the septum per se, or to an interruption of efferent fibers passing through the septum. Sprague-Dawley albino rats were used as subjects. Standard electrolytic lesions were produced which destroyed the entire septum as well as hippocampal efferents in the fornix. Infusion of kainic acid (.3 µg in .3 µl of vehicle) produced small lesions of the septum, involving only about 10% of the structure. These lesions were much smaller than anticipated, however, our previous experience with kainic acid (KA) indicated that dosages larger than this usually resulted in hippocampal and infratemporal cortical damage as well. Infusion of AF64A (1 nm in .5 µl of vehicle) resulted in lesions which more closely approximated the damage produced by electrolytic lesions. AChE staining clearly indicated the decreased presence of the enzyme in smaller lesions, but in most cases there was complete destruction of the septal area. However, in most cases the fornix was spared. Infusion of vehicle only did not produce any noticeable lesion. After a 1 month recovery period, subjects were trained in a two-way avoidance shuttle box for 30 CS-US trials each day. A 10 sec tone CS preceded a .7 mA grid-shock US. Subjects were trained to a criterion of 8 avoidances out of 9 consecutive trials, and then overtrained for an additional 30 trials. Active avoidance acquisition was markedly facilitated (Md=24 trials to criterion, N=8) by electrolytic lesions. The effect of the small KA lesions (Md=85 trials, N=9) was minimal when compared to the vehicle control group (Md=116 trials, N=6). Acquisition was moderately facilitated in the AF64A subjects (Md=55 trials, N=8). Only the electrolytic septal lesion effect was significant. After avoidance overtraining, subjects were trained to the same criterion on a passive avoidance task. The pattern of results was similar but the direction of effects was opposite. Electrolytic septal lesions markedly hindered passive avoidance acquisition (Md=49 trials to criterion vs. Md=8 trials for other groups). While we were not able to compare the effects of KA vs. AF64A in a meaningful way (KA lesions were small, AF64A lesions quite large), it is clear that AF64A destruction of the cells in the septum has a much smaller effect on both active and passive CAR learning than electrolytic septal lesions. These results would indicate that the bulk of the CAR electrolytic septal lesion effects may result from a disruption of fibers passing though the septum.

This research was supported by a grant from the Ohio University Research Foundation to D.A. Johnson.

188 SYMPOSIUM. PEPTIDE-AMINE INTERACTIONS IN THE NEURAL REGULATION OF CELL FUNCTION. A. Negro-Vilar, NIEHS-NIH, and B. McEwen, Rockefeller Univ. (Chairpersons); T.L. O'Donohue, NINCDS-NIH; R.E. Zigmond, Harvard Med. Sch.; H. Viveros, Wellcome Res. Labs.

Neurotransmission includes both excitation and inhibition of electrical activity, as well as involving activation of second messenger systems. However, neurotransmitters also produce effects which are not evident unless another excitatory substance is acting to depolarize or hyperpolarize. Other hormonal or neuroactive agents produce effects which alter, over a longer time course, the functional capacity of neurons to carry on neurotransmission. These latter two categories of action are often referred to under the heading of "neuromodulation" and will be the subject of the present symposium.

These general concepts of neuromodulation will be discussed, focusing particularly in peptide amine interactions as a key example of a modulatory mechanism within the nervous system (McEwen). The concepts of co-localization and co-secretion of peptides, or peptides and amines, will be analyzed using central proopiomelanocortin and dopaminergic neuronal interactions to illustrate regulation of hormone synthesis and secretion (O'Donohue). Several neuroendocrine examples of peptide-amine interactions will be described, including the potentiation of ACTH/β-endorphin secretion under combined stimulation by vasopressin and serotonin; the opiate-amine-LHRH interplay within the preoptic-hypothalamic system that provides a model regulatory system for gonadotropin secretion and gonadal function, and the possible intracellular messenger systems that may underlie these interactions (Negro-Vilar). The increased tyrosine hydroxylase (TH) activity in superior cervical ganglia observed after electrical stimulation is in part mediated by the cholinergic system and in part by a putative peptidergic transmitter related to the glucagon-secreting family. Additional data suggests that the resulting increase in TH activity is mediated via an enhanced production of cAMP, which may then represent the common link between these neuromodulators (Zigmond). Adrenal medulla chromaffin cells co-store large amounts of proenkephalin-derived peptides with catecholamines. Both neural agents are co-released upon neurogenic stimulation, and parallel or reciprocal changes in peptide and amine levels or biosynthesis can be observed under appropriate circumstances. Some of these parameters are also cAMP-dependent (Viveros). The evidence reviewed in this Symposium should provide some key examples of the neuromodulatory role played by peptide-amine interactions, both within the central and peripheral nervous system, and of the resulting modified cellular and hormonal responses observed under those influences.

189 Symposium. NEURAL BASIS OF LATERALIZED BEHAVIOR: FROM LABORATORY TO CLINIC. R. G. Robinson, Johns Hopkins Univ. Sch. Med. (Chairperson); S. Glick, Albany Medical College; V. Denenberg, Univ. of Connecticut; J. Lipsey, Johns Hopkins Univ. Sch. Med.; B. Kolb, Univ. of Lethbridge; A. Galaburda, Beth. Israel Hospital.

This symposium will present mechanisms underlying the behavioral expression of cerebral lateralization. Anatomical, biochemical, and physiological mechanisms involved in an asymmetrical elicitation of behavior or lateralized localization of behavior will be the focus of this symposium. The relationship between laboratory and clinical findings in humans will be emphasized.

Dr. Stanley Glick will present functional and neurochemical asymmetry in the dopaminergic nigrostriatal system. New data concerning mechanisms underlying interindividual differences and the degree and kind of asymmetry will also be presented. Furthermore, the relevance of brain asymmetry to the modes of action of psychoactive drugs will be discussed.

Dr. Victor Denenberg will discuss animals who have had extra stimulation in infancy who have been found to have more lateralized brains than non-stimulated controls. These differences have been found in adulthood using a variety of behavioral tests.

Dr. Robert Robinson will discuss findings in humans of anatomical specificity, including lateralized response, of mood disorders to stroke injury. The relationship between these clinical phenomenon and lateralized behavioral and biochemical response to brain injury in the rat will be presented as a possible basis for understanding the mechanism of mood disorders occurring in brain injured humans.

Dr. John Lipsey will discuss interhemispheric interactions in the lateralized affective and behavioral response to focal cortical brain injury. Data will be presented that support the view that poststroke depression following left anterior hemisphere injury in man and hyperactivity following right anterior hemisphere focal lesions in the rat do not depend on interhemispheric release or interaction.

Dr. Bryan Kolb will examine the effects of cortical lesions on the behavior of human and non-human species, with emphasis upon changes in praxic, spatial, and affective behavior; the relationships between behavioral changes and cortical site and side; and anatomical and neurochemical asymmetries related to the behavioral observations.

Dr. Albert Galaburda will describe his recent studies on the visual cortex of the rat which demonstrates that architectonic asymmetry reflects differences in total cell numbers. Furthermore, experiments aimed at specifying whether these differences result from asymmetrical cell production or asymmetrical cell death will be reviewed.

PAIN MODULATION: STIMULATION STUDIES

190.1 INHIBITION OF RAT SPINAL NEURONAL RESPONSES TO NOXIOUS SKIN HEATING BY STIMULATION IN HYPOTHALAMUS AND OTHER DIENCEPHALIC SITES. E. Carstens. Dept. Animal Physiology, Univ. Calif., Davis, CA 95616.

Electrical stimulation in the hypothalamus was reported to suppress responses of spinal dorsal horn neurons to noxious footpad heating in the cat (J. Neurophysiol. 48:809 and this volume). The present results confirm this in the rat, and provide additional quantitative analyses of hypothalamo-spinal inhibition as well as a map of the distribution of diencephalic sites at which stimulation inhibits or otherwise affects spinal neurons.

The responses of single lumbar dorsal horn units to noxious radiant heating of ipsilateral hindpaw skin were recorded with tungsten microelectrodes in adult male Sprague-Dawley rats deeply anesthetized with sodium pentobarbital. Unit responses to heat stimuli (e.g., 50°C, 10 s) repeated at 2 min intervals were stable; effects of bipolar stimulation (100 ms trains at 100 Hz, 3/s, 0-400 μA) applied during the unit's response to the next heat stimulus were expressed as a percentage of the unit's heat-evoked response in the absence of brain stimulation. To map inhibitory (or other) sites, stimulation was applied through each of 5 electrodes spaced 2 mm apart; the array was lowered in 1 mm steps. In each of 14 such experiments, constant stimulation (usually 200 μA) in the medial hypothalamus (from the level of mammilary bodies to just posterior to the optic chiasm) reliably suppressed heat-evoked responses (to 0-50% of control). Equally strong inhibition was usually generated from midline thalamus and lateral hypothalamus bilaterally and frequently from the ventrobasal thalamic complex bilaterally. Medial amygdalar stimulation frequently produced strong inhibition bilaterally, while more lateral amygdalar sites were less effective.

Responses of each of 30 units were inhibited by medial hypothalamic stimulation. This inhibition generally had a rapid time course, sometimes followed immediately by a post-stimulation rebound in unit firing. Inhibition increased with graded increases in stimulation intensity. The mean current at threshold for inhibition was 71 ± 43 μA for 13 units. Spinal unit responses generally increased linearly with graded increases in the temperature of noxious heat stimuli. The slopes of these linear stimulus-response functions were reduced, with no change in threshold, during medial hypothalamic stimulation in each of 9 units. The inhibition was reduced following systemic administration of the serotonin antagonist methysergide. These results are similar to our previous results in the cat, and indicate that a descending inhibitory system, possibly involved in analgesic mechanisms, can be activated from the medial hypothalamus in vertebrates.

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190.2 ELECTRICAL STIMULATION OF THE NUCLEUS TRACTUS SOLITARIUS (NTS) CAUSES OPIOID MEDIATED ANALGESIA IN THE RAT. J.W. Lewis, G. Baldrighi*, S.J. Watson, and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Recent evidence suggests that the NTS, a medullary nucleus known to be involved in autonomic control, may be an integral part of an endogenous opioid-mediated pain-inhibitory system. This nucleus is rich in opioid peptides and their receptors, and has anatomical connections with several brain regions involved in the control of pain. We now report that electrical stimulation of the NTS causes opioid mediated analgesia.

Rats were anesthetized with pentobarbital and mounted in a stereotaxic frame. Stimulating electrodes were lowered into the medulla, near the midline at the level of the obex, to reach the NTS. Pain sensitivity was assessed before and after 30 sec of stimulation using the tail-flick test. Tail-flick latencies exhibited by pentobarbital anesthetized rats are very similar to those recorded in awake animals.

Stimulation of the NTS, but not adjacent sites, caused significant elevations in tail-flick latencies. The analgesic response to NTS stimulation was nearly abolished by pretreatment with naloxone (10 mg/kg).

To assess the relationship between NTS stimulation-produced changes in hemodynamics and alterations in pain sensitivity, we measured simultaneously arterial blood pressure and tail-flick latencies. NTS stimulation typically caused hypertension, although some sites elicited hypotension and others had no effect. Overall, elevations in tail-flick latency and changes in blood pressure were not significantly associated. Furthermore, administration of a sympathetic ganglionic blocking drug prevented stimulation-produced hypertension without affecting analgesia, and treatment with naloxone blocked the analgesia without markedly affecting hypertension.

That stimulation of the NTS causes opioid mediated analgesia may alter conceptions of the organization of endogenous pain-inhibitory systems. Although these systems have been characterized as arising in the medial brainstem and descending to the spinal cord, the origin of the neural signals capable of energizing this system is not known. The NTS, a major recipient of somato/visceral sensory information with projections to many classical pain-inhibitory nuclei, is a likely candidate. Finally, since exposure to stress has been shown to cause analgesia and is accompanied by numerous autonomic sequelae, and because the NTS is known to be involved in these autonomic responses and NTS stimulation elicits pain-inhibition, it is reasonable to hypothesize that an important linkage between stressful stimuli and endogenous analgesia systems occurs via the NTS.

190.3 STIMULATION-PRODUCED ANALGESIA FROM VENTROLATERAL PONTINE TEGMENTUM IS NOT MEDIATED THROUGH THE NUCLEUS RAPHE MAGNUS. J. F. Miller & H. K. Proudfit, Dept. of Pharmacology, Univ. of Illinois at the Medical Center, Chicago, IL 60680.

Stimulation of the rat ventrolateral pontine tegmentum (VLPT) produces a potent analgesia on the tailflick test (Miller & Proudfit, Soc. Neurosci. Abstr., Vol. 10, Part 1, p. 201, 1984). The present experiments suggest that the stimulation-produced analgesia (SPA) obtained from VLPT sites is independent of the serotonergic raphespinal projection system arising from the nucleus raphe magnus (NRM).

In the first experiment, adult female Sprague-Dawley rats were implanted with both a unilateral twisted-wire bipolar stimulating electrode in the VLPT and an intrathecal catheter terminating in the lumbar subarachnoid space. Following a 4-5 day recovery period, baseline tailflick latencies (TFLs) were determined and the animals were screened for analgesic VLPT sites (stimulation parameters were: 0.1 msec square wave pulses, 50-200 uA, 60 Hz). Rats in which VLPT stimulation markedly elevated TFLs then received an intrathecal microinjection of either phentolamine (30 ug), methysergide (30 ug), or saline, and the effectiveness of VLPT stimulation in elevating TFLs was again assessed 15, 30, and 60 min after injection. In agreement with our previous report, intrathecal administration of the alpha-adrenergic antagonist phentolamine significantly attenuated VLPT SPA. By contrast, the serotonergic antagonist methysergide was without effect on SPA elicited from VLPT sites.

In the second experiment, adult female rats were implanted with both a unilateral bipolar stimulating electrode into the VLPT and a guide cannula aimed at the NRM. Rats in which electrical stimulation of the VLPT markedly raised TFLs subsequently received a microinjection of the local anesthetic tetracaine (5 ug/ 0.5 ul saline) directly into the NRM. The effectiveness of VLPT stimulation in elevating TFLs was found to be unimpaired by tetracaine microinjections into the NRM.

In summary, these experiments indicate that the SPA obtained from sites in the rat VLPT does not require the involvement of NRM serotonergic projections to the spinal cord, but rather depends on the activation of a descending noradrenergic spinal projection system. (This work was supported by USPHS Grant NS 18636).

190.4 REINVESTIGATION OF INHIBITION OF SPINAL NOCICEPTIVE NEURONS BY HYPOTHALAMIC STIMULATION IN THE CAT. J.M. Horowitz & E. Carstens. Dept. of Animal Physiology, Univ. Calif., Davis, CA 95616.

We previously reported that stimulation throughout the antero-posterior (AP) extent of the hypothalamus (J. Neurophysiol. 48: 808; 981; 50:192) suppressed responses of cat spinal dorsal horn neurons to noxious heating of footpad skin. These experiments were redone with the aim of correcting stimulation intensities which were inadvertently underestimated in the previous studies, and to quantitatively compare inhibition from multiple diencephalic sites.

In cats anesthetized with sodium pentobarbital and 70% N20, tungsten microelectrodes recorded responses of single lumbar dorsal horn units to noxious radiant heat stimuli (e.g., 50°C, 10 s) on glabrous footpad skin. Unit responses to heat during bipolar brain stimulation (100 ms trains at 100 Hz; 3/s; 0-600 μA monitored with a current probe) were expressed as a % of the unit's response without brain stimulation.

Responses of 36/39 units were reliably reduced during diencephalic stimulation. Inhibition (to 0-50% of control) was evoked by 400 or 600 μA stimulation throughout the AP and mediolateral extent of the hypothalamus bilaterally, as previously reported. Inhibition increased with graded increases in brain stimulation intensity. Mean values (± S.D.) for 3 parameters of inhibition are tabulated below according to medial posterior, mid- and preoptic, or lateral, hypothalamic stimulation sites. Recruitment index = slope of current-inhibition plot.

Site (N)	Threshold (μA)	Recruitment Index (%/100 μA)	Current at 50% inhibition (μA)
posterior (16)	109 ± 82	17 ± 6	323 ± 164
mid- (10)	54 ± 36	18 ± 5	291 ± 191
preoptic (10)	124 ± 87	13 ± 7	353 ± 158
all medial (36)	93 ± 74	16 ± 6	321 ± 167
lateral (5)	87 ± 53	28 ± 14	205 ± 117

We believe that these values are more accurate than those in our previous reports, which overestimated inhibition by 1.5-3 fold due to a ground loop in the brain stimulation circuit. Unit responses increased linearly with increasing stimulus temperature, and the slopes of these temperature-response lines were reduced during medial hypothalamic (N=7 units), preoptic-septal (N=4) or lateral hypothalamic (N=4) stimulation with no significant change in threshold, confirming our previous reports. Finally, we have confirmed our report (Soc. Neurosci. Abs. 9:788) that stimulation at more lateral sites including the internal capsule and amygdala produces varying degrees of inhibition of spinal units. Supported by NIH grant NS 19330.

190.5 ELECTRICAL STIMULATION OF THE RAT VENTRAL AND LATERAL MIDBRAIN SUPPRESSES THE TAIL FLICK REFLEX (SOMETIMES). J.M. Rothfeld*, M.J. Guinan*, S. Pretel, E.S. Culhane*, E. Carstens & L.R. Watkins (SPON: P.A. Pappone). Dept. of Animal Physiol., Univ. of Calif., Davis, CA, 95616.

Stimulation of ventral & lateral areas of the midbrain (VMB) strongly inhibit dorsal horn neuronal responses to noxious skin heating in anesthetized rats (Carstens and Watkins, Soc. Neurosci. Abs. 10:674, '84). The present study investigated VMB stimulation effects on the behavioral response of awake rats to noxious heat stimuli. The effects of two different stimulation parameters were compared: (A) the monophasic stimulation parameter (MPS) used in previous electrophysiological studies (monophasic 0.1 ms square waves at 100 Hz given in 100 ms trains 3/s, 50-1100 μA) and (B) a biphasic stimulation parameter (BPS) more commonly used in behavioral studies (20 Hz biphasic pairs of 0.05 ms square waves of opposite polarity & equal amplitude separated by 0.1 ms, 0.25-4.0 mA). The stability of the phenomenon and the effects of naloxone were also examined. Two bipolar electrodes separated mediolaterally by 1-2 mm were chronically implanted in the VMB of each of 130 male Sprague-Dawley rats. Two and 3 weeks after implantation, a system of ascending limits was used to determine the threshold stimulation current (TC), using either BPS or MPS, necessary to increase the tail flick latency (TFL) from control levels (~3 s) to 8 s. The TFL was measured immediately after 20 s of brain stimulation.

Stimulation in various areas in the VMB suppressed the TF reflex, but no area was consistantly effective. BPS was effective (mean TC = 2.1 mA) at 70 of 214 sites while MPS was effective (mean TC = 680 μA) at 72 of 215 sites. Both were effective at 33 of these sites. The effectiveness of a given site could not be correlated with either the intensity of other behaviors exhibited by the animal during the stimulation, or the anatomical location of the electrode. No differences were observed in the effects of naloxone (10 mg/kg i.p.) & saline on TF suppression.

In a second experiment the stability of the TC at 30 sites in 15 animals was examined by repeating the TC determination one week later. For both MPS & BPS, stability was poor; only 69% of the originally effective sites could suppress the TF when retested. Among these remaining effective sites the TC was seen to increase or decrease markedly. Four sites that were initially ineffective became effective in week 2. In these same 15 animals the short term stability was assessed by retesting the animal at the TC 30 min after the original TC determination. Short term stability was also poor; only 65% of the sites remained effective. These results indicate that apparent anti-nociception from the VMB is an extremely labile phenomenon and that great caution must be used in interpreting the results of such brain stimulation studies. Supported by NIH grant NS20037.

190.6 A REINVESTIGATION OF SEROTONIN INVOLVEMENT IN DESCENDING INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION PRODUCED BY STIMULATION OF MEDIAL FOREBRAIN. M.J. Guinan*, D.A. Van Alstine* and E. Carstens (SPON: L.R. Watkins). Dept. of Animal Physiology, Univ. Calif., Davis, CA 95616.

We previously reported that inhibition of dorsal horn neuron responses to noxious skin heating by medial forebrain stimulation was reduced by (1) the serotonin (5-HT) antagonist methysergide, or (2) pretreatment with the 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) (J. Neurosci. 3:10, 2112). It was later discovered that the current intensities used were underestimated due to a ground loop in the stimulation circuit. A re-evaluation of currents necessary for spinal inhibition appears in this volume (see Horowitz & Carstens). The present study was undertaken to re-evaluate the role of 5-HT in descending inhibition from the medial diencephalon and forebrain.

In cats anesthetized with sodium pentobarbital and 70% N_2O, we recorded responses of single lumbar dorsal horn neurons to noxious radiant heating (48-52°C, 10 s) of the hind footpad skin. Unit responses to heat during bipolar brain stimulation (100 ms trains, 3/s, of monophasic 0.1 ms pulses at 100 Hz; 100-800 μA) were expressed as a percentage of the response without brain stimulation.

Confirming our previous results, inhibition was greatly reduced or abolished in 6/7 units after methysergide (0.5-1.5 mg/kg, i.v.). For 14 stimulation sites, the average inhibition (21.9%) was significantly reduced (90.9%) after methysergide (paired t-test $p < .005$). Reduction of inhibition was comparable for rostral (medial preoptic) and caudal (periventricular gray) sites. In one unit, inhibition was seen to recover after 90 min. Preliminary results from 14 units in 4 cats pretreated with PCPA (500 mg/kg, 72 h prior to experiment) indicate there is no reduction in inhibition compared to non-pretreated cats.

In addition we have repeated previous experiments (with S. Pretel) describing spinal inhibition from stimulation of the caudate nucleus (Pretel et al., Soc. Neurosci. Abst. 9:788, 1983) and orthodromic activation of raphe-spinal neurons from forebrain stimulation (Guinan et al., Soc. Neurosci. Abst. 9:788, 1983) which were also performed with the flawed stimulation circuit. In 10 cases, contrary to our previous report, no inhibition could be demonstrated from stimulation of the caudate nucleus with bipolar currents of up to 1 mA, while inhibition was generated from more ventromedial sites in the same experiments. We also feel that in some cases the reported forebrain driving of raphe-spinal cells may have been due to current spread and that these latter experiments need to be repeated. Supported by NIH grant NS19330.

190.7 THE EFFECTS OF NEUROTENSIN AND MORPHINE ON BRAIN-STIMULATION ESCAPE. H.S. Wheeling*, T.L. Sullivan*, and A. Pert. (Spon. C.T. Bennett). NIMH, Biological Psychiatry Branch, Bethesda, Maryland, 20205.

Neurotensin, an endogenous tridecapeptide, displays antinocisponsive activity when tested with standard analgesic screening methods. Instrumental escape responding maintained by electrical stimulation delivered to the mesencephalic reticular formation (MRF) has been shown to be responsive to the anti-aversive properties of various opioids administered systemically. Thus, we decided to compare the effects of intracerebroventricularly administered neurotensin and morphine on brain-stimulation escape.

Rats were implanted with bipolar electrode aimed at the MRF and 22 ga. guide cannula aimed 1.5mm dorsal to the lateral ventricle. In a discrete trial paradigm, subjects were trained to press a lever in order to escape from 10s of continuous biphasic electrical stimulation (0.2ms p. width, 0.2ms delay, 0.5ms ISI) delivered to the MRF. Current amplitude was varied according to a modification of the psychophysical method of limits in order to establish an escape threshold. The major dependent variable was the difference between post-treatment and pretreatment escape thresholds.

The administration of morphine (5-15 nmoles, i.v.t.) produced elevations of escape thresholds, as has previously been demonstrated with systemically administered morphine utilizing the same paradigm. In contrast, neurotensin (5-15 nmoles, i.v.t.) not only failed to elevate escape thresholds, but actually lowered thresholds as compared with control injections (0.9% NaCl), suggesting a pronocisponsive effect.

Whether the observed enhancement of aversive brain stimulation by neurotensin is a function of the use of central stimuli or of the measurement of conditioned behavior is unclear. However, our results with this peptide contrast with those obtained by measuring unconditioned responses to peripheral noxious stimuli.

190.8 ELECTRICAL STIMULATION OF THE HABENULA REDUCES TONIC PAIN IN THE FORMALIN TEST. S. R. Cohen and R. Melzack*. Dept. of Psychology, McGill Univ., 1205 Docteur Penfield, Montreal, P.Q., Canada, H3A 1B1.

The habenula connects many limbic forebrain structures with brainstem nuclei known to play an important role in analgesia and is therefore ideally situated to modulate the affective component of pain. We have shown that microinjection of morphine into the area of the habenula/dorsal-posteromedial thalamus produces analgesia in the formalin test, which involves tonic, moderate pain (Pain, Supp. 2, 1984, p. 347). Furthermore, Benabid and Mahieux (Soc. Neurosci. Abst., 1984) report stimulation-produced analgesia from the habenula in the tail-flick test. This analgesia was not present during stimulation, developed slowly, reached a peak 60-80 min post-stimulation, and was reversed by naloxone.

To determine whether habenular stimulation produces analgesia in the formalin test, twisted bipolar electrodes were implanted unilaterally in the habenula of anesthetized rats at least 1 wk prior to testing. Stimulation consisted of monopolar, square-wave pulses of 0.5 msec duration at a frequency of 50 Hz, delivered for 30 sec either once or 10 times over a period of 10 min. Effective current intensities ranged from 50-300 μA.

Electrical stimulation of the habenula reduced pain in the formalin test both during and following stimulation. This effect was not blocked by administration of 5 mg/kg naloxone s.c. The duration and strength of post-stimulation analgesia varied from rat to rat and was also dependent on current intensity. Since testing was stopped 60 min following the formalin injection because baseline pain levels begin to drop after this length of time, it was not possible to determine the maximal duration of the post-stimulation analgesia. However, post-stimulation analgesia was recorded for up to 24 min. While pain was sometimes abolished by habenular stimulation, more frequently the pain was reduced but not eliminated. The unilateral electrode was able to reduce pain in either hindpaw. During the stimulation the rats usually placed the elevated paw on the floor and often became active, walking normally and rearing frequently. Occasionally the rats would urinate during the stimulation. No behavioral effects indicating aversiveness were noted.

These results indicate that habenular stimulation can reduce tonic, moderate pain as well as the phasic, threshold level pain measured in the tail-flick test by Benabid and Mahieux. While the analgesia produced in the formalin test was evident immmediately and was not affected by naloxone, the analgesia reported in the tail-flick test was delayed and completely reversed by naloxone. Whether this difference is due to the different types of pain studied or to different stimulation parameters remains to be determined.

190.9 INHIBITION OF TRIGEMINAL NOCICEPTIVE INPUT BY DORSAL COLUMN STIMULATION (DCst) IN DECEREBELLATE-DECORTICATE CATS. S.F. Atweh, N.E. Saadé*§ and S.J. Jabbur. Fac. of Med., Amer. Univ. of Beirut, Beirut and §Fac. of Sci., Lebanese Univ., Hadath-Beirut, Lebanon.

We have previously shown that DCst in decerebrate and decerebellate cats can modulate pain evoked activities in the spinal cord through a brainstem loop (Brain Res., 310:184, 1984; 335:306, 1985; and in Press 1985). We now demonstrate that ascending activation of the dorsal columns (DC) can also inhibit nociceptive evoked activity in the trigeminal nucleus caudalis (n. caud.) through a brainstem loop. Ten cats were anesthetized (Nembutal or chloralose), immobilized (Flaxedil) and artificially ventilated. They were decerebellated and decorticated through a large craniotomy. The upper cervical cord and lower medulla were exposed through a cervical laminectomy and the C_1-C_4 dorsal roots were sectioned. In two cats, the DCs were dissected as a strand and placed on stimulating electrodes after cutting their caudal ends. In eight cats, the ventral and lateral funiculi were bilaterally lesioned at both C_1 and C_4 levels, leaving the DCs intact; in this preparation (v-cut), the DCs were either stimulated directly through bipolar electrodes placed caudal to the cuts, or indirectly by stimulating the paws. The extracellular activity of 46 neurons in n. caud. were recorded and classified according to their sensory modalities (low threshold mechano-receptive-LTM or wide dynamic range-WDR neurons), their response to tooth pulp (TP) stimulation and their projection (relay or non-relay neurons) to the thalamus. Conditioning electrical stimulation, delivered directly to the DCs or to the paws in v-cut preparations, inhibited 40 (87% of the total) neurons. This inhibition was most marked on the late firing of WDR neurons evoked by nociceptive stimuli to the face or TP, but was also observed on LTM neurons evoked by just-suprathreshold stimuli. Both relay and non-relay neurons were inhibited. In v-cut preparations, stimulation of the forepaws was more effective than the hindpaws and ipsilateral side was more effective than contralateral side.

Thus, our findings show that somatic sensory input triggered by peripheral stimuli and ascending in the DCs can inhibit nociceptive input in trigeminal n. caud. No direct projections have been described between the DCN and the trigeminal nuclei. We have earlier demonstrated, however, DCN inputs into periaqueductal gray and raphe nuclei which have been implicated in stimulus produced analgesia. A DC-brainstem-trigeminal loop can, therefore, account for the findings of this report.

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190.10 **INHIBITION OF SPINAL DORSAL HORN RESPONSES TO NOXIOUS SKIN HEATING BY ELECTRICAL STIMULATION IN SUBCORTICAL FOREBRAIN REGIONS OF THE CAT.** J. Siegel[1], C. R. Morton*, J. Sandkühler*, H.-M. Xiao*, and M. Zimmermann. II Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg, FRG.

Electrical stimulation to regions of the subcortical forebrain produce EEG synchronization, inhibition of motivated behaviors, and inhibition of cellular activity of the rostral brainstem. Electrical stimulation to caudate and septal nuclei and the BFB area produce the above effects as well as analgesia in various species, including man. Antinociceptive effects to stimulation of brain stem structures are due, at least in part, to descending inhibition of spinal dorsal horn neurons. This experiment explored the extent to which descending inhibition also contributes to analgesia from stimulation in forebrain regions.

In 22 cats anesthetized with pentobarbital and N_2O, unit activity was recorded from lumbar dorsal horn cells responding to noxious levels of skin heating. Forebrain sites (A17.0 to 13.5; L0.5 to 6.0; H+8 to -5.0) were mapped for their effectiveness in inhibiting dorsal horn heat-evoked neuronal responses.

The BFB exhibited a rostro-caudal gradient of efficacy; the rostral BFB had fewer inhibitory sites (26% of sites) and weaker effects (inhibition to 80% of control activity) than did sites in the caudal BFB (74% of sites, 50% of control). However, the rostral BFB was more effective in generating EEG synchronization. The dissociation between EEG synchronization and descending effects on spinal neurons was most clear with caudate nucleus stimulation. The caudate was quite effective in generating EEG synchrony but was largely ineffective in inhibiting dorsal horn heat-evoked neuronal activity. Spinal neurons were sometimes inhibited for up to 30 minutes following a single trial of BFB stimulation (50 ms trains of 100 Hz pulses at a rate of 6 Hz for 35 sec).

We conclude that the BFB is the rostral-most extent of a diencephalic-brain stem-spinal nociceptive inhibitory system and that the caudate nucleus achieves its analgesic effects by modulating nociceptive signals at higher supraspinal levels.

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190.11 DISTRIBUTION OF NEUROTENSIN-LIKE IMMUNOREACTIVE FIBERS IN THE PERIACQUEDUCTAL GRAY OF THE RAT. M.T. Shipley, J.H. McLean* and M.M. Behbehani. Departments of Anatomy-Cell Biology and Neurosurgery and Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

It has recently been shown that injection of neurotensin (NT) into the periacqueductal gray (PAG) increases the firing rate of PAG neurons and produces a strong, long-lasting analgesia. Unlike morphine, the NT analgesia is not blocked by naloxone, but like the opiate effect, it is abolished by lesions of the nucleus raphe magnus (NRM) (Behbehani and Pert, Brain Res. 1984). Thus, it is hypothesized that there is a circuit comprised of NT terminals acting upon PAG neurons which in turn activates elements in NMR that project to the spinal cord to particiipate in pain modulation.

In an effort to further elucidate this pathway, we have studied the distribution of NT containing fibers and terminals in PAG using immunocytochemical staining. NT fibers in PAG are extremely fine caliber and in initial experiments we found that neither PAP nor "double" PAP methods provided adequate visualization of these elements. However, improved visualization was obtained when the "ABC" method was combined with osmication of the stained, defatted sections. Additional improvement in the detectability of the NT-like fibers was achieved with an image processing/analysis computer system (Magiscan IIA). Using software developed for this system, distribution maps of NT-like fibers in PAG are being constructed.

NT-like fibers have a markedly heterogeneous distribution in PAG. Although at least some elements are present throughout PAG, the dorsal and dorsolateral parts of the region are only sparsely innervated. The fibers are particularly heavily concentrated in the ventral half of PAG and it appears that the region of highest density corresponds well to the cytoarchitectural boundaries of the dorsal raphe nucleus (DR). The lateral parts of this nucleus have a particularly rich plexus of fibers. A prominent group of fibers courses from the ventral to the dorsal surface around the circumference of the acqueduct, although they fail to reach the most dorsal part of the canal. Fewer, but nonetheless significant, numbers of fibers are present in the mid-lateral parts of PAG. Material sectioned in the horizontal plain shows that the vast majority of NT fibers course longitudinally through PAG.

The regions of highest fiber density coincide with the sites where neurons are sensitive to exogenously applied NT (Behbehani et al., this meeting). Current experiments are aimed at identifying the sources of the NT terminals in PAG. This should provide an anatomical basis for further studies of the neural mechanisms involved in NT mediated central analgesia.

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190.12 SITE SPECIFICITY OF THE ACTION OF NEUROTENSIN IN THE PERIACGUEDUCTAL GRAY: AN IN VITRO STUDY. M.M. Behbehani, J.A. Eppley* and M.T. Shipley. Department of Physiology and Biophysics and Department of Anatomy and Cell Biology. University of Cincinnati College of Medicine, Cincinnati, Ohio 45267-0576.

Behavioral and physiological studies have shown that injection of neurotensin (NT) into the periacqueductal gray (PAG) increases firing rate of PAG cells and produces strong analgesia (Behbehani and Pert, Brain Res. 1984). Anatomical studies have shown that distribution of NT terminals and receptors in the PAG is not uniform. In this study, we examined the action of NT on different parts of the PAG area. Rats weighing 40 to 100 grams were used. Each animal was decapitated, its brain was removed and 400 micron sections were cut throughout the pontin region from intraculicular plane to the level of thalamus. The sections were incubated in a buffered physiological saline solution (PSS) that contained in mM: 124 NaCl, 5 KCl, 26 $NaHCO_3$, 1.2 Na_2CO_3, 2.4 $CaCl_2$, 1.3 MSO4, 10 glucose that was continuously oxygenated with 95% O2 and 5% CO2. After one hour of incubation, a single slice was sandwiched between two nylon meshes and placed into a perfusion chamber. The slice was perfused at a rate of 2 ml/min with oxygenated PSS at room temperature (22°C). Under direct visual observation, extracellular recordings were made from different PAG regions and 4 nmole of NT was bath applied in 10 seconds. In some experiments, after making a recording the cell was penetrated and was filled with HRP. Following these injections, the slice was incubated in oxygenated PSS for at least two hours and the DAB method was used to stain HRP filled neurons. In order to examine the presynaptic action of NT after recording from a cell, the tissue was incubated in PSS containing 3 mM $CoCl_2$ and the response of the cell to NT was measured again.

The responses of 46 neurons to NT were recorded. In the majority of these, NT caused an excitation that lasted for at least 4 minutes. In a few cells, the response to NT was short and lasted only 40 to 60 seconds. Eighty eight percent of the neurons in the ventrolateral part of the PAG responded to NT. Neurons adjacent to the aqueduct were mostly excited by NT (12/14). There was no correlation between the duration of response to NT and their location in the PAG. Both long-lasting and short-lasting excitation was observed at all locations in the PAG. When intracellular recordings were made, NT caused a 20 to 35% decrease in the resting potential. Incubation in $CoCl_2$ did not affect the response to NT.

These results indicate that: 1) the majority PAG neurons are excited by NT; 2) the action of NT involves a post-synaptic mechanism, and 3) there are long and short lasting responses to NT which imply that there may be more than one type of neurotensin receptor. This study was supported by grant NS 20643.

190.13 SUPRASPINAL CONTROL OF THE VISCERAL AFFERENT INPUT TO THE THORACIC SPINAL CORD OF THE CAT. F. Cervero*, B.M. Lumb* and J.E.H. Tattersall* (SPON: European Neuroscience Association). Department of Physiology, University of Bristol Medical School, Bristol BS8 1TD, England (U.K.).

Previous reports from this and other laboratories have shown that a large proportion (60-70%) of neurones in the cat's thoracic spinal cord receive convergent somatic and visceral inputs even though only 7% of all primary afferent fibres entering this part of the cord are of visceral origin. We suggested that the widespread actions of these few visceral fibres are mediated by extensive central divergence through polysynaptic pathways. We have now studied further the pathways that mediate the visceral input to some thoracic spinal cord neurones and the possible involvement of descending influences from the Nucleus Raphe Magnus (NRM) and adjacent areas of the Reticular Formation (Ret. F.).

Experiments were performed on choloralose anaesthetized cats. Extracellular single unit recordings were made in the T9-T11 segments of the cord from seventy four neurones driven by somatic inputs and by electrical stimulation of the splanchnic nerve and/or distension of the biliary system. Descending influences on these neurones were tested by reversible spinalisation with cold block at either the T7 or C3 segments and by electrical stimulation of locations within the NRM and Ret. F.

Sixty neurones (81%) showed changes in their responses to splanchnic nerve stimulation when the animals were spinalised. Two groups of neurones were distinguished:

i) twenty nine cells (39%) showed an increased response during spinalisation which suggests that their spinally mediated visceral inputs were under tonic descending inhibition. Sixteen out of seventeen of these neurones were phasically inhibited by electrical stimulation in the NRM and Ret. F. Nearly half of this group of cells (14/29) were recorded in the dorsal horn, particularly in Lamina V.

ii) thirty one neurones (42%) showed a reduction or complete abolition of their visceral response in the spinal state. This indicates that the visceral input to these neurones was partially or wholly mediated by supraspinal loops since the background activity of such cells was not reduced in a similar manner and in some cases was even increased during spinalisation. Most of these neurones (25/31) were located in the ventral horn. Nineteen out of twenty five were phasically excited by electrical stimulation in the NRM and Ret. F.

These results show that the visceral afferent input to the thoracic spinal cord is under considerable central control which involves tonic and phasic descending excitations and inhibitions from supraspinal regions.

190.14 NEUROPATHIC PAIN AND PARESTHESIAE: MICRONEUROGRAPHY OF ECTOPIC IMPULSE GENERATION FROM PRIMARY SENSORY UNITS IN PATIENTS. J. Ochoa, P. Marchettini*, M. Sivak*, and E. Torebjörk*. Dept. of Neurology, University of Wisconsin Medical School, Madison, WI 53792.

In previous reports we have documented anomalous generation of nerve impulses, from non-receptor sites, as the pathophysiological basis for paresthesiae induced by nerve compression-ischemia in healthy volunteers (Torebjörk et al., Acta. Physiol. Scand., 105:518, 1979; Ochoa and Torebjörk, Brain, 103:835, 1980). Hyperexcitability of comparable character has been reported more recently in a variety of neurological patients that exhibited either mechanosensitive or spontaneous paresthesiae (Ochoa et al., Muscle and Nerve, 5:S74, 1982; Nordin et al., Pain, 20:231, 1984). In such patients the abnormal nerve impulse activity was recorded intraneurally from the peripheral branch of primary sensory units and usually originated distal to the recording electrode. Examples of antidromic propagation from proximal sources were also reported, inclusive of mechanosensitive generator sources in the central branch of primary sensory units in dorsal columns (Sign of Lhermitte, Nordin et al., Pain, 20:231, 1984).

Here we report further microneurographic recordings obtained via intrafascicular tungsten semi-microelectrodes from nerves of patients with pain or paresthesiae of primary sensory unit origin. The impulse activity to be reported was abnormal in that it involved spontaneous bursting discharge in the absence of adequate natural stimulus to receptors, or involved discharge in response to mechanical stress applied midway along sensory axons in nerves or nerve roots. In patients with mechanosensitive paresthesiae the ectopic activity correlated optimally in time both with the delivery of the provocative mechanical maneuver (Sign of Tinel, Sign of Spurling) and with the verbalized sensory experience. In patients with spontaneous neuropathic pain it was exceptional to identify the source of ectopic discharge or the kind of nerve fibers involved. Despite a low yield, the microneurographic technique may provide a definitive document on the pathophysiology of positive phenomena of sensory unit origin.

191.1 BRAIN CELLS THAT COMMAND SEX IN THE SNAIL. R. CHASE (SPON: P.D. Evans). Department of Biology, McGill University, Montreal, Quebec, H3A 1B1, Canada.

The mesocerebrum of Helix aspersa is a locus for the integration of sensory information and the issuance of motor commands to the organs of reproduction. There is a substantial asymmetry of structure and function in the mesocerebrum on the right and left sides of the brain, consistent with the location of the reproductive organs on the right side. The population of neurons on the right side numbers 138 + 27 cells (N=7), with mean diameters of 77 + 13 µm (N=140). The left side has 19% fewer cells and they are 19% smaller. Spatial convergence of excitatory sensory inputs, from as many as 7 peripheral nerves, is the dominant feature of synaptic organization for neurons on the right side and, to a lesser extent, for those of the left side . The rapid temporal depression of synaptic responses is also characteristic. All neurons in the right mesocerebrum send an axon into the ipsilateral cerebropedal connective, but the trajectory of axons on the left side is more variable, with many traveling in the contralateral connectives.

Intracellular stimulation of neurons in the right mesocerebrum can produce contractions of the "love dart" sac, the penis sheath, or both (17% of cells tested). In an intact animal, these movements would result in the release of darts and the eversion of the penis. The latencies of the evoked responses are 6-12 secs. In some cells, stimulation for 5 sec causes repeated complex contractions lasting over several minutes. Extracellular stimulation of the right mesocerebrum, but not the left, causes vigorous concurrent contractions of both the dart sac and the penis. All the evoked motor responses are mediated through the right cerebropedal connective and the pedal nerve, NCPD (Schmalz, 1914). Neural activity in NCPD is rhythmically excited for several minutes following a 0.5 sec stimulus to the right mesocerebrum. The motorneurons for the described behaviors are presumed to reside in the right pedal ganglion.

191.2 OPTICAL MEASUREMENTS, USING ABSORPTION, OF ACTIVITY FROM NAVANAX BUCCAL GANGLIA DURING SPONTANEOUS PHARYNGEAL EXPANSION AND DURING FEEDING. D. Zecevic, J.A. London and L.B. Cohen. Dept. of Physiology, Yale Univ. School of Medicine, New Haven, CT 06510, U.S.A.

We are investigating the use of optical methods to simultaneously measure action potential activity from many cells in the nervous system of a minimally dissected opisthobranch mollusc, Navanax inermis. Using a modified whole animal preparation we were able to monitor activity in the buccal ganglion, a ganglion with about 200 neurons. Animals in this preparation exhibited spontaneous and food-induced expansions of the pharynx. A 1 cm slit was made in the ventral body wall and in the pharynx immediately under the buccal ganglion. One end of a light pipe was inserted through a small hole in the dorsal side of the pharynx and dorsal body wall. The preparation was positioned on the stage of a microscope so that the light guide brought the incident light from the microscope condenser to the buccal ganglion. An enlarged image of the ganglion was formed on a 124 element photodiode array. The ganglia were stained with a 0.5 mg/ml solution of the pyrazo-oxonol dye RH155, kindly provided by R. Hildesheim and A. Grinvald. Action potential signals were obtained in experiments where no significant pharmacologic effects or photodynamic damage was observed. We monitored activity in the buccal ganglion during spontaneous expansions and during feeding, and detected activity of between 10 and 30 neurons.

In the best analyzed experiment, 23 different neurons were found to be active during feeding with phases of the neuronal activity clearly correlated with specific feeding movements. Combined morphological evidence and optical monitoring during nerve stimulation suggests that these recordings were at least 70% complete. It appears that more neurons are active during feeding than during spontaneous expansions. In addition, in repeated recordings of the same preparation, it appears that there is a core of neurons consistently active and another population of neurons, putative sensory neurons, which in general is active, but specific neurons in this population may or may not be active. The activity of relatively large neurons was detected on several adjacent photodetectors. In some cases this activity could be associated with identified neurons in the ganglia by superimposing an outline of the array elements with the activity on a photograph of the ganglion.

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191.3 A VOLTAGE CLAMP ANALYSIS OF EGG-LAYING HORMONE AND SEROTONIN EFFECTS ON THE IDENTIFIED MOTONEURON B16 OF APLYSIA. M.D. KIRK, S.H. THOMPSON AND R.H. SCHELLER. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

The identified buccal ganglion motoneuron, B16, innervates the accessory radula closer muscle of the buccal mass in Aplysia. Previously, it had been shown that the neuropeptide egg-laying hormone (ELH) excites B16 directly at nanomolar concentrations in a dose-dependent manner. Bath application of ELH causes the B16 membrane potential to slowly depolarize, and the neuron begins to fire tonically with a latency of less than four minutes. The B16 membrane potential usually returns to the control condition within 30 minutes after washing away the ELH. Serotonin (5-HT), at micromolar concentrations, excites B16 in a manner similar to that of ELH, and forskolin mimics both of the transmitters' actions suggesting that these effects may be mediated by cyclic AMP.

We have performed a voltage clamp analysis of the ELH and 5-HT effects on B16. The membrane poential was clamped to various holding voltages (V_H) and ten millivolt hyperpolarizing pulses (2 sec duration) were repetitively applied during bath application of ELH (50-500 nM) or 5-HT (50-100 µM). Little or no response was evident with V_H below -50 mV. However, for V_H between -40 mV and -15 mV, bath application of the transmitters caused a 1-2 nA slow inward shift in holding current. Within this range of V_H, the current responses to the ten millivolt hyperpolarizing pulses changed from inward, in the control condition, to a steady state outward current during the action of ELH or 5-HT. Thus, a negative resistance region was induced within this range of V_H. Steady state I-V curves revealed that a slow inward current (SIC) appeared within the membrane potential range where the maximal effects of ELH and 5-HT were found. Difference I-V curves reflected this increase in inward current, however, above -15 mV the I-V curves did not differ. The SIC was seen with reduced amplitudes before the transmitters were applied. When Na^+ was replaced with equimolar TRIS, the SIC was nearly eliminated, and the response to the transmitters was reduced or eliminated. The response to ELH and 5-HT was not due to effects on I_A or $I_{K'}$ and the response persisted in solutions with 0 mM Ca^{++}, 10 mM Co^{++}.

We have not eliminated the possible contribution of the I_S potassium channels or other voltage-dependent potassium channels in the response of B16 to ELH and 5-HT. Presently, we are testing the reversal potential of the B16 response for its sensitivity to K^+, Cl^- and Na^+.

191.4 CEREBRAL-BUCCAL INTERNEURONS WHICH INITIATE FICTIVE FEEDING IN LIMAX MAXIMUS. K. Delaney* and A. Gelperin, Dept. Biology, Princeton University, Princeton, NJ 08544 and Dept. Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974.

We are characterizing interneurons in the cerebral ganglion (CG) of the land slug Limax maximus which respond to lip chemostimulation and are capable of triggering feeding motor program (FMP) in vitro. We have identified several putative 'trigger interneurons' whose somata are located near the insertion points of the lip nerves which convey afferent taste and mechanoreceptive input.

All the feeding interneurons we have studied belong to a population of about 16 cells in the lateral CG which have a single descending axon in the ipsilateral cerebro-buccal connective. We call these cells cerebral-buccal interneurons (CBIs). These CBIs have extensive branching in ipsilateral lobes of the CG and varicose branches throughout the ipsi- and contralateral buccal ganglia. One CBI, CBI_2, also crosses the midline to branch in the area of the insertion points of the contralateral lip nerves. Using a lip-brain preparation, the activity of CBIs was monitored and controlled by an intracellular electrode while FMP responses were measured by extracellular recordings from multiple buccal nerve roots. CBI_1 and CBI_2 respond with a 15-20mv short latency depolarization to applications of carrot juice to the ipsilateral lip. This depolarization produces a several second burst of action potentials which is interrupted during FMP by a strong hyperpolarization in phase with the protraction burst in buccal roots. The activity of the CBIs declines even while the food extract is still on the lip and while the bite rate is increasing. Depolarizing current injection of .3-.6nA produces rapid firing of CBI_1 and CBI_2 and generates FMP. As with lip stimulation the CBIs receive a strong hyperpolarizing input which interrupts their firing during the phase-locked bursting seen in buccal roots during protraction. The CBIs are not normally active during later parts of the FMP bout but receive cyclical depolarizations and hyperpolarizations in phase with the buccal rhythm. Short bursts of CBI action potentials interpolated between bites can phase advance the bite rhythm while prolonged activation over several cycles first excites, then slows FMP, which speeds up when stimulation ends.

Thus, CBI_1 and CBI_2 are excited by taste stimuli applied to the lips and are capable of initiating FMP in vitro. These cells are most active shortly after chemostimulation of the lips and although they can influence the rate of FMP they are not necessary for the production of bites later in a bout of FMP. The CBIs thus appear to serve an initiating or 'trigger' function. We intend to examine the responses of these cells to applications of a taste paired with an aversive stimulus in vitro to see if they represent a locus for change following associative conditioning. (Supported by NIMH 39160 and an NSERC, Canada, postgraduate scholarship to KD.)

191.5 SIMULTANEOUS OPTICAL RECORDING FROM MANY CELLS FROM APLYSIA ABDOMINAL GANGLIA DURING THE GILL-WITHDRAWAL REFLEX. J.A. London, D. Zecevic and L.B. Cohen, Dept. of Physiology, Yale Univ. School of Medicine, New Haven, CT 06510, U.S.A.

We are studying the possibility of using optical measurements for monitoring membrane potential changes in many neurons from Aplysia abdominal ganglia. We began with fluorescence measurements from ganglia stained with several styryl dyes. In experiments, with RH386 and RH725 (provided by R. Hildesheim and A. Grinvald) the signal-to-noise ratios during individual action potentials were about 10. Measurements of bleaching indicated that illumination of the ganglion for approximately 60 seconds would reduce the signal size by 50%. This period of illumination also led to a reduction in the number of neurons that could be activated by nerve stimulation. In absorption experiments similar signal to noise ratios were measured using the merocyanine dye RGA525 (provided by A.S. Waggoner) or the pyrazoloxonol dye RH155 (Nippon Kankoh-Shikiso Kenkyosho, Co.). With RH155 neither bleaching nor photodynamic damage were detected after 300 seconds of illumination.

An isolated siphon preparation (Kupfermann, Carew and Kandel, J. Neurophysiol.,1974) was modified for use in the optical monitoring system with the intent of observing simultaneous activity from a large number of neurons from the abdominal ganglion during behavior. An isolated siphon, gill and abdominal ganglion were placed in a recording chamber positioned on a microscope stage. The ganglion was stained with a 1 mg/ml solution of RH155 for 30 minutes. In a preliminary experiment, electrical stimulation of the siphon skin was followed by gill contraction. Simultaneous optical measurements from the left side of the abdominal ganglion (dorsal side up) indicated that many neurons were actived by the stimulation. Repeated stimulation of the siphon seemed to reduce this activity. After a short wait, the activity partially recovered. We hope that optical methods can be a useful tool in studying the neuronal interactions involved in the gill-withdrawal reflex.

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191.6 NEURALLY ACTIVE PEPTIDES ACTIVATE CENTRAL MODULATORS OF THE GILL WITHDRAWAL RESPONSE IN APLYSIA. Ken Lukowiak* and A. Don Murphy (SPON: W.D. Ruwe) I.O.M. Tribhuvan University, Kathmandu, Nepal and Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Neurally active peptides endogenous to Aplysia's CNS affect the amplitude of the gill withdrawal reflex (GWR) evoked by tactile stimulation of the siphon in vitro. The superfusion of arginine vasotocin (AVT) and Met-enkephalin over the abdominal ganglion cause a significant reduction in the amplitude of the evoked GWR, while FMRFamide and SCP_B facilitate the GWR. These substances may, therefore, determine the preparation's level of arousal. The state of the animal just prior to dissection affects non-associative and associative learning of the preparation; it is, therefore, important to understand how these peptides affect the GWR and associated behaviors. Among many possibilities, these peptides may: 1) directly influence the sensory-motor neuron synapse or 2) act on central cells exerting either facilitatory or suppressive control over the PNS of the gill. Evidence for the first hypothesis is available, but possibility 2) has not yet been tested. This series of experiments tests the second hypothesis and shows that the neurons which exert both facilitatory and suppressive control over the PNS are affected by these substances. If the peptides alter the balance of facilitatory and suppressive control the CNS exerts over the PNS, then the ability of a central motor neuron to elicit a gill movement will be affected. Motor neurons L_7, LDG_1, or L_9 were impaled and the amplitude of a gill movement, elicited by a constant number of action potentials evoked by depolarizing current (ISI = 20 min), was measured before, during, and after superfusion of the test peptide over the abdominal ganglion. Two peptides were tested: SCP_B and AVT.

Superfusion of the abdominal ganglion with SCP_B (10^{-6}M) increased the L_7-dependent gill movement to 315% (n=9). Following 1 hr washout, the response was still 147%. Stimulation of LDG_1 in the presence of SCP_B elicited an increase of 312% (n=3) and recovered to 126% following 1 hr washout. L_9's efficacy in eliciting contractions was not affected by SCP_B (n=2). By contrast, superfusion of 10^{-6}M AVT reduced contractions elicited by L_7 to 20% of control (n=3) and by LDG_1 to 29% of control (n=3). One hour washout was sufficient to completely reverse AVT's effect in all cases. Again, L_9 was unaffected by this peptide.

Thus, the superfusion of these peptides affected the ability of a central gill motoneuron to move the gill. Because only central motor neurons were depolarized in these experiments, the most likely explanation for these results is that the central neurons modulating the PNS are affected by these two peptides, independent of the peptides' effects on the central sensory-motor synapse. (Supported by the MRC.)

191.7 THE FORMS OF SPONTANEOUS AND EVOKED GILL MOVEMENTS IN APLYSIA. J.L. Leonard* and K. Lukowiak* (SPON: L.E. Muske). Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alberta T2N 1N4, Canada.

The amplitude of the gill-withdrawal reflex (GWR), the response of the gill of A. californica to a mechanical stimulus to the siphon, is, in in vitro preparations, dependent on the behavior of the animal prior to dissection and can be modulated by a number of endogenous neuropeptides (Lukowiak and Murphy 1985). In order to understand the neural control of the GWR, it is necessary to characterize the form(s) of evoked and spontaneous movements of the gill.

We have identified four types of movement of the whole gill. These are: 1) Roll. The gill rotates along its longitudinal axis, so that the tips of the pinnules describe an arc away from the mantle and toward the midline of the body. The maximum amplitude of Roll is about 90 degrees. 2) Shorten. The base of the gill contracts slightly. 3) Lift. The distal end of gill is brought away from the mantle. 4) Pinnule Contraction. This was described by Kupfermann et al (1974) and consists of a coordinated reduction in length of the pinnules. In addition, we have confirmed the observations of Kupfermann et al. (1974) on the movements of gill halves and pinnules. Any of these movements occurs in a graded fashion and the movements occur in a variety of combinations.

The gill can respond to siphon stimulation in two distinct ways; directly, with a short latency response, or with a long-latency response which closely resembles spontaneous gill movements (SGMs) and which is presumably mediated by the Interneuron II network. The usual short-latency response of the gill to a mechanical stimulus to the siphon involves a Roll, often in combination with Shorten and some degree of Pinnule Contraction. In some cases, particularly in suppressed preparations, Roll may be the only response. The form of response in a particular preparation is the same with or without the abdominal ganglion present. The largest and most common (seen in the greatest number of preparations) spontaneous gill movement involves a tightly coordinated combination of Roll, Shorten, Lift, and Pinnule Contraction. We suggest that the term SGM be reserved for this movement. It is always large but does vary somewhat in amplitude primarily due to a variable degree of Pinnule Contraction. Such movements can occur in response to siphon stimulation. A variety of other movements can occur spontaneously, including Rolls, Lifts, either of the those combined with Shorten, and/or the types of pinnule movements described by Kupfermann et al. (1974).

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191.8 INTERNEURONS CONTRIBUTING TO THE MEDIATION AND MODULATION OF THE TAIL WITHDRAWAL REFLEX IN APLYSIA. L.J. Cleary and J.H. Byrne Dept. of Physiol. and Cell Biol., Univ. of Texas Med. Sch. Houston, Texas 77225.

Tail withdrawal is one of several defensive behavior in Aplysia that have been utilized to study the synaptic changes underlying behavioral modifications. The sensory and motor neurons which conduct the tail withdrawal reflex have been identified, yet little is known of the contributions made by interneurons.

We have begun to study a population of interneurons in the pleural ganglion which form connections with motor, sensory and other interneurons. Target neurons are located in the pedal, pleural and cerebral ganglia. Recordings were obtained from 288 pleural interneurons. Of these, 78 are excitatory to putative tail motor neurons in the pedal ganglion, and 48 are inhibitory. Seventeen cells produce a mixed response.

Two types of responses in the motor neurons are produced by the excitatory interneurons. The first type is a fast, short latency EPSP evoked by a single spike in the interneuron. The second is a slow, long latency EPSP evoked by a brief burst of spikes. On average, this response peaks at 2.3 sec, lasts for 42 sec and is associated with a decreased input conductance. The slow EPSP can modulate the effect of sensory input to the motor neurons. Weak nerve shocks which normally generate subthreshold EPSPs can trigger spikes during the slow EPSP. In some cases, the slow EPSP itself produces a prolonged discharge of spikes in the motor neuron.

Excitatory interneurons share several characteristics. First, they receive excitatory input from sensory neurons. Second, they inhibit sensory neurons. Third, they excite neurons in the cerebral B cluster. Fourth, after HRP injection, three major branches are observed: one to the motor neuron region of the pedal ganglion, a second to the cerebral ganglion, and a third to the pleural-abdominal connective. There is also a small dendritic arborization within the pleural ganglion itself.

The excitatory interneurons may act via a feed-forward pathway to amplify the withdrawal response to noxious stimulation of the tail. In addition, they may provide a means by which a brief afferent volley is transformed into the prolonged motor neuron discharge and consequent tail withdrawal that is typically observed in response to brief tail stimulation. The connections between excitatory interneurons and motor neurons may also be an additional site for the synaptic plasticity underlying behavioral sensitization and associative learning. Finally, excitatory interneurons may be responsible for the integration of tail withdrawal with other defensive responses triggered by stimulation of the tail such as locomotion, inking and gill and siphon withdrawal.

191.9 CHANGES IN CELLULAR EXCITABILITY IN A NEW CLASS OF SIPHON MOTOR NEURONS DURING SENSITIZATION IN APLYSIA. W. N. Frost , G. A. Clark, and E. R. Kandel. H. Hughes Medical Institute and Ctr. for Neurobiol. & Behav., Columbia Univ., P & S, and NYS Psychiatric Instit., New York, NY 10032.

Sensitization and classical conditioning of the gill and siphon-withdrawal responses in Aplysia involve a cAMP-dependent decrease in a specific K^+ channel in siphon sensory cells. Closure of the K^+ channel can broaden each action potential and thereby enhance transmitter release at the terminals (Hochner et al., 1985; Belardetti et al., 1985). In addition, K^+ channel closure can result in an increase in the probability and frequency of action potential discharge (Klein and Kandel, 1978; Hochner et al., 1985).

If similar mechanisms were to occur in postsynaptic cells, one might expect such cells to exhibit an increase in tonic firing rate and increased discharge to a given synaptic input. Previous studies of the several known classes of gill and siphon motor neurons did not reveal changes in excitability. We have recently discovered in the abdominal ganglion a new group of siphon motor neurons (the LF_s cells) whose interneuronal connections differ from other previously identified motor neurons. At rest, these cells are tonically active at rates below threshold for eliciting siphon contraction. Sensitizing stimuli to the tail or connective stimulation produce, in most of these cells, an increase in tonic firing lasting several minutes as well as an apparent increase in input resistance (Clark & Kandel, 1984). The increase in tonic firing rate can amplify siphon contractions produced by constant spike trains in the LF_s cells, perhaps due to the facilitating effect of tonic firing rate on release from the motor neuron terminals (Jacklett & Rine, 1977).

To compare the mechanisms of the excitability change in the LF_s siphon motor neurons to those of the sensory neurons, we applied either 8-benzylthio cAMP (a membrane-permeable cAMP analog) or IBMX (a membrane-permeable phosphodiesterase inhibitor) extracellularly. We also injected cAMP intracellularly. All of these treatments produced a depolarization and increase in tonic firing rate in the LF_s cells, thereby simulating sensitizing stimuli. We are now exploring whether this depolarization is due to a decreased K^+ conductance, as occurs in siphon sensory cells.

Although the evidence for cAMP mediation is not complete, these preliminary data suggest that the mechanisms underlying plasticity in the sensory cells may contribute to changes in excitability of a subgroup of siphon motor neurons. Therefore, sensitization of this reflex may involve the coordinated regulation of cellular excitability at several different circuit elements -- facilitation of sensory neurons, disinhibition of excitatory and facilitatory interneurons (Frost and Kandel, 1984), and increased excitablity of LF_s motor neurons -- perhaps using a common molecular mechanism: cAMP-dependent protein phosphorylation of ion channels.

191.10 **DEVELOPMENT OF LEARNING AND MEMORY IN *APLYSIA*: I. FUNCTIONAL ASSEMBLY OF THE GILL AND SIPHON WITHDRAWAL REFLEX.** T. J. Carew, C. H. Rankin* and M. Stopfer* Dept. of Psychology, Yale University, New Haven, CT 06520

Learning and development are two areas of major importance in Neurobiology. We have combined these two areas in an analysis of the development of learning and memory in *Aplysia californica*. We have focussed on the defensive withdrawal reflex of the gill and siphon, for it displays associative and nonassociative learning, both of which can exist in short and long-term forms.

In adults the gill and siphon show two kinds of contractions: (1) endogenous and (2) elicited. To permit developmental comparisons we first characterized the quantitative relationship between gill and siphon withdrawal in the adult. Animals were restrained and both spontaneous and elicited contractions were videotaped and digitized for subsequent computer analysis. During elicited and spontaneous contractions the siphon and gill always contracted together and the responses were highly correlated both in amplitude and duration.

We next examined the assembly of the siphon and gill withdrawal components in 3 early juvenile developmental stages: 9, 10, and 11 (Kriegstein, 1974). The siphon first appears in Stage 9, the gill in Stage 10. Animals of these stages (.05 - 4 mm in length) were restrained with suction electrodes and videotaped under a stereomicroscope. As soon as the siphon develops in Stage 9, it exhibits both elicited contractions to brief tactile stimuli and spontaneous contractions.

The gill emerges in Stage 10 and immediately exhibits spontaneous contractions. In early Stage 11 two types of gill contractions are evident: (1) high frequency twitches (40/min); and (2) lower frequency spontaneous contractions (2 - 4/min). The twitches appear to be the passive result of blood-flow since they are correlated 1:1 with heart beat and are abolished by surgically disconnecting the heart. Interestingly, although spontaneous contractions of both gill and siphon are present at this stage, they appear to be relatively independent, occurring synchronously only 20% of the time. In contrast, in late Stage 11 animals spontaneous contractions resemble the adult form (*ie* siphon and gill co-contract). These data suggest that early in development there are independent oscillators for the siphon and gill (perhaps sub-components of INT. II), which become coupled in late Stage 11. Elicited gill contractions to siphon stimulation also progressively develop during this stage.

In conclusion, both spontaneous and elicited siphon and gill components of the withdrawal reflex are functionally assembled in Stages 9-11 of development. In the two companion abstracts behavioral and neuronal studies of the development of learning and memory in this reflex system are described.

191.11 **DEVELOPMENT OF LEARNING AND MEMORY IN *APLYSIA*: II. HABITUATION, SENSITIZATION AND SHORT TERM MEMORY** C. H. Rankin* and T. J. Carew (Spon: M.Constantine-Paton) Dept. of Psychology, Yale University, New Haven, CT. 06520

The developing gill and siphon withdrawal reflex of *Aplysia* provides an excellent system in which to analyze the development of a variety of forms of learning and memory. In our initial studies of this system we have examined the development of two forms of nonassociative learning, habituation and sensitization, in the siphon component of the reflex in early juvenile *Aplysia* (Stages 9-11; Kreigstein, 1974; Carew *et al*, this vol.).

Animals (0.5-4 mm in length) were restrained under a stereomicroscope with suction electrodes and the siphon was stimulated with a brief quantifiable water-jet. A suction electrode was attached to the tail to deliver a sensitizing stimulus. The behavior was measured by videotaping and digitizing the response for subsequent computer analysis. In these studies we examined whether different forms of learning (habituation and sensitization) and different forms of memory emerge at different stages of development. To examine short-term memory we used three different inter-stimulus intervals (ISIs: 10, 5 and 1 sec) during habituation.

Stage 11 animals exhibited clear habituation at all 3 ISIs, with progressively greater decrement with shorter intervals. As in adults, sensitization was evident at this stage. Stage 10 animals showed little habituation at a 10 sec ISI, but clear and progressively greater decrement with 5 and 1 sec ISIs. Sensitization was also evident at this stage. Finally, Stage 9 animals showed no habituation at either 10 or 5 sec ISIs; only a 1 sec ISI produced decrement. Moreover, in contrast to Stages 10 and 11, sensitization was completely absent at Stage 9.

Thus our results show that different forms of learning emerge at different stages of development: habituation is present in the youngest animals we tested (Stage 9), while sensitization emerges at a distinct and later stage (Stage 10). Memory also appears to develop systematically: greater habituation occurs at progressively longer intervals during development, suggesting that at successive stages short-term memory is progressively more mature.

In addition to nonassociative learning, *Aplysia* has been shown capable of classical and, quite recently, operant conditioning (Cook and Carew, this vol.). Therefore it will now be possible to determine the developmental timetable of associative learning in this system, as well as the emergence of long-term memory for these various forms of learning. Thus development in *Aplysia* can serve as a valuable tool for analyzing the nature of different components of learning and memory as they emerge and are assembled into their adult form.

191.12 **DEVELOPMENT OF LEARNING AND MEMORY IN *APLYSIA*: III. CENTRAL NEURONAL CORRELATES.** T.G. Nolen, E. Marcus*, T.J. Carew. Dept. of Psychology, Yale University, New Haven, CT 06520.

The defensive gill and siphon withdrawal reflex of *Aplysia* has been useful for gaining insights into the neural mechanisms of different forms of learning and memory. Since it is now possible to study the development of learning and memory in this reflex (Rankin and Carew, this vol.), we have begun to explore neural correlates of learning at different stages of development. As a first step in this analysis, we examined the central control of the reflex in two preparations: (1) semi-intact, and (2) isolated central nervous system (CNS).

Semi-intact: the gill of Stage 11 animals (2-4 mm in length) was removed leaving it attached to the abdominal ganglion by the branchial nerve. The afferent siphon nerve was electrically stimulated (with a single 5 ms pulse) by a suction electrode and the amplitude of the evoked gill contraction was measured by videotaping and digitizing the response for subsequent computer analysis. A 2.5 sec train of facilitating stimuli was delivered by a suction electrode to one of the pleuroabdominal connectives. Stimulating the siphon nerve reliably evoked a contraction of the gill which completely decremented within 15 successive stimuli (ISI=30 sec). Stimulating the connective facilitated the evoked gill response. Behavioral experiments in intact Stage 11 animals show that habituation is more profound with shorter ISIs (Rankin and Carew, this vol.). Similarly, the evoked gill response decremented more rapidly with a shorter ISI (10 sec). These results show that both centrally-projecting siphon afferents and facilitatory inputs make functional connections with gill motor neurons in Stage 11 animals.

Isolated CNS: we have identified neural correlates of habituation and sensitization in the isolated abdominal ganglion by recording the efferent output (spike number and PST histogram) of the branchial nerve in response to siphon nerve stimulation. Using the same parameters as in the semi-intact preparation, the evoked branchial nerve response decremented with repeated stimuli and was facilitated following stimulation of the connective. As in the behavior and the semi-intact preparation, decrement was greater with a 10 sec than with a 30 sec ISI.

In summary, our data show that in Stage 11 *Aplysia* a component of the withdrawal reflex, as well as its habituation and sensitization, is centrally mediated. Similar studies are being carried out in Stages 9 and 10. These studies, coupled with an intracellular analysis in the CNS of juvenile *Aplysia* at different stages, will permit us to investigate the developmental changes which underlie the emergence of different forms of learning and memory in an identified neural circuit.

192.1 A SET OF PROTEINS ENRICHED IN SMALL SYNAPTIC VESICLES FROM MAMMALIAN BRAIN. E. Floor, S.F. Schaeffer*, and S.E. Leeman. Dept. of Physiology, Univ. Massachusetts Med. Sch., Worcester, MA 01605.

Small synaptic vesicles were substantially purified in modest quantities (3-4mg) from mammalian brain. Rat or cow brain (~75g) was homogenized in a 0.3M sucrose buffer, the homogenate osmotically shocked by 1:1 dilution with water, and the solution returned to isotonicity with KCl after 1min. Debris was removed by centrifugation at 7,500xg and particles in the resulting supernate pelleted at 50,000xg. The particulate material was resuspended in a 0.15M KCl buffer and sedimented to equilibrium on an isoosmotic 10-30% linear Nycodenz/KCl gradient. Material just denser than the bulk membrane bands was collected and fractionated by size on a calibrated, 400ml column of CPG-3000 controlled-pore glass beads.

Particles ~50nm in diameter based on the size calibration contained a distinctive set of about a half dozen major proteins of molecular weights about 33, 38, 41, 57, 62, and 76kd by SDS gel electrophoresis. These proteins were present in similar relative abundances in ~50nm vesicles from rat or cow brain or from rat brain synaptosomes but were not present in rat liver membranes purified by the same protocol. By electron microscopy >50% of the particles in column fractions richest in these proteins were spherical vesicles about 50nm in diameter. These vesicles were bounded by a trilamellar unit membrane and were electron lucent. A synaptic vesicle-specific monoclonal antibody (antibody 48) isolated by W. D. Matthew et al. (J. Cell Biol. 91, 257) selectively immunoprecipitated vesicles with this set of proteins in a procedure utilizing second antibody-coated beads. When large membranes from the void volume of the CPG column were added to the small vesicle preparation, they were not immunoprecipitated by antibody 48. In summary, the ~50nm vesicles studied here were identified as synaptic vesicles by their size, tissue specificity, presence in nerve endings, and synaptic vesicle-specific surface antigen.

At present the identities of the small synaptic vesicle proteins are not known. By molecular weight or immunoblotting these proteins are not actin, synexin, or the synaptic phosphoproteins synapsin Ia or Ib, or protein IIIa or IIIb. The possible identities of the 62kd protein with the antigen recognized by antibody 48 or of the 57kd protein with tubulin have not yet been tested. The 41 and 62kd synaptic vesicle proteins appear to be integral membrane proteins as indicated by partitioning into the detergent-rich phase of Triton X-114 solutions. The 33, 38, 57 and 76kd proteins distributed between the two detergent phases.

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192.2 ARE PRESYNAPTIC CALCIUM STORES PRESENT IN CENTRAL NERVOUS SYSTEM SYNAPSES? S.B.Andrews*, R.D.Leapman*, D.M.D.Landis and T.S.Reese (SPON: J.Fex). NINCDS and DRS, NIH, Bethesda, MD 20205 and The Marine Biological Laboratory, Woods Hole, MA 02543.

We have used quantitative elemental imaging and microanalysis of freeze-dried cryosections of selected, rapidly-frozen, synaptic preparations to address the question of the presence, identity, and activity of putative calcium storage organelles in nerve terminals. This approach permits an exhaustive, unbiased sampling of the calcium distribution in synaptic structures without the complicating influences of chemical fixation or organelle isolation.

In vitro preparations of two diverse central nervous system synapses were studied, namely, the synapses of parallel fibers with Purkinje cell spines within excised hemispheres of the molecular layer of mouse cerebellar cortex, and the predominantly cholinergic presynaptic synaptosomes from the optic lobe of the squid. Following incubation under control (normal Ringer) or stimulating (50 mM K^+) conditions, samples were rapidly frozen against a liquid helium-cooled copper block. The outer layer of well-frozen material was then prepared for imaging and analysis by ultracryomicrotomy at -135°C followed by controlled freeze-drying, or for structural analysis by freeze-substitution or by freeze-fracture. Analytical electron microscopy was carried out by means of a recently developed, computer-controlled instrument that is capable of simultaneously acquiring digital scanning transmission (STEM) and quantitative elemental (x-ray) images.

Imaging and microanalysis of unstimulated cerebellar slices revealed no pre- or postsynaptic organelles larger than 40 nm in diameter that contained calcium at a concentration greater than 5 mmol/kg wet weight; this conclusion extends to morphologically identified parallel fiber synapses. However, following a brief (less than two min) stimulation of resting cerebellar slices with a depolarizing, high-potassium solution, numerous sites of focal calcium accumulation (typically less than 200 nm diameter and containing ca. 30 mmol/kg wet weight Ca) were evident. Thus, calcium is detectable under these preparative conditions and a calcium-sequestering system is present which is loaded by neuron activity. Similar results were obtained with squid synaptosomes; synaptosomes that had enough internal potassium to qualify as functional models of presynaptic endings had calcium concentrations less than 5 mmol/kg in synaptic vesicles and in other organelles larger than 40 nm diameter. Thus, the concentration and the distribution of calcium do not support the notion that synaptic vesicles function as a primary calcium storage site. However, the results are consistent with the presence of a calcium sequestration system as an integral part of synaptic transmission.

192.3 SYNAPSIN-I-LIKE IMMUNOREACTIVITY IN THE NERVOUS SYSTEM OF APLYSIA CALIFORNICA. M.E. Bongiðvi, R.T. Ambron, A.-J. Silverman, M. Flaster and P. Greengard. Dept. of Anatomy and Cell Biology, Columbia Univ., NY, NY 10032 and Dept. of Molecular and Cellular Neuroscience, Rockefeller Univ., NY, NY 10021.

Synapsin-I, a phosphoprotein associated with synaptic vesicles in the mammalian nervous system is postulated to play a role in synaptic vesicle fusion and neurotransmitter release (De Camilli, et al., JCB, 1983). We have investigated the distribution and biochemical nature of synapsin-I-like antigens (SILA) in the nervous system of the marine invertebrate Aplysia californica. Polyclonal antibodies directed against bovine brain synapsin-I combined with light microscopic peroxidase cytochemistry was used to localize SILA in vibratome sections of the abdominal ganglion and body wall. SILA are present in neuronal cell bodies and axons and at presumptive terminals in the neuropil. Immunoreactive neurons displayed various staining patterns. In some cells the entire cytoplasm of the soma and axon hillock contained reaction product, while in others, discrete perinuclear staining was observed. Still other neurons exhibited punctate staining on the periphery of their soma, a pattern expected of axo-somatic synaptic contacts. In the neuropil, the major site of synaptic transmission in invertebrates, punctate staining was present. In order to determine the subcellular localization of SILA, EM immunoperoxidase studies were initiated. Preliminary results indicate that SILA are associated with vesicles, both in axons and in varicosities in the neuropil.

To associate SILA with identified cells, we first examined the body wall which contains gland cells innervated by the giant neuron, R2 of the abdominal ganglion (Rayport, et al., J. Neurophysiol., 1983). Patches of staining are present on some glands. Others were enveloped completely by immmunoreactive axons. Differences in staining intensity and pattern may represent underlying differences in the patterns of innervation of the glands by R2. The pair of serotonergic giant neurons of the cerebral ganglion (GCN) were also found to be immunoreactive.

The molecular weights of the immunoreactive species were determined by immunoprecipitation of ^{32}P- or ^{35}S-methionine labelled Aplysia nervous tissue with the anti-synapsin-I antibodies. Proteins of 57kD and 67kD were found by SDS-PAGE analysis to be precipitated selectively. These are in the M_r range reported for synapsin-I-like molecules by Goelz and colleagues (J. Neurochem., in press) in a survey of various vertebrate and invertebrate species. Our biochemical and immunocytochemical studies suggest, therefore that a synapsin-I homologue is present in Aplysia neurons.

192.4 ASSOCIATION OF SYNAPSIN I WITH NEURONAL CYTOSKELETAL PREPARATIONS. J. R. Goldenring, Lasher, R. S., Vallano, M. L., Ueda, T., and DeLorenzo, R. J. Dept of Neurology, Yale Med. School; Dept. of Anatomy, U. Colorado Med. School; Dept. of Psychiatry, U. Michigan

Calcium and calmodulin are important regulators of cytoskeletal dynamics. A calmodulin-dependent kinase purified from rat brain cytosol (CaM Kinase II) phosphorylates several cytosketal proteins including MAP-2, synapsin I and tubulin. CaM Kinase II is associated with both microtuble preparations (Larson, et al., J. Neurochem, In Press) and neurofilament preparations prepared from in vitro polymerized microtubules (Vallano, et al., PNAS, In Press). CaM Kinase II in these preparations phosphorylates endogenous MAP-2, tubulin and an 80,000 dalton phosphoprotein doublet. We now report that this 80,000 dalton phosphoprotein doublet is identical to synapsin I.

The 80,000 dalton phosphopeptide (pp80) demonstrated identical migration in SDS-PAGE gels with authentic synapsin I. Phosphorylation of pp80 by both cAMP-dependent and calmodulin-dependent kinases yielded identical phosphopeptide maps to those seen with synapsin I. pp80 failed to focus on equilibrium isofocusing gels but ran as a rapidly migrating basic doublet in non-equilibrium pH gradient electrophoresis (NEPHGE). pp80 comigrated with authentic synapsin in NEPHGE. Like synapsin I, pp80 was highly susceptible to digestion with collagenase. Affinity-purified anti-synapsin I antibodies specifically labelled an 80,000 dalton doublet co-migrating with pp80. Anti-synapsin I antibodies also labelled neurofilaments when examined under electron microscopy.

These results suggested that synapsin I was tightly associated with neurofilaments. The distribution of synapsin I in tissue sections under both light and electron microscopy was also studied with affinity purified anti-synapsin I antibodies. We found that the results were dependent on the presence of Triton X-100 in the incubation solutions. When Triton was absent, strong cytoplasmic staining was evident with labelling of neurofilaments, microtubules, the presynaptic grid, post-synaptic densities as well as dense labelling of synaptic vesicles. In the presence of Triton, staining of microtubules and neurofilaments was markedly reduced.

These results indicate that synapsin I is associated with the cytoskeleton and may be an important link between cytoskeletal elements as well as between the cytoskeleton and membrane.

192.5 IMMUNOCYTOCHEMICAL EVIDENCE FOR THE ASSOCIATION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II WITH THE CYTOSKELETON. R. S. Lasher, J. R. Goldenring, M. L. Vallano* and R. J. DeLorenzo. Dept. of Anatomy, Univ. Colorado Med. Sch., Denver, CO 80262, and Dept. Neurology, Yale Univ. Sch. Med., New Haven, CT 06510.

Recent studies have demonstrated that calcium/calmodulin-dependent protein kinase II (CaM kinase II) activity co-purifies with in vitro preparations of neurofilaments and microtubules (Larson et al., 1985. J. Neurochem., in press; Vallano et al., 1985, PNAS 82, in press). This association of CaM kinase II with cytoskeletal elements has now been studied by immunocytochemical methods employing a monoclonal antibody (MAb) 39-29:x which reacts with the α/ρ subunit of CaM kinase II and with highly purified major postsynaptic density protein (Lasher et al., 1985, Anat. Rec. 211:105A). Light and electron microscopic evaluation of vibratome sections of rat brain fixed in 5% paraformaldehyde and processed by indirect immunoperoxidase methods indicates that the MAb binds to both synaptic and cytoplasmic structures in axons and dendrites, including intense labeling of neurofilaments and microtubules as well as of amorphous material bridging these structures. This cytoplasmic labeling is especially apparent in Purkinje cells and pyramidal cells, but is also present in other neurons and in Bergmann glial cells. In some sections, antigen appears to be associated with microtubules in the form of 25nM diameter spheres having a center-to-center spacing of 35nM. Fixation of the brain tissue in 5% paraformaldehyde + 0.2% glutaraldehyde and use of 0.3% Triton X-100 in the incubations with antibody resulted in the loss of most of the antigen associated with the cytoskeleton, but the labeling of postsynaptic densities and presynaptic dense projections appeared to be enhanced.

In order to confirm observations made using tissue sections, we examined isolated cytoskeletal preparations by electron microscopy coupled with indirect immunogold methods. MAb 39-29:x was found to specifically decorate neurofilaments isolated from multiply-cycled microtubule preparations. The microtubules were also specifically decorated by the MAb. The degree of MAb binding to neurofilaments and microtubules appeared to correspond to the amount of endogenous kinase II activity present.

The presence of CaM kinase II on cytoskeletal elements may provide a mechanism for rapid modulation of the interaction of these structures by autophosphorylation or phosphorylation of associated proteins.

192.6 INHIBITION OF PURIFIED CALMODULIN KINASE II BY AUTOPHOSPHORYLATION. J.M.Bronstein*, C.G.Wasterlain*,and D.B.Farber. UCLA School of Medicine, Depts. of Neuroscience, Neurol., and Opthal., Los Angeles, CA.

Calmodulin kinase II (Calm-K II) is a calcium/calmodulin dependent protein kinase which is highly concentrated at synaptic junctions. It is ideally located to mediate some of calcium's postsynaptic actions and may have a presynaptic role in transmitter biosynthesis and release. Calm-K II is composed of 50 and 60 Kilodaltón subunits which undergo autophosphorylation. We examined the effect of autophosphorylation on purified Calm-K II activity and its role in determining substrate specificity. Phosphorylation of Calm-K II was found to dramatically inhibit kinase activity and the degree of inhibition was closely correlated with the degree of autophosphorylation. Maximal inhibition resulted in a 12.6 fold decrease of ^{32}P incorporation into glycogen synthase, 7.1 fold decrease into lysine rich histone, and 3.4 fold decrease into synapsin I. Inhibition of Calm-K II by autophosphorylation was observed over calmodulin concentrations ranging from 50-1000nM. Synapsin I and glycogen synthase were found to be excellent substrates for Calm-K II possibly because they are rapidly phosphorylated prior to autophosphorylation of the enzyme which greatly reduces kinase activity. Lysine rich histone was a poorer substrate possibly because autophosphorylation of Calm-K II preceeds phosphorylation of this protein. These data suggest that autophosphorylation of Calm-K II maybe a rapid means of terminating some of calcium's actions in synaptic events.

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192.7 **REGULATION OF TYPE II CaM KINASE ACTIVITY BY AUTOPHOSPHORYLATION.** S.G. Miller* and M.B. Kennedy. Div. of Biology, 216-76, Caltech, Pasadena, CA 91125.

Type II CaM kinase is an oligomer of ~ 12 homologous 50 and 60 kDal catalytic subunits. In the presence of Ca^{2+} and calmodulin (CaM), it is rapidly autophosphorylated at several sites on both subunits. We have investigated the effects of this autophosphorylation.

Kinase was first autophosphorylated with cold ATP in the presence of Ca^{2+} and CaM, then diluted 500-fold and assayed for ability to phosphorylate synapsin I in the absence and presence of Ca^{2+}. Autophosphorylation had two effects. The initial rate of phosphorylation of synapsin I was reduced to 20-30% of controls. However, most of the reduced activity no longer depended on the presence of Ca^{2+} and CaM. Thus, authophosphorylation produced a significant Ca^{2+}-independent kinase activity. Addition of phosphate to 3 or more subunits per oligomer apparently produced an allosteric change in the conformation of all the subunits since it was sufficient to cause the complete reduction of initial rate. The reduced rate resulted from a decreased V_{max} rather than decreased affinity for synapsin I. In vivo, this mechanism could produce Ca^{2+}-dependent bursts of high kinase activity followed by low levels of Ca^{2+}-independent activity outlasting the initial Ca^{2+} transient.

Rapid autophosphorylation of an entire oligomer was initiated by autophosphorylation of only a few of its subunits. The initial "trigger" autophosphorylation depended on Ca^{2+} and CaM, while autophosphorylation of the rest of the subunits continued at the same rate after removal of Ca^{2+} by addition of EGTA. The final number of phosphates incorporated per mole of kinase was higher in the presence of Ca^{2+} suggesting that a portion of the autophosphorylation sites are strictly Ca^{2+}-dependent. Multiple site phosphorylation of the kinase may prolong its Ca^{2+}-independent state by prolonging the time required for removal of phosphate by phosphatases.

The rate of Ca^{2+}-dependent autophosphorylation of native kinase was unchanged over an 80-fold range of kinase concentration, indicating that this reaction is primarily intramolecular. To test whether the Ca^{2+}-independent phospho-kinase is capable of activating native kinase by intermolecular phosphorylation, maximally phosphorylated kinase was incubated with native kinase at various ratios in the presence of ATP. Under these conditions, intermolecular autophosphorylation occurred at < 5% of the intramolecular rate. No increase in Ca^{2+}-independent activity of the native kinase was observed. It is therefore unlikely that, in vivo, phospho-kinase is able to transmit its activated state by triggering Ca^{2+}-independent activity of native kinase.

192.8 CHARACTERIZATION OF A SYNAPTIC MEMBRANE-ASSOCIATED PROTEIN PHOSPHATASE. L.A. Dokas, L. Jeziorowski* and D. Ondrejka*, Depts. of Neurosciences and Biochemistry, Medical College of Ohio, Toledo, OH 43699.

Rat brain synaptic plasma membranes (SPM) enriched in presynaptic elements, contain an endogenous protein phosphatase that will dephosphorylate ^{32}P-labeled B50. The latter protein is a substrate for protein kinase C and its phosphorylated state correlates with the activity of membrane-bound diphosphoinositide kinase. Moreover, this protein phosphatase can be inhibited by a somatostatin analog ([D-Trp^8]-somatostatin) which has a high affinity for brain membrane binding sites. Determination of the activity of this protein phosphatase, therefore, could be one means by which presynaptic functions are regulated. Based on these functional considerations, we have begun to characterize this SPM-associated protein phosphatase. Crude rat brain membranes were treated with 0.5% Triton X-100 - 75 mM KCl and the extract was chromatographed on a DEAE-cellulose column with a 0-400 mM NaCl gradient. Using ^{32}P-labeled B50 as a substrate, the major portion of protein phosphatase activity elutes at 260 mM NaCl and can be further enriched by fractionation between 0 and 55% ammonium sulfate. A heat-stable protein inhibitor does not alter the activity of the protein phosphatase. Addition of 20 mM EDTA to the dephosphorylation assay medium (containing 10 mM Mg^{2+} and 1 mM Ca^{2+}) inhibits the column-purified protein phosphatase, as well as the endogenous activity found in SPM. A similar concentration of EGTA has relatively little effect on this enzyme. Protein staining of the ammonium sulfate fraction demonstrates a number of protein bands, with one major species migrating at about 43,000 daltons. In the classification of Ingebritsen and Cohen (Science 221:331, 1983), protein phosphatase 2C is insensitive to the heat-stable inhibitor, dependent upon Mg^{2+}, but not Ca^{2+} and composed of a single protein subunit with a molecular weight of 43,000 daltons. The correspondence between the characteristics of protein phosphatase 2C and the SPM-associated protein phosphatase suggest a common identity between the two forms. Supported by Grants AG04190 from the National Institute on Aging and 84-1472 from the Alzheimer's Disease and Related Disorders Association.

192.9 EFFECTS OF CALCIUM ON THE BINDING OF CALMODULIN (CM) TO POSTSYNAPTIC DENSITY (PSD) PROTEINS AND ITS ACTIVATION OF A PROTEIN KINASE. L. Sachs*, R. Carlin*, and P. Siekevitz, Lab. of Cell Biology, Rockefeller University, N. Y., N. Y. 10021

We have examined the concentration dependence of Ca^{2+} to promote the binding of CM to specific proteins in cortical PSDs. Two techniques were used. The first employed the binding of 10 μg of ^{125}I-CM to 200 ug of EGTA treated PSDs in the presence of 40 mM HEPES/KOH, pH 6.8, 2 mM EGTA/KOH, pH 6.8, and varying $[Ca^{2+}]$ for 2 hours at 25° C, with subsequent centrifugation and washing to remove unbound ^{125}I-CM. The second was the gel overlay method (Carlin, R., et al., J. Cell. Biol., 89, 449, (1981)), where ^{125}I-CM was bound in the presence of increasing Ca^{2+} to proteins on SDS gels and examined by autoradiography. The first method revealed that 50% binding of CM to PSD proteins occurred at a free Ca^{2+} conc. of 0.6 μM. The second method indicated that CM became bound abruptly to all known CM binding proteins of the PSD at a Ca^{2+} conc. of 0.5 μM, with the 51 kD protein, the major PSD protein and presumably the Ca^{2+}/CM-dependent protein kinase, being the major CM binding protein.

Next examined was the effect of phosphorylation conditions on the Ca^{2+} conc. necessary for binding and phosphorylation. Using slightly different conditions (40 mM PIPES/KOH, pH 6.8, 2 mM EGTA/KOH, pH 6.8, 1 mM $MgCl_2$, and 1 mM DTT), it was found that the addition of CM stimulated phosphorylation but the stimulation was independent of Ca^{2+} above 0.08 μM. In addition, we used binding conditions as immediately above but without DTT, and with the addition of the bifunctional and photoactivatable crosslinking agent (cysteamine-^{35}S)N-succinimidyl-3-((2-nitro-4-azidophenyl)-2-aminoethyldithio)proprionate to identify CM binding proteins of the PSD. Again, binding was independent of Ca^{2+} above 0.08 μM, becoming abruptly bound to the known CM binding proteins of the PSD (Carlin, et al., as above).

The most significant difference between the phosphorylation and non-phosphorylation experiments is the presence of high Mg^{2+} conc. in the former, a concentration where Mg^{2+} is known to bind to the 4 Ca^{2+} binding sites of CM (Haiech, J., et al., Biochem., 20, 3890, (1981)). This might explain the difference in Ca^{2+} conc. necessary for CM binding between the two conditions. It is known that the free intracellular Mg^{2+} conc. in brain is 0.6 to 1.0 mM (Veloso, D., et al., J. Biol. Chem., 248, 4811, (1973)). This suggests that Mg^{2+} might be involved in modulation of interaction between Ca^{2+}, CM, and CM binding proteins. Further experiments will be done to determine how Mg^{2+} quantitatively and qualitatively modifies the interaction of Ca^{2+} and CM in binding to appropriate proteins of the postsynaptic density.

192.10 EXISTENCE OF A VOLTAGE-DEPENDENT Ca^{2+} CHANNEL PROTEIN IN SYNAPTIC MEMBRANE (SM) AND POSTSYNAPTIC DENSITY (PSD) FRACTIONS ISOLATED FROM CEREBRAL CORTEX (CTX) AND CEREBELLUM (CL) OF CANINE BRAIN, AS DETERMINED BY NITRENDIPINE BINDING. P. Siekevitz, R. Carlin* and K. Wu* (Spon: D.R. Griffin). Lab. Cell Biology, The Rockefeller University, 1230 York Ave., New York, NY 10021.

We have used (^3H)-nitrendipine, a specific blocker of voltage-dependent Ca^{2+} channel, to study its binding to SM and PSD fractions of canine brain. After incubation under dim red light (in 20 mM Tris, 50 mM choline-Cl^-, 1.5 mM $CaCl_2$, pH 7.4) at 0-4°C for 90 min., the reaction mixtures were immediately filtered through Whatman GF/B filters, washed with 3 x 5 ml. of ice cold wash buffer (20 mM Tris, 0.2 M choline-Cl^-, 1.5 mM $CaCl_2$, pH 7.4) and the bound radioactivity on the filters determined. Specific binding was defined as the difference in binding between the absence and presence of 1 μM nifedipine. The specific binding constants thus obtained were: CTX-SM, KD=110 pM (Bmax=126 fmol/mg protein); CTX-PSD, KD=207 pM (Bmax=196 fmol/mg); CL-SM, KD=100 pM (Bmax=65 fmol/mg); CL-PSD, KD=189 pM (Bmax=80 fmol/mg). No significant difference was found between fresh and frozen samples. All fractions showed one single class of nitrendipine binding sites. The values of 110 pM and 126 fmol/mg for canine CTX-SM were similar to those obtained with a rat SM fraction (98±25 pM and 160±9 fmol/mg) (H. Schoemaker et. al., in Nitrendipine, 1984, p.133). Calmodulin may be important in the interaction between nitrendipine and its receptor since the calmodulin inhibitor R24571 (calmidazolium) at 5 μM was found to inhibit by about 80% of the binding of nitrendipine to CTX-SM or CTX-PSD fractions; similar results were reported on binding of nitrendipine to a rat brain membrane fraction. (S.A. Thayer and A. S. Fairhurst, Mol. Pharmac., 24(1983)6). Treatment of CTX-PSD with 0.5% deoxycholate, 1% N-lauroyl sarcosinate, 4 M guanidine-HCl, pH 7.0 and 1.0 M KCl resulted in removal of the receptors from the PSD by 42%, 51% 51% and 16%, respectively. However, the two detergents solubilized about 90% of the receptor from CTX-SM, indicating the presence of the Ca^{2+} channel also in non-synaptic membranes and in accord with its presence in erythrocyte membranes (H. Glossmann et. al., T I P S, 3(1982)431). The data suggests that at the synapse there is about a 3-fold concentration of the Ca^{2+} channel protein in the CTX-PSD fraction. The findings also indicate that the nitrendipine binding protein, which spans the postsynaptic membrane, is an intrinsic PSD component. The presence of the voltage-dependent calcium channel, of a Ca^{2+}-dependent K^+ channel (K. Wu et. al., Brain Res., in press) and of various neurotransmitter receptors (by ourselves and by others) in the isolated PSD fraction suggest that the PSD may play an important role in the mediation of neurotransmission.

192.11 COMPARISON OF BINDING OF L-GLUTAMATE TO SYNAPTIC MEMBRANE (SM) AND POSTSYNAPTIC DENSITY (PSD) FRACTIONS ISOLATED FROM CEREBRAL CORTEX (CTX) AND CEREBELLUM (CL) OF CANINE BRAIN. K. Wu*, R. Carlin* and P. Siekevitz (Spon: N.E. Miller). Lab. of Cell Biology, The Rockefeller University, 1230 York Avenue, New York, N.Y. 10021

The Na^+-independent binding of the excitatory neurotransmitter, L-glutamate, to SM and PSD fractions of canine brain was studied using L-(^3H)-glutamate. With 10 mM HEPES/KOH, pH 7.4 as the assay buffer, the specific binding constants were (A) fresh fractions (fresh tissue):CTX-SM, KD_1=20.1 nM ($Bmax_1$=0.8 pmol/mg protein) and KD_2=792.8 nM ($Bmax_2$=22.0 pmol/mg);CTX-PSD, KD=400.0 nM (Bmax=35.6 pmol/mg);CL-SM, KD=869.0 nM (Bmax=56.4 pmol/mg);(B) frozen fractions (fresh tissue):CTX-SM, KD_1=18.9 nM ($Bmax_1$=0.8 pmol/mg) and KD_2=874.1 nM ($Bmax_2$=23.6 pmol/mg);CTX-PSD, KD=456.0 nM (Bmax=32.1 pmol/mg);CL-SM, KD=704.0 nM (Bmax=52.8 pmol/mg);(C) frozen fractions (frozen tissue):CTX-PSD, KD=417.7 nM (Bmax=28.9 pmol/mg);CL-PSD, KD=500.3 nM (Bmax=92.0 pmol/mg). Under these conditions, there was a concentration of glutamate binding sites in CTX-PSD and CL-PSD over the respective membrane fractions. When the binding was assayed with 10 mM HEPES/KOH, pH 7.4, containing 1 mM Ca^{2+} and 40 mM Cl^-, fresh CTX-SM (fresh tissue) had KD_1=10.8 nM ($Bmax_1$=1.2 pmol/mg) and KD_2=709.9 nM ($Bmax_2$=55.6 pmol/mg), whereas fresh CL-SM (fresh tissue) had a KD=789.4 nM (Bmax=139.1 pmol/mg). The results, together with those of others, suggest that the thin CL-PSD are probably derived from the excitatory synapses in the molecular layer. The ion dependency of glutamate binding to CTX-SM was similar to that to rat brain SM (A. Foster and G. Fagg, Brain Res. Rev. 7(1984)103):(a) Cl^- increased number of glutamate binding sites, and the effect was enhanced by Ca^{2+}; Ca^{2+} alone had no significant effect;(b) both amino-phosphono-butyrate (APB) and freezing abolished Cl^-/Ca^{2+}-dependent glutamate binding;(c) low concentrations of Na^+ (< 5 mM) specifically depressed Cl^-/Ca^{2+}-dependent glutamate binding. Na^+-dependent glutamate binding to CTX-SM was inhibited by K^+ and was reduced by 50 % upon freezing and thawing. CL-SM showed similar ion dependency except for the absence of Na^+-dependent glutamate binding sites. CTX-PSD had neither Na^+-dependent nor APB-sensitive binding sites and its glutamate binding was unaffected by freezing and thawing, in agreement with the findings using rat PSD (G. Fagg and A. Matus, PNAS, 81(1984)6876). Glutamate binding to CTX-SM or CTX-PSD was not affected by pretreatment with 10 mM L-glutamate, nor by simultaneous incubations with calmodulin. Also, phosphorylation of the CTX-SM or CTX-PSD proteins had no effect on glutamate binding. Treatment of CTX-PSD with 0.5 % deoxycholate, 1 % N-lauroyl sarcosinate, 4 M guanidine-HCl pH 7.0 and 1.0 M KCl removed the receptor from the PSD by 25 %, 44 %, 40 % and 11 %, respectively. The respective percentages of protein solubilized by these reagents were similar, indicating no preferential dissociation of the receptors, and suggesting that the glutamate receptor is an intrinsic PSD protein.

192.12 TRANSCELLULAR FILAMENTS AT SYNAPSES: A STRUCTURAL CONTINUUM LINKING SYNAPTIC MEMBRANES AND CYTOSKELETONS. Mark H. Ellisman, R.D. Fields, Karen L. Anderson*, Thomas J. Deerinck*. Lab. for Neurocytology, Dept. Neurosci., UCSD, La Jolla, CA 92093.

We recently reported observations on an extensively distributed but previously undescribed pattern of filamentous structures within cells of tissues. These structures form a structural continuum between cells linking together cytoskeletons and extracellular matrices. We have referred to this network as a transcellular filament system (TCFS). Some constituents of the TCFS have been identified by immunoelectron microscopy as intermediate filaments (Ellisman, JCB 99:195a, 1984). We have now examined several different types of synaptic complexes and observed filamentous structures crossing synapses, interconnecting the cytoskeletons of pre- and post-synaptic cells. These structures have been observed by several methods including 3-D imaging by high voltage EM of thick sections, contrast enhancement by computer aided image analysis, platinum shadowing of resinless sections and freeze-etching. Trans-synaptic fibers have been observed in all synaptic specializations examined in detail thus far. These have included synapses at sensory receptors, motor nerve terminals and between neurons within the CNS. At ribbon synapses of the ampulla of Lorenzini (an electrosensory receptor of sharks, skates and rays) filaments course across the synaptic cleft connecting the presynaptic ribbon with filaments of the postsynaptic cytoskeleton. At electromotor synapses on eel (Electrophorus electricus) electric organ, stain resistant fibers within the nerve terminal are structurally continuous with cytoskeletal elements within the electrocytes. In the rat CNS, trans-synaptic filaments are observed linking pre- and post-synaptic structures. Clusters of ribosomes characteristic of some synaptic specializations appear to associate with the stain resistant fibers. We consider the trans-synaptic complex of fibers linking pre- and post-synaptic structures across the synaptic cleft to represent focal differentiations of the TCFS.

Immunolabeling studies to identify constituents of the TCFS indicate that this network, although forming a structural continuum, may be composed of many different macromolecules. These macromolecules probably include a group of cytoskeletal proteins, membrane proteins and extracellular matrix components that can co-polymerize or otherwise link together. A primary characteristic shared by components of the TCFS is resistant to staining with most procedures used for electron microscopy. Description of this type of structure has become possible in part due to new developments in electron microscopic imaging technology. The distribution and likely molecular constituents of this system indicate possible roles in cell-cell recognition, differentiation, ion regulation, and the regulation of cell form.

193.1 A cDNA PROBE TO STUDY CELLULAR INTERACTIONS DURING CNS DEVELOPMENT. D. Goldowitz, M. Vijh* and J. Rossant*. Daniel Baugh Institute of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107; and Dept. Biol. Sci., Brock Univ., St. Catharine, Ontario, Canada.

A cell marking system is described that can be used to monitor the interactions of differentially-derived cells in transplant studies, experimental mouse chimeras and cell culture work. The marking system is based upon a sequence of satellite DNA that is highly abundant in Mus musculus cells but not in the cells of another mouse species, Mus caroli (Siracusa et al., 1983, J. Embryol. exp. Morphol. 73:163). Thus, hybridization of the cloned cDNA (pMR 196) to cell or tissue mixtures can detect cells of M. musculus origin vs. M. caroli origin. For several important reasons this is an ideal marker for the numerous cell interactions involved in CNS development.

The cDNA is labeled with either ^{3}H-nucleotides or biotinylated-d-UTP with an in vivo nick translation system. The labeled cDNA probe is applied to fixed tissue or cells. Several fixatives were examined with the aim of optimizing tissue DNA preservation and subsequent hybridization to the cDNA probe. Perfusion fixation with acetic acid:95% ethanol (1:3) or 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) was found to give the best results for both large and small neurons. Other fixatives such as Bouin's, 4% paraformaldehyde, Carnoy's and periodate polylysine paraformaldehyde (PLP) gave inferior results. Tissue pieces were routinely embedded in paraffin. Sections of tissue mounted on poly-L-lysine coated slides or cells on coverslips were treated for 10 minutes at 80° C in 2 x SSC to denature DNA in preparation for in situ hybridization. Throughout denaturation and hybridization formamide was found to be unnecessary as long as temperature parameters were properly adjusted. The hybridization solution of 10% dextran sulfate, 2.5 x SSC, 250 ug/ml sonicated salmon sperm DNA worked at least as well as more complex hybridization formulations. Hybridization was at 60°C for 12-16 hr. After thorough rinsing in successively lower concentrations of SSC and lower temperatures, tissue or cells were either coated with NTB-2 emulsion for autoradiography or processed for ABC (Vectastain)-diaminobenzidine (DAB) reaction. Detection could easily be amplified by longer exposure for autoradiograms or heavy metal intensification of the DAB reaction product. Studies of CNS development using this marking system will be discussed.

193.2 NEURONAL DEGENERATION MUTATIONS THAT SPARE PRIMARY NEURONS IN THE ZEBRAFISH. C.B. Kimmel, D.J. Grunwald*, C. Walker*, M. Westerfield and G. Streisinger[1]. Institute of Molecular Biology, Univ. of Oregon, Eugene, OR 97403.

To learn how genes control the development of functional neuronal circuits in a vertebrate we are inducing mutations in lethal-free clonal lines of zebrafish and systematically screening for mutations that perturb the nervous system of the embryo. We describe a set of independent zygotic recessive lethal mutations (2 induced by γ-rays, 1 by UV, and 1 by ethylnitrosourea) that specifically produce massive cell death in the CNS shortly after its formation. However, the mutations appear to spare large cells we call "primary" neurons. These neurons are generated during the first half-day after fertilization, and are subsequently assembled into a functional network that mediates the first motile behaviors of the embryo.

We have characterized one γ-ray induced mutation, let(b39), in some detail. It segregates as a single Mendelian gene. Mutant embryos appear unaffected during most of the first day of development. Spontaneous motile behaviors at 24 hr, and responses to tactile stimulation at 32 hr, are normal. Degenerating single cells and clusters of cells become evident in the CNS during the second day, and by the third day cell death is widespread; only a few viable cells remain in some CNS regions such as central retina and dorsal brain. By this stage embryonic behaviors are grossly abnormal, and within the next 2-3 days the larvae die. Primary neurons, including Rohon-Beard neurons, identified reticulospinal interneurons and primary motoneurons were identified by HRP labeling, acetylcholinesterase histochemistry, staining with a monoclonal antibody, and inspection with Normarski optics of living embryos. These neurons appear unaffected by the mutation. Furthermore, the connections between primary motoneurons and muscle cells appear physiologically normal.

The existence of genes that appear not to be required for the development and maintenance of primary neurons but that are essential for survival of neurons that develop later could mean separate genetic control of these two classes of cells.

([1]Deceased.) (Supported by NIH grant GM 22731 and NSF grant PCM 8317049.)

193.3 RETINAL CHIMERAE OF XENOPUS: ANALYSIS OF NEURAL GRAFT INTEGRATION AND GROWTH USING A CELL AUTONOMOUS GENETIC MARKER. S. O'Gorman*, J. Kilty*, and R.K. Hunt* (SPON: W.M. Cowan). The Salk Institute, La Jolla, CA 92037.

Compound eyes, formed by surgical fusion of half eye rudiments of Xenopus embryos, have long been used to study the formation of retinotectal projections without an independent means of assessing the retinal areas occupied by graft- and host-derived neurons. In quinacrine-stained paraffin sections of compound retinae formed by fusion of X. laevis (Xl) and X. borealis (Xb) half eyes, graft- and host-derived neurons can be reliably distinguished because of striking differences in the patterns of nuclear chromatin staining in the two subspecies. Compound eyes were constructed by replacing the excised right temporal half eyes of albino Xl hosts with stage-matched, pigmented Xb half eyes. Orthotopic compound eyes were formed by replacement with right temporal half eyes, heterotopic compound eyes were formed by replacement with either left temporal, left nasal or right dorsal half eyes. The grafts included recently postmitotic neurons and pigment epithelial cells from the back of the eye (BOE) and mitotically active cells from the germinal zone at the front of the eye (FOE) that generate neurons and pigment epithelial cell during continued growth of the eye. Donor pigment epithelium could be seen in the intact embryo, and cases showing robust growth of graft-derived pigment epithelium were processed for histologiccal analysis of the neural retina at St 50-57.

In both orthotopic (N=15) and heterotopic (N=18) compound eyes, grafted neurons and neurons derived from grafted germinal zone occupied large contiguous sectors whose angular positions were conserved from the BOE to the FOE. In most cases Xb neurons comprised 40% to 50% of the retina; in no case did they comprise less than 25%. In chimerae sacrificed at St 50-52, the graft-host borders of all three retinal nuclear layers were in close register with one another and there was little evidence of cell mixing across borders. In chimerae sacrificed at later stages, the registration of graft-host borders was less precise. Additionally, individual neurons along the inner surface of the inner nuclear layer and in the ganglion cell layer were often seen on the "wrong" side of the graft-host borders, separated from other members of their genetic cohort by many cell diameters. Anomalies of initial graft integration, including laminar faulting, hypoplasia, and tubular rosettes, were present at the BOE of many cases and were propagated during initial postoperative growth in some of these. These were resolved during further growth of the eye so that in sections closer to the FOE the angular positions of these malformations were occupied by normally laminated, well integrated retina derived from the grafted Xb germinal zone. Host-derived tissue did not extend into angular territories initially occupied by graft-derived tissues. Supported by NSF Grant PCM83-11082 and by the Keck Foundation.

193.4 MITOTIC WITHDRAWAL AND GERMINAL CELL FATE IN DEVELOPING NEURAL RETINA: GENETIC CHIMERAE IN XENOPUS. R.K. Hunt* and S. O'Gorman* (SPON: D.D.M. O'Leary). The Salk Institute, La Jolla, CA 92037.

The back of the eye (BOE) becomes mitotically quiescent at embryonic St 28-38 (2.5-4 days) in Xenopus, and cell division is confined thereafter to a bilayered germinal ring on the front of the eye (FOE). This germinal ring has an outer pigmented layer, which adds post-mitotic melanocytes to the margin of the pigment epithelium (PRE), and an inner neuroepithelial layer, which adds annuli of neurons to all layers of the neural retina (NR). We have analyzed the fate of small patches of embryonic eye cells transplanted from St 36-38 donor embryos (pigmented X. borealis and X. borealis/X. laevis hybrids) into St 32-36 albino X. laevis host eyes. Orthotopic FOE transplants successfully healed into the host germinal ring in about 70% of cases and adopted a germinal cell fate. New 'arcs' of black PRE cells were added to the rim of the PRE to yield an elongating black sector visible in the living albino eye; and cases fixed at St 50-57 (2-7 mo. after surgery) and stained with quinacrine to demonstrate X. borealis (Xb) chromatin, showed a grossly corresponding sector of Xb neurons in all layers of NR. By contrast, although orthotopic BOE grafts healed into the mitotically quiescent region of the host eye, thereafter they remained as a tiny black spot on the back of the host eye; and sectioned material at St 50-57 revealed a tiny cluster of Xb neurons in the back of the host retina (N=4). Following heterotopic transplantation (FOE-into-BOE or BOE-into-FOE), the pigmented cells of the graft adapted quickly to their new environment; and sectioned material at St 50-57 revealed a similar modulation of neural cell fates. In 11 FOE-into-BOE cases, where germinal cells had been introduced into the mitotically quiescent BOE, Xb neurons were confined to a small cluster at the back of the host retina. The exact size (and precision of integration) of these clusters varied from case to case and may reflect varying residua of mitotic activity in the first days after the surgery. The 13 BOE-into-FOE cases, in which mitotically quiescent PRE and NR cells had been transposed into the host germinal ring, gave mixed results. Four showed little or no growth of either PRE or NR cells; the grafted cells were quickly displaced from the host germinal ring, leaving a small spot of black PRE and a small cluster of Xb neurons at the back of the St 50-57 eye. One graft grew out a black sector of PRE, but the final NR (and, by inference, the original graft) contained no Xb neurons. In the remaining 8 cases, both PRE and NR showed a robust sector of graft-derived cells; and, in 5 of these, marked neuroepithelial cells persisted on the germinal ring and continued to support new growth up to the stage of sacrifice. We infer that local positional cues modulate the fate of neurogenic cells to withdraw from cell division or to become germinal cells that support the larval growth of the retina. Supported by NSF grant PCM83-11082 and by the Keck Foundation.

193.5 *SEVENLESS*, A CELL-SPECIFIC HOMEOTIC MUTATION THE *DROSOPHILA* RETINA. A. Tomlinson* and D. F. Ready (SPON: C. Sahley) Dept. of Biology, Princeton University, Princeton, N.J. 08544

During development cells select pathways of specialization which lead to the differentiation of specific cell types. Lesions in the genetic elements mediating these selections can cause cells to follow inappropriate pathways, resulting in homeotic transformations. For example, in *Drosophila*, the homeotic mutant *bithorax* causes mesothoracic structures to produce their methoracic counterparts (Lewis,E.B., Nature 276:565,1978). The developing compound eye of *Drosophila* offers several advantages for studying pathway selection at the single cell level. Each eye is a several hundred-fold reiteratior of a stereotyped cellular pattern, the ommatidium. Within each ommatidium, four lens-secreting cone cells overlie a core of eight photoreceptors. Ommatidia assemble in a choreographed sequence of cell movements. The photoreceptor core is assembled apically in the prospective retinal epithelium, and then sinks basally. Concomitant with this basal shift, four surrounding non-photoreceptor cells rise along the flanks of the cluster to meet over the cluster apically. Later in development this quartet of cells secretes a refractile extracellular lens, the cone, above the photoreceptors.

In *sevenless*, cell clustering proceeds normally and early clusters contain the full complement of eight cells. As the mutant photoreceptor cluster sinks, the cell in the R7 position is left behind, remaining apically. Subsequently, it is joined by three cells which rise along the flanks of the descending cluster to produce a typical cone cell quartet. *sevenless* is thus a cell-specific homoeotic mutation, causing a cell to adopt a path of differentiation inappropriate for its postion.

Mosaic analyses indicate that the developmental pathways taken by retinal cells are directed by their environment, rather than their ancestry (Ready, D.F., Hanson, T.E. and Benzer, S., Dev. Biol., 53:217,1976). Separate experiments also indicate that *sevenless* is cell-autonomous (Harris, W.A., Stark, W.S. and Walker, J.A., J.Physiol. (Lond.) 256:415, 1976; Campos-Ortetga, J.A., Jurgens,G. and Hofbauer, A., Wilhelm Roux Archiv. 186:27,1979). Together with the present results, these observations suggest that *sevenless* cells "misread" the R7 position. Whether the mutation specifically redirects prospective R7 cells into a cone cell pathway, or represents a "default" pathway for a defective R7 remains to be discovered.

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193.6 NEURAL PLATE MORPHOGENESIS AND CELL LINEAGES DURING NEURULATION IN THE ASCIDIAN EMBRYO. D. Nicol and I.A. Meinertzhagen. Dept. of Biology, Dalhousie Univ., Halifax, N.S., Canada B3H 4J1.

The determinate cleavage and mosaic development of the ascidian embryo combine with its chordate ancestry and the apparent eutely of its larval nervous system to offer a favourable model of vertebrate neurogenesis. Embryos of Ciona intestinalis have been reconstructed at 12-min intervals from 7.9 - 11.7 hr (16°C), using serial semithin sections to analyse the morphogenetic events which produce a neural tube from a superficial plate of neurectoderm. We have previously confirmed the cell lineage originally described by Conklin (J. Acad. Nat. Sci. Phila., 13:1-119, 1905) by which, at the end of gastrulation (7.0 hr: 16°C), the neural plate comprises 40 cells. The cells are arrayed over the dorsal surface of the embryo in regular rows, 2 posterior rows each of 8 cells and 4 anterior ones each of 6.

Between 7.9 and 9.5 hr, neurulation follows a postero-anterior wave of transversely-directed cleavages which advances the lineage from these 40 to at least 74 cells arranged in their 10th generation into 12 rows. The morphogenesis of cells of the posterior rows occurs in three consecutive steps which resemble the amphibian pattern of neurulation but are analysed here at single-cell level. Each cell: 1. decreases its apical surface area and changes from columnar- to wedge-shaped; 2. elongates, the neural plate lengthening from 50µm at 7.0 hr to 120µm at 11.7 hr, along with the rearrangement and elongation of the underlying presumptive notochord cells; 3. undergoes shearing movements with respect to its neighbours, so as to disrupt their orderly rows. In each row, the pair of midline cells shears, each to straddle the ventral midline of the neural tube, with one (either left or right) in front of the other; two pairs of medio-lateral cells shear to form the walls of the tube, while the pair of lateral cells meets and intermingles at the dorsal midline. This gives the characteristic arrangement of presumptive ependymal cells in cross section. Anteriorly, the cell rows merge into each other increasing the number of cells in any cross section, with each cell remaining columnar and surrounding a large central lumen. Between this region, which becomes the brain, and the ependymal region is a transitional zone.

The cell lineage has been followed through the 11th generation, the first cells to enter which (at 10.5 hr) are ventral ependymal cells, originally the midline cells in the most anterior of the posterior rows of 8. These divide symmetrically, other ependymal cells following suit non-synchronously till the end of neurulation (11.7 hr). In the brain, synchronous cleavages occur between 11.3 and 11.7 hr to produce a total of 134 cells for the entire nervous system at the end of neurulation. Divisions occur later in the transition zone between ependyma and brain.

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193.7 CENTRIFUGATION AFFECTS CELL FATE IN THE LEECH EMBRYO S.Astrow* and D.A. Weisblat, Dept. Zoology, Univ. of Calif., Berkeley, CA 94720

One mechanism thought to be important in the specification of distinct cell lines during development is the localization of cytoplasmic factors that restrict or direct developmental potential. In the leech, domains of yolk-deficient cytoplasm, called teloplasm, become segregated into one cell, D, of the 8-cell embryo. Cell D is unique; it generates the five bilateral pairs of stem cells (teloblasts) that give rise to all segmental tissues of the leech. We have tested the hypothesis that D owes its unique character to factors associated with the teloplasm, by centrifuging embryos to effect changes in teloplasm distribution and in cell fate.

At the 2-cell stage, the teloplasm is localized in two caps at the dorsal and ventral poles of cell CD. Mild centrifugation just prior to the second cleavage partially stratifies the cellular contents so that the yolk-free cytoplasm is displaced centripetally. Most spun embryos cleave on schedule, generating 4-cell embryos. Normal 4-cell embryos undergo a round of asymmetric divisions (micromere production), then cell D cleaves equatorially, producing teloblast precursors DM and DNOPQ while A,B, and C produce another round of micromeres. While most spun embryos (75%) cleave normally, in approximately 20% of spun embryos cells C and D cleave simultaneously and equatorially. These abnormal progeny of C appear mirror-symmetric to DM and DNOPQ and are called CM and CNOPQ.

Evidence that redistribution of teloplasm may be responsible for the fate change is that the fates of spun embryos correlate with the distribution of yolk-deficient cytoplasm between C and D at the second cleavage. Embryos in which D inherits most of the teloplasm usually cleave normally (95%). But embryos in which cells C and D receive roughly equal portions of teloplasm cleave symmetrically. Rarely, the prospective C cell receives a larger portion of teloplasm than D. In these cases, C cleaved equatorially.

Observation of symmetrically cleaving embryos, stained at stage 8 with Hoechst 33258 to label nuclei, suggests that supernumerary germinal bandlets are generated. This possibility has been investigated using microinjected lineage tracers to label descendents of early blastomeres. We find that cells CM and CNOPQ are capable of giving rise to teloblasts and germinal bandlets which may contribute to the germinal bands. Cells DM and DNOPQ in spun embryos follow the same fate as in controls.

These initial results are consistent with two alternate hypotheses: 1)the difference in fate between cells C and D depends on some component of the cytoplasm normally being segregated exclusively to cell D; 2)the fate of cells C and D is governed by a disproportionate distribution of teloplasm between the two cells.

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193.8 Protein Differences Between Sister Cells of Different Fates in Leech Embryos. B. Holton and D. Weisblat. Dept. of Zoology, Univ. of Ca. Berkeley, Berkeley, CA 94720.

Embryonic cells of different fates must exhibit qualitative or quantitative biochemical differences either as a cause or as an effect of individual fate determination. We have begun to study such differences in embryos of the leech, Helobdella triserialis, where cell fates can be observed and manipulated. Cell CD divides into cells C and D which normally follow different fates. C forms transient larval epithelium and some nonsegmental cells of the adult, including neurons of the supraesophageal ganglion. D cell gives rise to all of the segmental ectodermal and mesodermal derivatives. Cells C and D are visibly different. D contains a region of yolk-deficient cytoplasm (teloplasm) and C does not; D produces one micromere then divides equally to form cells DM and DNOPQ, whereas cell C produces two micromeres. Cells C and D are large (250µm diam.) and can be dissected apart in quantities sufficient to make biochemical comparisons feasible.

We used SDS polyacrylamide gel electrophoresis (PAGE) coupled with a sensitive silver stain for proteins to compare cells C and D. Before comparing these cells we sought to distinguish between yolk-associated proteins and those associated with yolk deficient cytoplasm. To do this we centrifuged 4-cell embryos to stratify their intracellular components (see preceding abstract). Each embryo was cut into two pieces: the light part contained predominantly yolk-deficient cytoplasm and the heavy part contained the yolk-rich cytoplasm. Equal amounts of protein from each fraction were compared by 1-dimensional PAGE. The yolky fraction yielded only about a dozen prominent protein bands whereas the yolk-deficient fraction separated into over one hundred bands. When the C and D cells were compared, cell C proved to be greatly enriched in yolk associated proteins. Cell D contained relatively more of the non-yolk proteins which we expect are primarily associated with the teloplasm. In particular, two protein bands (50 kdaltons and 80 kdaltons molecular weight) appeared in much higher (greater than ten fold) amounts in D than in C cells. S. Astrow (see preceding abstract) is able to redistribute cytoplasmic components in the 2-cell embryo so that cell C later undergoes D cell-like cleavages. The protein pattern from such modified C cells was indistinguishable from that of D cells, including the presence of the two D cell-enriched proteins. We are now using 2-dimensional PAGE to further examine differences between the C and D cells.

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193.9 UNEQUAL CELL DIVISION IN LEECH DEVELOPMENT. S. A. Settle* and D. A. Weisblat. (Spon: M. Law). Dept. of Zoology, Univ. of CA Berkeley, CA 94720

Cell divisions which produce daughters of unequal size are observed at various stages of development in leeches and other animals. To determine how the asymmetry of unequal cell divisions arises we have observed the position of nuclear DNA in embryonic cells of the glossiphoniid leech Helobdella triserialis. Leech embryos were fixed with formaldehyde, stained with the DNA stain bisbenzimide (Hoechst 33258), and viewed by fluorescence microscopy. A novel approach was used for observing the nuclei in the large cleavage blastomeres. Daughter blastomeres were separated along their cleavage plane and mounted with the flat face of the cleavage plane facing the microscope objective. This provided a rapid method for viewing nuclei located near the center of the spherical embryo. We have observed that large cleavage blastomeres (200um in diameter) and small ectodermal blast cells (25um in diameter) differ in their mechanisms of unequal cell division. In large cleavage blastomeres the prophase nucleus moves toward the cortex of the cell, and metaphase occurs in a an eccentric location. This premitotic nuclear movement accounts for most but not all of the asymmetry in the division of a blastomere into a micromere and a macromere. Further inequality in blastomere cell division arises from an asymmetrical anaphase which projects the future micromere nucleus into a small cytoplasmic bud protruding from the surface of the blastomere. Unequal divisions of small n_f, o, q_f, and q_s ectodermal blast cells may be mechanistically less complex than those of the large blastomeres. In the unequal divisions of ectodermal blast cells the metaphase DNA is located centrally within the cell. An asymmetrical anaphase ensues, as in the budding process of the blastomere division. One daughter nucleus moves into a cortical region of the blast cell and the blast cell cortex deforms into a bud which becomes the smaller daughter of the unequal division. The combined process of asymmetrical anaphase and cytoplasmic budding account for all of the asymmetry in the unequal blast cell divisions. These findings indicate that both movement of the premitotic nucleus and an asymmetrical anaphase contribute to the asymmetry of the unequal division of the large yolk-filled blastomeres. In contrast, asymmetrical anaphase is the predominant mechanism of unequal divisions of the small ectodermal blast cells.

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193.10 DEVELOPMENTAL COMMITMENT OF ONLY ONE OF THE TWO DAUGHTER CELLS DURING SERIAL BLAST CELL DIVISIONS IN THE LEECH. M. Shankland. Dept. of Molecular Biology, U. of California, Berkeley, CA 94720.

The o and p bandlets of the leech embryo are parallel columns of ectodermal blast cells which arise from the egg via equivalent cell lineages. The blast cells in these two bandlets are born with comparable developmental potential, but undergo an interaction which brings about subsequent divergence in their fates. The O and P fates have been distinguished both by the set of identified pattern elements which comprise the blast cell's descendant clone, and by the sequence of blast cell divisions in the lineage leading to that clone.

Previous work has shown that the o blast cells, although initially capable of following either the O or P pathway, become committed to the O pathway 20-35 hr after their birth. This commitment is not a single event, but rather a sequence of at least three separate events which determine the fates of different pattern elements within the clone. These three commitment events occur around the time when the o blast cell experiences its first few cell divisions, and I propose that each partial commitment is (i) associated with a different division in the o blast cell's descendant lineage, and (ii) determines the fate of only one of the two daughters of that division.

If this model is correct, then those O pattern elements whose fates are determined in unison at a particular commitment event should arise from a single o blast cell daughter, and should be more closely related to one another than to elements whose fates are determined at other events. This prediction has been tested by injecting different progeny of the o blast cell with rhodamine-conjugated dextran, and then scoring the cellular composition of the labeled descendant clone at later times in development.

The normal o blast cell clone contains 7 unambiguously identifiable O pattern elements. The fate of 2 of these elements is determined at the first commitment event, and injections reveal that these 2 elements arise from cell o.aa, a granddaughter of the primary o blast cell, while the other 5 elements arise from the sister cell o.ap. Within the latter group of 5 pattern elements there are 4 whose fates are determined at the second commitment event--and which arise from the anterior daughter of cell o.ap--and 1 whose fate is determined at the third commitment event and which arises from the posterior daughter of cell o.ap. Thus, there appear to be at least two sequential divisions in the o blast cell lineage where one of the two daughter cells becomes committed to the O pathway, while the other daughter remains uncommitted.

193.11 EVIDENCE FOR TWO ALTERNATING CLASSES OF N BLAST CELLS IN THE LEECH EMBRYO. S.T.Bissen* and D.A.Weisblat (Spon: G.S.Stent). Dept. of Zoology, Univ. of California, Berkeley, CA 94720.

In the embryo of the leech Helobdella triserialis there are five bilateral pairs of teloblasts (M, N, O/P, O/P, Q) that generate bandlets of blast cells; blast cells in turn proliferate to form the segmental tissues. Each half segment contains cells derived from two n blast cells. Alternate blast cells (designated n_f and n_s) can be distinguished early in development by the timing and geometry of their first mitosis. Eventually one n blast cell gives rise to cells in the anterior portion of the ganglion and the other gives rise to cells in the posterior portion of the ganglion, plus a few peripheral cells.

As a preliminary to testing the hypothesis that these two alternating classes of n blast cells are formed with a single developmental potential, we first determined the correspondence between the two classes of early n blast cells and the two classes of N progeny. Fluorescein dextran (FDX) and HRP were injected into N teloblasts that had already begun making blast cells. The embryos were later separated into two groups on the basis of the first labeled blast cell, which was identified as either n_f or n_s using a combination of bright field and fluorescence optics. After further development, the embryos were fixed and stained for HRP. It was found that the n_s blast cell gives rise to the anterior portion of the ganglion and the n_f blast cell gives rise to the posterior portion of the ganglion and the peripheral cells.

To determine whether the n blast cells are developmentally equivalent, several FDX-labeled n blast cells in the germinal band were ablated by laser microbeam irradiation before their first division, and the fate of the first surviving blast cell posterior to the lesion was examined. If the n_f and n_s blast cells form an equivalence group at this stage, one fate should be dominant. It was found, however, that the first cell posterior to the lesion gives rise to either the n_f or n_s progeny. These results suggest that, unlike the o/p blast cells, the alternating n blast cells are determined to be different well before their first mitosis, and that they may, in fact, be intrinsically different at birth.

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193.12 MICROMERE DERIVATIVES IN THE EARLY LEECH EMBRYO. R.K. Ho* and D.A. Weisblat (Spon. J. Weeks) Dept. of Zoology. Univ. of California, Berkeley, CA 94720

Previous studies have revealed that segmentally iterated cells in the leech Helobdella triserialis arise by the proliferation of longitudinally arrayed columns of blast cells produced by the five teloblast pairs. But the contributions of a set of much smaller cells, the micromeres, have remained obscure. Because of their size, these cells have been difficult to inject with lineage tracers, or even to see in the intact embryo. The present study was undertaken to determine the normal fate of these cells as the first step in determining their role in various morphogenetic and determinative events in which they are thought to participate.

It was previously shown that the progeny of the first quartet of micromeres a, b, c and d, derived from cells A, B, C and D, respectively of the four cell embryo, give rise to non-metameric prostomial structures including the supraesophageal ganglion and body wall tissues. They also contribute cells to the epithelium of the provisional integument, a transient tissue that arises between the germinal bands during stages 5-8 and covers the surface of the embryo during stages 5-10. In this study, the existence of other micromeres has been confirmed and their fates followed by lineage tracer injection. The following micromeres have been examined: a", b" and c", produced in the second micromere division by cells A, B and C; dm' and dnopq' produced by proteloblasts DM and DNOPQ; left and right nopq', produced by the left and right NOPQ cells; and left and right n' and opq', produced from the left and right N and OPQ cells. These cells complete the set of micromeres described on other leech species and we have found no other micromeres in Helobdella.

We have found that progeny from identifiable micromeres give rise to reproducible, stereotypic and lineage specific patterns of epithelial cells within the micromere cap of the stage 8 embryo. The mitoses of the micromeres are highly sensitive to -amanitin treatment. Moreover, we are able to inject micromeres with photo-sensitizing tracers and specifically ablate labeled micromere progeny to ask what roles these cells play in the early morphogenisis and organization of the leech embryo.

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194.1 THE CHOLINERGIC SYSTEM IN THE RAT VISUAL CORTEX: INTRINSIC NEURONS AND AFFERENT PROJECTIONS. J.G. Parnavelas* and F. Eckenstein* (SPON: J.K. McDonald). Dept. of Anatomy and Embryology, University College London, London WC1E 6BT, U.K. and Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The morphology and distribution of cholinergic neurons and the distribution and synaptic organization of cholinergic axons were examined in the rat visual cortex by immunocytochemical visualization of choline acetyltransferase (ChAT). The preparation and characterization of the monoclonal antibody to ChAT have been described previously (Eckenstein and Thoenen, EMBO J., 1: 363, 1982). ChAT-labelled perikarya were observed in Tayers II through VI with the majority situated in layers II and III. They were all nonpyramidal neurons displaying chiefly bipolar morphology. Their ultrastructural features were consistent irrespective of layer or dendritic geometry: they showed relatively large nuclei and a thin rim of perinuclear cytoplasm containing clusters of ribosomes, abundant mitochondria and sparse granular endoplasmic reticulum. ChAT-labelled fibers, most arising in the nucleus basalis, formed an elaborate network throghout all cortical layers. Although the preponderance of fibers appeared to have no definite orientation, many in layers I and VI and in the subcortical white matter showed a horizontal preference. Examination of this dense fibre system with the electron microscope revealed that in a given section only a small proportion of axons and axon-terminals formed synapses (Gray type II) with dendrites and somata of pyramidal and nonpyramidal neurons.

194.2 VISUAL CORTICAL AFFERENTS AND EFFERENTS WITH POSSIBLE EXCITATORY NEUROTRANSMITTERS IN THE RAT: IPSILATERAL PATHWAYS. G. A. Looney and A. J. Elberger, Dept. of Anatomy, Div. of Neuroscience, Univ. of Tennessee Ctr. for Health Sci., Memphis, TN 38163

Evidence from several species suggests that excitatory amino acids are used as neurotransmitters by many efferent and afferent neocortical pathways. Studies in the cat show selective uptake and transport of excitatory amino acids by corticogeniculate and thalamocortical pathways (Baughman and Gilbert, 1981; Hicks et al, 1981). Similarly, studies in the rat show that excitatory amino acids are transported by corticotectal, corticogeniculate, corticostriatal and claustrocortical pathways (Divak et al, 1977; Fischer et al, 1982; Kvale and Fonnum, 1983). The present study attempted to confirm these findings, and elaborate on them further by defining afferents and efferents of visual cortex in the albino rat that selectively transport [3H] D-Aspartate (Asp).

Male rats weighing 175-200 gr were given 0.1-0.3 ul injections of 0.7-3.6 mM Asp in buffered saline along the medial, lateral and posterior borders of Area 17. With 24 hr survival the rats were perfused with a mixed aldehyde fixative; brains were frozen sectioned at 30um and coated with Kodak NTB-2 emulsion. After exposure times ranging from 3-6 months the sections were developed and Nissl stained. Sections were examined for the presence of retrogradely labeled cell bodies, and regions of distinctly increased grain concentrations indicating anterograde transport.

Four separate ipsilateral regions had labeled cell bodies: dorsal lateral geniculate nucleus (dLGN), superior colliculus (SC), lateral posterior nucleus of the thalamus (LP), and claustrum (Cl). In the dLGN and Cl these cells were uniformly distributed throughout the nucleus; in the SC and LP they were found in segregated areas. These four regions have been shown to reciprocally connect with the cortical areas injected in the present study (Carey and Neal, 1985; Hughes, 1977; Lund, 1978). In addition, dense concentrations of grains were noted throughout the dLGN, SC, and Cl, more so in the dLGN and Cl than in the SC. The results indicate: 1)Both afferent and efferent visual cortical pathways selectively take up and transport Asp, suggesting that they utilize excitatory amino acids as a neurotransmitter. 2)The present study's results confirm the more infrequently demonstrated phenomenon of anterograde transport of Asp (Baughman and Gilbert, 1981; Cuenod et al, 1981). 3)Specific uptake and transport of Asp is demonstrated by the absence of retrograde or anterograde transport to other regions of the brain connected with the cortical regions injected in this study (Rieck and Carey, 1984).

In conclusion, we have demonstrated the specific transport of Asp by reciprocal pathways between the rat visual cortex and the dLGN, SC and CL, and by visual cortical afferents from LP.

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194.3 VISUAL CORTICAL PATHWAYS VIA THE CORPUS CALLOSUM WITH POSSIBLE EXCITATORY NEUROTRANSMITTERS IN THE RAT. A. J. Elberger and G. A. Looney, Dept. of Anatomy, Div. of Neuroscience, Univ. of Tennessee Ctr. for Health Sci., Memphis, TN 38163

Several cortical regions in the rat are connected by the corpus callosum with contralateral cortex. Studies using retrograde and anterograde tracers show that the borders of Area 17 have abundant callosal connections with contralateral visual cortex (Cusick and Lund, 1981; Miller and Vogt, 1984; Olavarria and Montero, 1984). Callosally connected cortical regions have been examined for specific uptake of putative neurotransmitters. Homotopic and heterotopic callosal pathways of sensorimotor and frontal cortex in the normal rat, and heterotopic pathways of visual cortex in neonatally lesioned rats had selective uptake of [3H] L-Glutamate (Glu) or [3H] D-Aspartate (Asp) (Cuenod et al, 1981; Divak et al, 1977; Fischer et al, 1982; Kvale and Fonnum, 1983). Physiological evidence indicated that excitatory amino acids mediate callosal transmission between contralateral visual cortices in the cat (Hicks and Guedes, 1981). The present study attempted to correlate the callosal connections at the borders of area 17 with selective uptake of an excitatory amino acid in the normal rat.

Adult male albino rats were given 0.1-0.3 ul injections of 0.7-3.6 mM Asp along the medial, lateral and posterior borders of Area 17 and perfused 24 hr later. Brains were frozen sectioned at 30um and coated with Kodak NTB-2 emulsion, exposed for 3-6 months and then developed and counterstained. The contralateral cortex was examined for evidence of retrograde or anterograde transport.

Retrogradely labeled cell bodies were found; there was no clear evidence of anterograde transport. Asp labeled cell bodies were found in seven subregions of contralateral cortex. They are: Area 29, Area 29/18b border, Area 18b, Area 18b/17 border, Area 17/18a border, Area 18a, Area 18a lateral margin. (Area 18b has also been referred to as 18, medial to Area 17.) In these seven subregions many more labeled cell bodies were seen in the supragranular than in the infragranular layers, although the difference was less in Area 29. The number of labeled cells was far less than that of HRP labeled callosal cell bodies (Miller and Vogt, 1984) showing that the Asp labeled a specific segment of the callosal population.

The Asp labeled cells are found in visual Areas 18b, 17 and 18a which have abundant reciprocal callosal connections (Cusick and Lund, 1981; Miller and Vogt, 1984; Olavarria and Montero, 1984). The labeled cells in Area 29 of cingulate cortex also are located in regions with reciprocal callosal connections (Cusick and Lund, 1981; Miller and Vogt, 1984). Thus, the results of the present study suggest that in all regions giving rise to callosal cells, some of these cells use aspartate/glutamate as a neurotransmitter. This parallels other reports of glutamatergic or aspartatergic callosal pathways. Supported by Grant NS20597 awarded to A.J.E.

194.4 RESPONSE VARIABILITY OF NEURONS RECORDED FROM THE VISUAL CORTEX OF MONKEY AND CAT. D.B. Hamilton,* E.T. Vu* and D.G. Albrecht. Department of Psychology, University of Texas, Austin, Texas 78712.

Any systematic interpretation of the signaling properties of visual cortical neurons must take into account the variability associated with responses; such variation clearly sets the limits of reliable information transmission. To quantitatively assess the response variability of neurons recorded from the cat and monkey striate cortex, we examined the relationship between the standard deviation and the amplitude of the response for 617 neurons as function of spatial contrast, spatial frequency and temporal frequency.

We found that when the standard deviation is plotted as a function of response on double logarithmic axes, giving a response-standard deviation (R-SD) function, the data points were best fitted by a straight line (i.e. a power function) with a slope of 0.67 (s.e. 0.01) and an intercept of 2.19 (s.e. 1.02). The R-SD function for simple cells was significantly different from that of complex cells, with the former showing less variability than the latter. Further, a multiple regression analysis showed that response variability was not actually determined by the contrast level per se, but rather the amplitude of the response. Finally, an analysis of the R-SD function measured at optimal vs nonoptimal spatial frequencies revealed no significant differences for the simple cell group, but a very interesting difference for the complex cell group. Specifically, for complex cells, as the spatial frequency is shifted away from the optimal value, the slope of the R-SD function increases monotonically.

194.5 RESPONSES OF SIMPLE AND COMPLEX CELLS IN THE CAT VISUAL CORTEX TO VISUAL DOT STIMULI. Bernt C. Skottun*, David Grosof* and Russell L. De Valois* (SPON: Theodore E. Cohn). Department of Psychology, University of California, Berkeley, CA 94720.

It has previously been claimed that random dots are potent stimuli for complex cells but fail to activate simple cells. This would imply, among other things, that simple cells cannot provide input to complex cells as was originally proposed by Hubel and Wiesel. We have quantitatively compared the responses of simple and complex cells to drifting dot patterns.

Extra-cellular recordings were made from neurons in Area 17 and in the 17/18 border region in anaesthetized and paralysed adult cats. Stimuli were presented on a high resolution video monitor and consisted of either drifting sinusoidal luminance gratings or drifting patterns of random, bright dots (9 min diam) on a dark background. Responses to both sine gratings and dot patterns were determined for drift directions at 15 deg intervals around the clock. Units that responded to gratings with a modulated discharge were classified as simple cells and those that responded with a uniformly elevated firing were classified as complex cells.

Some simple as well as some complex cells were found to respond to random dot patterns; others from each class did not. Typically, those simple and complex cells that showed directional selectivity to sine gratings also showed directional selectivity to dot patterns. In order to make sure that a response was not due just to some local feature in a particular dot pattern, we frequently repeated the experiment using different patterns with identical statistics. Responses from both simple and complex cells were fairly reproducible and have been examined over a wide range of dot densities and drift velocities. To patterns of dots covering 0.5 % of the screen and drifting at 3 deg/sec, complex cells responded on average with a 3-4 times higher firing rate than simple cells. However, complex cells also fire more to conventional stimuli. In our sample, sine gratings of optimal orientation and spatial frequency were found to elicit firing rates from complex cells that are on average more than double those of simple cells. We calculated the relative response to dots vs. gratings for each cell: (response to dots)/(response to gratings). We find that simple cells have relative dot responses that averages 2/3 of those of complex cells. Therefore, one may attribute much, but not all of the complex vs. simple cell differences in dot response to differences in general responsiveness. The finding that many simple cells respond well to dot stimuli contrasts with previous claims that all simple cells are unresponsive to such stimuli. We are currently investigating possible sources for this discrepancy. Supported by NSF grant BNS 78061 & NIH grant EY 00014.

194.6 SEPARABILITY ASSUMPTIONS IN SIMPLE RECEPTIVE FIELDS R. Szulborski*, R. Stepnoski*, J. Jones* and L. Palmer. Dept. of Anatomy, Univ. of Penn. Sch. of Med., Phila., Pa. 19104

Conventional experimental methods, such as generation of length and width tuning curves or orientation (OR) and spatial frequency (SF) tuning curves, implicitly assume that the receptive fields (RFs) of simple cells are Cartesian separable in the two dimensional (2D) space domain and polar separable in the 2D SF domain. For Cartesian separablilty, the response function is given by: $r(x,y) = l(x)w(y)$, where l and w are independent 1D length and width response functions, respectively. Similarly, for polar separability, the response is given by: $R'(OR,SF) = G(OR)H(SF)$, where G and H are independent 1D OR and SF response functions, respectively. If simple cells sum spatially distributed inputs linearly, a well supported notion, these assumptions are mutually inconsistent since the Fourier transform of a Cartesian separable function is Cartesian separable but not polar separable.

In an attempt to resolve this inconsistency, we examined simple RFs using methods which make no assumptions about separability. In the space domain, the responsive region was enclosed by a 16x16 grid which defined 256 unique stimulus locations. In the spatial frequency domain, a 16x16 grid defined 256 unique SF/OR pairs, each pair determining the parameters of a drifting sinosoidal grating. These stimuli were randomly presented, the response amplitudes plotted as surfaces, and the surfaces analyzed for separability. Cartesian separability in the space domain was demonstrated when sections through the surface parallel to the width (length) axis differed only by scale factors. Analogously, polar separability in the SF domain was established when iso-OR (iso-SF) sections differed only by scaling.

We found that more than half of the RFs studied were Cartesian separable in the 2D space domain. As expected, their RFs were also Cartesian separable in the 2D SF domain. Since they are not polar separable, they are poorly characterized by single SF/OR tuning curves. The remaining RFs were not Cartesian separable due to oblique staggering of individual subregions in space (ARVO Abs. 26:265). In the SF domain, the RFs were neither Cartesian nor polar separable and thus are inadequately characterized by length/width or SF/OR tuning curves.

These results are consistent with our recent demonstration that simple cells are well described by Gabor functions (Neurosci. Abs. 11:800). These functions can be, but are not required to be, Cartesian separable in the space domain. However, they can never be polar separable in the SF domain. Clearly, determination of separate tuning curves for length and width in the space domain and SF and OR in the SF domain constitute an incomplete description of simple cell behavior. Methods making no assumptions about separability better characterize simple RFs.

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194.7 A GENERAL METHOD FOR MODELING RECEPTIVE FIELDS COMPOSED OF GAUSSIAN SUBUNITS, AND ITS APPLICATION TO ORIENTATION TUNING IN LGN AND CORTICAL NEURONS. R. E. Soodak* and R. M. Shapley* (SPON: J. Gordon). The Rockefeller University, New York, NY 10021.

When modeling receptive fields in the visual system, it is natural to assume that at any given level of integration, the receptive fields are composed of subunits from previous levels. Since the introduction by Rodieck of the difference of gaussians model for retinal ganglion cell receptive fields, it has become standard practice to explain the response properties of LGN and cortical neurons in terms of convergence of multiple gaussian inputs. Such models have proven extremely useful in explaining major qualitative aspects of neuronal responses, such as spatial filtering properties and the existence of orientation and directional tuning.

We will present a method for quantitative prediction of the behavior of model neurons made up of any arbitrary number of linearly summing gaussian subunits. These subunits can assume any position in the X,Y plane with individually specified amplitudes and widths. In addition, the temporal phase of the response of each subunit can have any value between 0 and 360 deg., allowing the construction of receptive fields which are not spatio-temporally separable. With the parameters thus specified, the model will predict the response to drifting gratings at any spatial frequency and at any orientation. Both response amplitude and response phase are given.

In brief, the method is based on the fact that the response amplitude of an individual subunit depends on its height and width, and the spatial frequency of the grating, but is independent of its position and the grating orientation. The response phase of a single subunit, on the other hand, depends on its position, and both the spatial frequency and orientation of the grating, but does not depend on its height or width. After calculation of the individual amplitudes and phases, the subunit responses are summed vectorially.

With this approach we have successfully modeled the unusual, "four-leaf clover" orientation tuning curves seen in the cat LGN by Soodak, Shapley and Kaplan (Invest Ophthalmol Vis Sci 26(suppl):264, 1985). We will also compare and contrast the predictions of various models of simple cortical cell orientation tuning. Copies of a PASCAL source will be made available.

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194.8 VELOCITY-TUNING MECHANISMS OF DIRECTION-SELECTIVE NEURONS IN STRIATE CORTEX OF THE CAT. Curtis L. Baker, Jr., Department of Psychology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada.

Flashed and continuously moving bar-shaped stimuli were used to study direction-selective neurons in striate cortex of the N_2O/barbituate-anaesthetized cat. Velocity-tuning was measured with stimuli moving continuously through the receptive field at velocities ranging from 1 to 200 deg/sec, both in preferred and null directions. The difference in peak spike frequency for preferred vs null directions always showed a characteristic optimal velocity, V_{opt}. Measured values of V_{opt} ranged from 2 to 150 deg/sec.

Stimuli flashed sequentially at two adjacent points in the receptive field produced direction-selective behaviour comparable to that observed with continuously moving stimuli. Independent variation of the positions of the 2 flashes allowed determination of the optimal displacement, D_{opt}, to produce direction-selectivity. Measured values of D_{opt} ranged from .2 to 2.0 deg for receptive field sizes of .5 to 10 deg.

The optimal velocity, V_{opt}, for a given neuron was systematically related to the optimal displacement, D_{opt}, for 2-flash motion: $V_{opt} = D_{opt}/T_c$. The temporal proportionality coefficient, T_c, represents the time lag for a stimulus moving at a velocity, V_{opt}, to move a distance, D_{opt}. Consequently, T_c can be interpreted as the time delay in a Reichardt-type correlation model of motion detection, for detectors spaced at a distance of D_{opt}.

The histogram of T_c values measured on all sampled neurons showed a peak at 40-80 msec, comparing well with previous estimates of optimal temporal delay for human motion perception.

This temporal delay, T_c, was found to vary inversely with D_{opt}, typically over a range of 20-160 msec. This co-variation of temporal and spatial factors allows a 2-3 octave range of variation of spatial properties (i.e., D_{opt}) to provide a 6-7 octave range of optimal velocities. Thus, striate visual cortex neurons achieve higher values of optimal velocity by making correlations over larger spatial distances as well as over smaller time lags.

Also, since previous findings imply that D_{opt} is inversely proportional to the optimal spatial frequency for a given neuron, these results suggest a direct relationship between velocity-tuning and spatial frequency-tuning.

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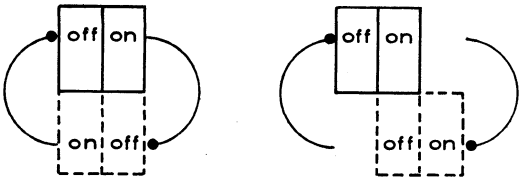
194.9 DIRECTION SELECTIVITY OF VISUAL CORTICAL NEURONS EXAMINED WITH A STROBOSCOPICALLY ILLUMINATED LIGHT BAR. J. Duysens, G.A. Orban and H. Maes*. Laboratorium voor Neuro- en Psychofysiologie, K.U.L., Campus Gasthuisberg, B-3000 Leuven (Belgium).

Despite numerous studies it is still unclear whether the interactions underlying direction selectivity of S cells occur within subregions (e.g. in-field inhibition) or between subregions (i.e. facilitation through superposition in the preferred direction; lateral inhibition in the non-preferred direction). To distinguish between these alternatives one needs to know the spatial and temporal spread of the direction selective mechanisms. We investigated direction selective S (or A) cells, having receptive fields with a wide range of eccentricities, in areas 17 and 18 of anesthetized and paralyzed cats, using a light bar which was moved at speeds between 0.5 and 900°/sec and illuminated stroboscopically (90 μsec) at frequencies between 2 and 50 Hz.

At 2 Hz the responses were never direction selective and thus could be used as a reference to evaluate suppression or facilitation of responses obtained at higher flash frequencies. The maximum interflash distance over which direction selective responses were obtained equalled on average the subregion width and varied little with interflash time interval. The largest effective distances were encountered in cells which were more direction selective at high speeds than at low speeds. The increase in direction selectivity at high speeds in the latter cells was due to an increase both in facilitation in the preferred direction and in suppression in the null direction. Most commonly these cells lost their direction selectivity at interflash intervals of more than 65 msec. In contrast, cells with strong direction selectivity at low but not at high speeds, often tolerated interflash intervals as large as 250 msec. It is concluded that facilitatory and inhibitory interactions between subregions underly direction selectivity in S cells. A model is proposed in which direction selectivity depends on the mutual inhibition of pairs of S cells with receptive fields which are either partly or completely overlapping with subregions of opposite polarity.



194.10 INFLUENCE OF A MOVING NOISE BACKGROUND ON THE AREA 17 CELL RESPONSES TO A MOVING LIGHT BAR. G.A. Orban, B. Gulyás* and J. Duysens. Laboratorium voor Neuro- en Psychofysiologie, K.U.L., Campus Gasthuisberg, B-3000 Leuven (Belgium).

Modulation of responses to a narrow (0.3°) moving light bar by motion of a random noise background (grain size 0.04°; mean luminance 2.75 or 0.35 cd/m^2) was investigated in area 17 cells recorded in paralyzed and anesthetized cats.Preliminary to the testing of the interactions, the optimal velocity for slit motion was determined with a velocity-response curve (Orban et al., J. Neurophysiol., 45:1043, 1981). Also the slit contrast producing 50% of the maximum response when the slit moved over the background held stationary was determined. The interaction series was run with a fixed slit velocity (usually the optimal one) and 7 background motion conditions : in- and antiphase motions at 3 speeds (same speed as slit, 2 octaves slower and 2 octaves faster) and no motion. Fifty-one cells were successfully tested : 95% of them had receptive fields within 10° from the fixation point. Four patterns of direction selectivity were observed under relative motion conditions : (1) cells were direction selective for all background motion conditions (n=7); (2) cells preferred a fixed slit direction, but direction selectivity was clearcut only for antiphase background motion (n=9); (3) cells were direction selective only for 2 or 3 background velocities close to zero (n=16) and (4) cells preferred the direction of slit motion opposite to background motion (n=4) The latter cells which exhibited a relative direction selectivity, switched their preferred velocity when the direction of background motion was reversed. They were all 4 endstopped layer VI cells and were not direction selective when tested with a slit alone. With respect to the influence of background velocity on the slit response, many cells (n=14) were inhibited for background motion faster than 1 or 2°/sec, resulting in a narrow tuning for near zero background velocities, 7 cells were tuned to zero background velocity, 4 cells to a low inphase background velocity and 3 cells to a low antiphase background velocity. These tunings were not invariant with slit velocity showing that these were tunings for background velocity as such and not for the relative velocity between slit and background. These tunings indicate that these cells will be inhibited by any background movement above 1 or 2°/sec and thus will operate only during fixation. The sharpness of the tuning suggests that these cells may encode depth relationships specified by the optical flow. These cells would not directly encode the distance between the slit and the background surface, but rather specify the position of the background surface with respect to the plane of slit motion and the fixation plane (the cells cannot distinguish whether the slit moves behind or in front of the background). Depending on the value of the background velocity yielding optimal slit response, the cell signals that the background is located at the fixation point (when zero background velocity is optimal), between the slit and the fixation point (when a low inphase background velocity is optimal), or just at the other side of the fixation plane (when a low antiphase background velocity is optimal).

194.11 CAT CORTICOTECTAL CELLS: STANDARD COMPLEX AND SPECIAL COMPLEX CELLS HAVE ORTHOGONAL ORIENTATION ANISOTROPIES. T.G. Weyand, J.G. Malpeli and C. Lee. Dept. Psychology, University of Illinois, Champaign, IL 61820.

We have determined the optimal stimulus orientation and length response characteristics of 68 antidromically identified corticotectal (CT) cells in area 17 of the cat. Following Gilbert (J. Physiol., 268: 391-421), those cells whose response increased with increased stimulus length were classified as standard complex (n=41), while those that responded best to short stimuli were classified as special complex (n=27). Virtually all of our sample is from cells whose receptive field centers were within 6 degrees of the horizontal meridian, and the distributions of receptive field positions for both standard and special complex cells are similar. The distribution of orientation preferences for the special complex cells is skewed toward horizontal and vertical orientations, whereas standard complex cells have a strong preference for stimuli with oblique orientations. Vertical orientation preferences are particularly uncommon among standard complex cells. For the oblique 45 degree sectors the ratio of standard to special complex cells is 2.9, while for the horizontal and vertical sectors this ratio is 0.8.

We conclude that in layer 5 of area 17, the orientation hypercolumn is internally heterogeneous. The relative contributions of standard complex and special complex CT cells vary cyclically and orthogonally within the hypercolumn. We have previously shown that standard complex CT cells are dependent upon layer A of the lateral geniculate nucleus, whereas special complex CT cells do not depend upon any single geniculate subdivision (Weyand et al., Neurosci. Absts., 10: 727, 1984). Thus, within an orientation hypercolumn, those orientation columns tuned to vertical and horizontal edges differ from those tuned to oblique orientations both in receptive field properties and in controlling thalamic inputs. Since the receptive field locations of our sample were predominately from the horizontal meridian, we cannot as of yet determine if the orientation anisotropies are actually related to absolute orientation or to orientation relative to polar angle of receptive field position. (This research was supported by NIH grants RO1 EY32114, T32 EY07005, K04 EY00229, and grants from the University of Illinois Research Board).

194.12 A NEW STEREO ILLUSION INDUCED BY BINOCULARLY PRESENTED GRATINGS: EFFECTS OF DISTANCE, AND FIELD SIZE. J.V. Odom and G.M. Chao*. Department of Ophthalmology, West Virginia University, Morgantown, WV 26506.

We have previously reported the parameters under which a new stereo illusion may be observed. If a single grating is viewed with both eyes, the grating appears to have several levels of depth. The sensation of several levels of depth disappeared if one 1) viewed the patterns monocularly, 2) oriented the pattern horizontally, or 3) changed the spatial frequency from an optimum of 3-4 cpd. The effects of distance and field size on the number of levels of depth seen were determined in two experiments with the authors as subjects. Photographic prints of vertical square wave gratings were viewed binocularly. The prints had a mean luminance of 150 cd/m^2 and a contrast of 75%. The subjects task was to state the number of levels of depth seen. The percept was classified as a single level even if the surface was wavy, had individual bars which tilted, or was visible through an indistinct layer without a clear boundary separating levels. To be classified as having two or more levels, a percept of several levels had to be simultaneously visible and have clear, distinct boundaries between the levels. In experiment 1 the effects of field size were studied. At a standard distance of 35 cm field size varied from 5° to 40° for spatial frequencies of 1.5-5.7 cpd. The stereo illusion was not present if field size was 10° or less. As field size increased more levels of depth were seen. In experiment 2 distance was varied from 20 to 140 cm as three levels of spatial frequency (3.2-5.7 cpd) and three field sizes (14°-26°) were held constant. As the viewing distance increased the number of levels decreased. The distance at which only one level was seen increased as field size increased and spatial frequency decreased. Although the illusion is observed when a single grating is viewed binocularly, we believe that it can be accounted for, in part, by the same mechanisms which cause stereo illusions when two gratings of differing spatial frequencies are presented dichoptically (e.g., Blakemore, Vis. Res., 10, 1181-1199). A discussion on these mechanisms and a demonstration of the illusion will be prsented. (Supported by NIH Grant EY 04806 and an unrestricted departmental grant from Research to Prevent Blindness).

194.13 BINOCULAR SPATIO-TEMPORAL INTERACTIONS IN CAT VISUAL CORTEX: A NEUROPHYSIOLOGICAL CORRELATE OF THE PULFRICH EFFECT. T.Carney*, M.A.Paradiso and R.D.Freeman. Neurobiology group, Minor Hall, University of California, Berkeley, CA 94720.

A pendulum moving in the frontoparallel plane of an observer is perceived to follow an elliptical path in depth when a light attenuating filter is placed in front of one eye. In 1922, Pulfrich described the effect and suggested that the filter delays neural processing such that the signal from the filtered eye refers to an earlier moment in time than the signal of the other eye. For objects in motion, this temporal delay is equivalent to adding an interocular spatial disparity to the object's retinal images, hence the perceived shift in depth. In fact, when moving stimuli are presented dichoptically, the shift in depth can be canceled by introducing an appropriate spatial shift to the stimulus being presented to the filtered eye.

It is often assumed that stereopsis involves binocular neurons, of the type observed in the cat visual cortex, which are selective for interocular spatial disparity. We examined the effect monocular light attenuation has on the disparity sensitivity of cat striate cortex neurons. The responses of single units were recorded during dichoptic presentation of drifting sinusoidal luminance gratings at various interocular spatial phases, with and without filters in front of one eye. As previously reported, most cells were selective for interocular spatial phase. When we introduced the filters however, the optimal phase (disparity) shifted. The magnitude of this phase shift was correlated with the degree of filter - induced light attenuation.

The response of disparity selective simple cells has been modeled as a simple linear summation of the temporally varying signals from the two eyes. At some particular spatial disparity (depth) the temporal signals from the two eyes will be in phase and maximally excite the cell. If the filter introduces a delay in one signal the response after binocular summation should decrease. To again elicit a maximal response one must compensate for the spatial shift that the temporal delay mimics by making the stimulus enter the neuron's receptive field in the filtered eye earlier. We accomplished this by changing the spatial disparity (phase) of the stimulus in the two eyes. Therefore, the optimal interocular spatial disparity changed as a function of the temporal delay introduced by the neutral density filter and the velocity of the stimulus.

Although we don't actually know if cats can perceive the Pulfrich Effect, it is a reasonable supposition if the observed change in optimal spatial disparity with filter density is essentially the physiological substrate for this perceptual illusion in man. (EY01175 & EY05636)

CHARACTERIZATION OF CHOLINERGIC RECEPTORS IV

195.1 MOLECULAR STRUCTURE OF THE GLYCINE RECEPTOR Heinrich Betz, Bertram Schmitt*, P. Knauss*, and K. Beyreuther* (SPON: ENA). Institute for Neurobiology, ZMBH, University of Heidelberg, D-6900 Heidelberg, FRG, and Institute for Genetics, University of Cologne, 5000 Köln 41, FRG

The glycine receptor of mammalian spinal cord is an oligomeric membrane glycoprotein which, after affinity purification, contains three polypeptides of 48K, 58K, and 93K. The strychnine binding site of the glycine receptor is located on the 48K subunit and partially overlaps to the 58K polypeptide. The function of the 93K polypeptide is unknown; immunological and peptide mapping experiments have provided evidence of homologies between all three glycine receptor polypeptides.

Two conditions were found which separate the 93K polypeptide from the two smaller glycine receptor subunits: i) purification of cholate-solubilized receptor in the absence of the putative protease inhibiting agent, iodoacetamide, gave receptor preparations containing only the 48K and 58K polypeptides; ii) upon prolonged centrifugation in sucrose gradients, the 93K polypeptide migrated more rapidly than the other glycine receptor subunits. Also, the biochemical properties of the 93K polypeptide differ from those of the smaller ones (concanavalin A binding, microheterogeneity etc.). Immunocytochemistry indicates that all three receptor polypeptides are co-located at spinal cord synapses, the main immunogenic region of the 93K polypeptide being exposed at the cytoplasmic face of the membrane (Triller et al., submitted). These data assign a special role to the 93K polypeptide; it may serve in the ionophore function or postsynaptic anchoring of the glycine receptor.

In order to obtain primary structural information, the N-terminal region and a V8 protease-derived peptide of the 48K-polypeptide were subjected to gas phase microsequencing. The N-terminal information was used for the synthesis of a decapeptide which then served for the production of antibodies. The resulting antiserum bound exclusively to the 48K polypeptide in immunoblots of purified rat glycine receptor. Also, it precipitated [^{3}H]-strychnine binding sites from detergent extracts of rat spinal cord membranes. The N-terminal portion of the 48K polypeptide thus is accessible to antibody binding in the receptor's native conformation.

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195.2 LOW AFFINITY ACETYLCHOLINE BINDING TO TORPEDO POST-SYNAPTIC MEMBRANES? Nina P. Strnad & Jonathan B. Cohen. Dept. of Anatomy & Neurobiology, Washington Univ. Med. Sch., St. Louis, MO 63110.

Interactions of acetylcholine (AcCh) with the membrane-bound AcCh receptor from Torpedo electric tissue have previously been characterized in terms of ligand binding parameters and the AcCh-induced permeability response. [3H]-AcCh binds at equilibrium with high affinity (Keq=10nM) to a binding site associated with the α-subunit of the receptor, and there are two such sites per receptor monomer. Two observations raise the possibility that additional low affinity binding sites might exist that have not been detected by radioligand binding assays. First, the AcCh concentration producing half-maximal permeability response (Kap~70μM) is considerably higher than the transient low affinity binding (K~1μM) observed in studies of [3H]-AcCh binding kinetics. Second, extensive sequence homologies exist between each of the four distinct receptor subunits. An equilibrium binding assay has been developed to evaluate whether additional low affinity sites for [3H]-AcCh exist in Torpedo membranes.

Equilibrium binding of [3H]-AcCh at concentrations up to 300μM was examined at 23°C by ultracentrifugation of receptor-enriched membranes in physiological saline. The binding of [3H]-AcCh was determined from [3H]-AcCh retained in the pellet, and the water content in each pellet was determined by a dual label procedure. Apparent non-specific binding of [3H]-AcCh was accounted for by the pellet aqueous volume. For membranes from T. californica and T. nobiliana, all of the observed specific binding was of high affinity, characterized by a Keq=10nM.

To ascertain the limits of the assay, various sulfhydryl reduction and alkylation conditions were used to modify the equilibrium binding properties of the high affinity sites. For example, reduction by 1mM DTT followed by alkylation with 10mM [14C]-iodoacetamide (IAA) at pH 8 results in alkylation of 80 to 100% of the α-subunit cysteine residues. Following such alkylation by IAA, [3H]-AcCh equilibrium binding was characterized by Keq=15μM and a Hill coefficient of 1. Alkylation of reduced receptors by 5mM N-ethylmaleimide produces similar results.

With the sensitivity of this assay it is possible to conclude that any additional low affinity sites present at the same concentration as the observed high affinity sites must be characterized by Keq > 1mM. This establishes that the AcCh receptor does not contain sites binding AcCh at equilibrium with an affinity similar to the Kap for channel activation and that any additional sites on other subunits must bind AcCh 10^5 -fold more weakly than sites on the α-subunit.

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195.3 PURIFICATION AND GENERATION OF MONOCLONAL ANTIBODIES AGAINST THE NEMATODE LEVAMISOLE RECEPTOR. J.A. Lewis, S. McLafferty*, J. Skimming*, J. Prenger*, and T. Hodgkiss. Dept. of Biological Sciences, Univ. of Missouri, Columbia, MO 65211.

Mutants of an apparent juvenile acetylcholine receptor in the nematode Caenorhabditis elegans can be isolated by selection for resistance to the nicotine-like compound levamisole. We have previously shown that mutants of 7 genes associated with extreme resistance to levamisole all have abnormal receptor activity as defined by (^{3}H)meta-aminolevamisole binding and thereby define a set of genes needed for the proper expression of the nematode receptor. To better define the role of these genes in receptor expression than possible by binding assay alone, we have devised a method of highly purifying the receptor to generate monoclonal antibodies as specific probes of receptor structure. A several fold receptor-enriched starting material was obtained by using dauer larvae of the receptor mutant lev-1(x61). Receptor was purified by extraction into 1% Triton X-100 solution, by chromatography on an affinity column derivatized with trimethyl-aminocaprylic acid (a choline analog) attached to a long spacer arm, and finally by chromatography on Concanavalin A-agarose. The final preparation was purified to a specific activity 30,000 to 60,000 times that of receptor found in crude extracts of wild type worms but the material was still estimated to be less than 10% pure. Triton X-100 was removed with Amberlite XAD-4. Monoclonal screening was done by first adsorbing any monoclonal to goat anti-mouse IgG-derivatized Sepharose beads and then assaying the ability of the monoclonal-coated beads to remove detergent-solubilized receptor from solution. Three anti-native receptor monoclonals were found from 392 hybridoma wells screened. One monoclonal identifies a receptor peptide of 43,000 daltons M.W. on Western blots. A second monoclonal antibody when coated on beads removes both the 43,000 dalton peptide and additionally another peptide of 38,000 daltons M.W. from highly purified receptor preparations, implicating both peptides as receptor peptides. Our purification method may provide a generally useful paradigm for the purification of cholinergic binding proteins.

195.4 THE EFFECTS OF INTRACELLULARLY INJECTED ACETYLCHOLINE ON CULTURED MESENCEPHALIC NEURONS. K.L. Futamachi* and J.H. Peacock (SPON: C. Wakefield). Division of Neurology, University of Nevada School of Medicine, Reno, NV 89557.

In view of recent findings that acetylcholine (ACh) exists in the cytoplasm as well as in the vesicles of presynaptic terminals of the Torpedo, we decided to investigate the consequences of ACh in the cytoplasm of cultured mesencephalic neurons.

ACh was injected intracellularly into presumed substantia nigra (SN) cells obtained from Swiss Webster mouse fetuses of 18-19 days gestational age and cultured for 24-70 days. Recordings were obtained in Dulbecco's Modified Growth Medium containing 5% fetal bovine serum, 5% horse serum and 6.8mM $CaCl_2$ at 37°C and pH 7.2-7.6. The 300MOhm ACh filled electrodes, with which the intracellular recordings were obtained, contained 2M ACh and were pretested on myotube cultures to determine the negative holding current levels which prevented ACh leakage. After a stable intracellular recording was obtained, the holding current was turned off and the ACh was either pulsed or allowed to diffuse into the cells.

The time required for ACh to diffuse into a cell varied from 10 minutes to over 3 hrs. However, in each case EPSP amplitudes were enhanced from 5 to 10mV to values up to 50mV, often leading to increased action potential frequencies. Action potential durations increased from a normal 1-2 msec to 20-30 msec and developed waveforms similar to those of Ca^{++} spikes. Cobalt blocked both the EPSP and the broad spike activity. Preliminary results suggest that the agonist carbamyl choline chloride also has similar effects when injected into these cells.

These results are consistent with the notion that potassium conductance, gK, is decreased by ACh. Present experiments are designed to elucidate whether this K-current is muscarinic (I_M), delayed rectifier (I_K), Ca^{++} activated (I_C) or transient outward (I_A).

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195.5 ACETYLCHOLINE RESPONSES IN SYNAPTICALLY ACTIVE NEURONS IN MOUSE SUBSTANTIA NIGRA CULTURES. J.H. Peacock and K.J. Futamachi*. Division of Neurology, University of Nevada School of Medicine, Reno, NV 89557.

A definitive cholinergic function for substantia nigra (SN) has not been established, despite intense staining of SN neurons for acetylcholinesterase, because of low levels of choline acetyltransferase in SN and lack of an identified, cholinergic, afferent pathway. We have addressed the issue of putative cholinergic function using electrophysiologic techniques in dissociated cell cultures from ventral mesencephalon; many neurons in these cultures have dopamine histofluorescence and are probably from SN.

Acetylcholine (ACh) was applied iontophoretically to neurons in 24-50 day old cultures prepared from Swiss Webster mouse fetuses of 18-19 days gestational age. Cells were studied in Dulbecco's Modified Growth Medium containing 5% fetal calf serum, 5% horse serum, 6.8mM $CaCl_2$ at 37.5°C and pH 7.2-7.6. ACh micropipettes containing 1-2M ACh were each pretested on myotube cultures to determine negative holding current levels which prevented ACh leakage. Responses were recorded from intracellular micropipettes containing KCl (3M) or KOAc (4M, pH 7.0).

Data are reported here for 60 neurons of which 29 had intracellular recordings and the other 31 were synaptically coupled to them. ACh caused either a slow depolarization which returned slowly to baseline after ACh application (9/29) or altered the synaptic input to the recorded neuron (20/29). Some neurons (19/29) demonstrated both response types. Membrane conductance increased little if any during the slow depolarization. Synaptic alteration included activation of postsynaptic potentials (PSPs) either excitatory (30/60), inhibitory (10/60), or both (2/60), as well as inhibition of spontaneously occurring excitatory PSPs (4/60) and inhibitory PSPs (5/60). Responses were predominantly muscarinic and blocked by atropine (1-2 μM), although a nicotinic action has not been entirely excluded.

Our findings are consistent with separate pre- and postsynaptic effects of ACh. Experiments are in progress to determine the mechanism of each.

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195.6 DISTRIBUTION OF CEREBRAL METABOLIC EFFECTS OF NICOTINE IN THE RAT. E.D. London, R.J. Connolly*, M. Szikszay*, J.K. Wamsley and M. Dam*. Neuropharmacology Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224, Dept. of Psychiatry, Univ. of Utah Med. Ctr., Salt Lake City, UT 84132.

Autoradiographic studies have demonstrated specific D,L-[^{3}H]nicotine binding sites in the rat brain (Clarke, P.B.S. et al., Brain Res., 323:390, 1984; London, E.D. et al., Neurosci. Lett., 53:179, 1985). To test the functionality of these sites, we used the 2-deoxy-D[1-^{14}C]glucose ([^{14}C]DG) method to measure the in vivo effects of nicotine on local cerebral glucose utilization (LCGU), an index of cerebral functional activity (Sokoloff, L. et al., J. Neurochem., 28: 897, 1977).

Male Fischer-344 rats were prepared with indwelling femoral arterial and venous catheters. After recovery from halothane anesthesia and at various times (2-60 min) before the injection of [^{14}C]DG (125 μCi/kg, intravenously), they were injected (1 ml/kg, subcutaneously) with 0.9% NaCl or an equal volume of D,L-nicotine bitartrate (0.05-1.75 mg/kg free base) in 0.9% NaCl. In some rats, mecamylamine HCl (2.5 mg/kg free base) was injected subcutaneously 10 or 20 min before nicotine.

Nicotine produced time- and dose-dependent, heterogeneous increases in LCGU, reflecting varied sensitivities to nicotine among different functional systems. Marked increases were noted in the medial habenula, anteroventral thalamic nucleus, interpeduncular nucleus and superior colliculus. Moderate increases were seen in the retrosplenial cortex (layer I, but not deeper layers), caudate-putamen, interanteromedial thalamic nucleus, and lateral geniculate body. No significant effects were observed in the frontoparietal cortex, lateral habenula or central grey matter. LCGU responses were antagonized by mecamylamine, indicating the specificity of nicotine's effects.

These LCGU findings correlate well with the reported distributions of [^{3}H]nicotine binding sites. In general, those areas which showed dense labelling with [^{3}H]nicotine (i.e. thalamic nuclei, interpeduncular nucleus, medial habenula, superior colliculus) also manifested moderate to marked LCGU increases which persisted for up to 2 hr. Areas with less binding (i.e. lateral geniculate body, caudate-putamen) showed lesser LCGU responses to nicotine. The central grey matter, which lacked specific [^{3}H]nicotine binding, demonstrated no LCGU response.

These results indicate that the [^{3}H]nicotine binding sites visualized autoradiographically are functional nicotine receptors, and may direct further anatomical investigations to elucidate the behavioral and physiological effects of nicotine.

195.7 Mr 72,000 AND Mr 86,000 FORMS OF MUSCARINIC ACh RECEPTORS IN AVIAN CNS: SHIFT IN PREDOMINANT FORM DURING DEVELOPMENT. T.H. Large, J.J. Rauh, N.J. Cho‡ A.F. Skorupa‡ and W.L. Klein. Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.

Two forms of muscarinic acetylcholine receptors from the avian central nervous system have recently been reported (Large, et al., Proc. Natl. Acad. Sci. in press). Receptors from embryonic chick retina, cerebrum, optic tectum and cerebellum, covalently labeled with (3H)PrBCM and subject to SDS/urea/PAGE, showed two electrophoretic forms with apparent molecular weights of 86,200 ± 400 and 72,200 ± 300. Each form was present, although decreased in mass by 6,000 daltons, after treatment with deglycosylating enzymes, consistent with molecular differences occurring in the protein portions, rather than carbohydrate portions, of the molecules. Pulse-chase labeling of receptors on cultured retina cells demonstrated that both forms were present on the cell surface; the labeled Mr 86,000 population had a half-life of 5 hours while the Mr 72,000 population had a half-life of 19 hours. Incubation of retina membranes with proteolytic enzymes indicated that Mr 72,000 receptors were more resistant to degradation than Mr 86,000 receptors. The two molecular weight forms appear to be unrelated to M1 and M2 pirenzepine subtypes since pirenzepine binding in the retina is exclusively low affinity (M2)(Large, et al. J. Biol. Chems. in press).

The relative proportions of the Mr 86,000 and Mr 72,000 receptors in retina showed a striking inversion during development. Before synaptogenesis, receptors were mainly of Mr 86,000, while after synaptogenesis receptors were mainly of Mr 72,000. The increase in Mr 86,000 receptors was not due simply to elevated proteolytic activity. The apparent molecular weight and relative proportion of receptor forms from embryonic membranes were unchanged by incubation with crude homogenate from hatched chick retina. Development of a predominantly low molecular weight receptor population also occurred in aggregate, but not monolayer, retina cell culture suggesting a possible role for cell-cell interactions in triggering the change. Two molecular weight forms of muscarinic receptors may represent products of differentially-regulated gene transcription, products of a single RNA transcript subject to differential processing, or post-translational conversion of the Mr 86,000 form to the Mr 72,000 form. The novel change in size of muscarinic acetylcholine receptors supports the hypothesis that molecular modification of neurotransmitter receptors plays a role in the development and regulation of synapses. (This work was supported in part by NIH grants NS18490 and DA02950 to WLK, NIMH post-doctoral grant MH08904 to JJR and NIMH pre-doctoral grant MH09228 to THL)

195.8 HYDROXYEICOSATETRAENOIC ACIDS (HETES): BLOCKADE OF MUSCARINIC RECEPTOR-MEDIATED CYCLIC GMP FORMATION AND ESTERIFICATION INTO PHOSPHOLIPIDS IN MURINE NEUROBLASTOMA CELLS (CLONE N1E-115). Michael McKinney. Dept. Pharmacology, Mayo Foundation, Rochester, MN 55905.

Muscarinic receptors in N1E-115 cells mediate increased cGMP formation and decreased hormone-induced cAMP formation. A biochemical and pharmacologic study of the relationship between arachidonate metabolism and muscarinic responses in N1E-115 cells was initiated. The cGMP response, but not the cAMP response, was blocked by lipoxygenase but not cycloxygenase inhibitors. Certain perturbants of the cellular oxidation state (e.g., methylene blue) also inhibited the cGMP response. The cyclic GMP response was blocked by arachidonate itself (IC_{50}=45 μM), while agents like stearate and lysophosphatidylinositol did not inhibit this response at concentrations up to 100 μM. The potency of arachidonate was increased 10-fold by the air-oxidation of the fatty acid. These findings are consistent with the idea that the oxidative metabolism of arachidonate is involved in the cyclic GMP response. In support of this, enzymatically-synthesized 15-HETE and 12-HETE were found to block the cyclic GMP response (IC_{50}'s 8 μM and 18 μM, respectively). [^{3}H]Arachidonate was metabolized by N1E-115 cells, primarily by rapid esterification into phospholipids and neutral lipids, but small amounts of prostaglandins and other unidentified products were also formed. ETYA and 15-HETE elevated free arachidonate levels in N1E-115 cells, consistent with the blockade of metabolic routes for the fatty acid. ETYA and 15-HETE both reduced the labeling of phospholipids by [^{3}H]arachidonate. However, 15-HETE was more selective than ETYA in decreasing the label in phosphatidylinositol (PI), suggesting the 15-HETE was an inhibitor of the acyl CoA-PI transferase or was a substrate for this enzyme. Further, [^{3}H]15-HETE itself was shown to be esterified into N1E-115 phospholipids; 65% of the esterification was into PI and 24% into the neutral lipids, with much lower amounts appearing in other lipids. [^{3}H]15-HETE also labeled the polyphosphatidylinositides. [^{3}H]5-HETE and [^{3}H]12-HETE were also esterified into N1E-115 lipids, but with profiles different from that of [^{3}H]15-HETE or [^{3}H]arachidonate. 15-HETE was shown to decrease the esterification of [^{3}H]arachidonate into PI with an IC_{50} of 7 μM, a value similar to the IC_{50} of 15-HETE for blockade of cyclic GMP formation. Thus the possibility exists that 15-HETE and other lipoxygenase inhibitors could block the muscarinic receptor-mediated cyclic GMP response by a mechanism involving the redistribution of arachidonate or the substitution of the inhibitor for the fatty acid in the cellular phospholipids. This may occur in addition to or exclusive of the inhibition of a putative lipoxygenase. (Supported by NIH grant NS21319 and the Mayo Foundation)

195.9 PURIFICATION OF MUSCARINIC RECEPTORS FROM RAT BRAIN. J. Baumgold. Clinical Neurogenetics Branch, DIRP, NIMH, Bethesda, Md. 20205

Since muscarinic receptors represent less than 0.01% of rat brain membrane protein, the purification of this and other receptor proteins from brain is impractical using conventional biochemical techniques. Successful purifications of this magnitude require use of specific affinity chromatography steps. I have taken advantage of a specific affinity chromatography gel first developed by Haga and Haga (J. Biol. Chem. 258 13575, 1983) which, when used in conjuction with conventional purification steps, was able to purify the muscarinic receptor to near homogeneity in substantial yield. This approach is similar to that recently taken by Peterson et al (PNAS 81 4993 1984) for purification of atrial muscarinic receptors.

Muscarinic receptors were solubilized at a 25-35% yield using the detergent digitonin. The solubilized receptor was first purified 4-6 fold by ion-exchange chromatography on DEAE-TRISACRYL gel. A further 4-6 fold purification was obtained on a hydroxylapetite column. The final 300-500 fold purification was obtained using the following affinity chromatography steps: 1) the receptor protein bound specifically to an aminobenzhydrol tropine (ABT) sepharose affinity gel and could be specifically eluted with either carbachol or scopolamine; 2) a wheat germ agglutinin sepharose affinity gel was used to concentrate the receptor and separate it from the cholinergic ligands used to elute it from the ABT affinity gel. This entire procedure resulted in the purification of a 75,000 dalton protein which bound 3H-QNB and could be affinity labeled with 3H-propylylbenzyl choline mustard. This protein was purified 5000-10,000 fold at an overall yield of 10-20% with respect to solubilized material. Work is currently underway in our laboratory to characterize the purified receptor.

195.10 CHOLINERGIC REM SLEEP INDUCTION: EFFECTS OF ARECOLINE IN MAN AND IN RAT BRAIN. J.I. Nurnberger, Jr., W.H. Berrettini*, W. Mendelson*, T.I. Soncrant*, D. Sack* and E.S. Gershon*. Clinical Neurogenetics Branch and Clinical Psychobiology Branch, DIRP, NIMH and Laboratory of Neuroscience, NIA, Bethesda, MD 20205

Various brainstem areas may be involved in the generation of REM (rapid eye movement) sleep, including the dorsal and lateral pontine tegmentum, the pontine and midbrain reticular formation, the locus coeruleus and the parabrachial area. Neurochemical control is also complex, probably involving NE, 5-HT, and Ach at various sites.

Pharmacologic manipulation of muscarinic receptors has been reported to affect the timing of REM sleep in animals and man. We have confirmed this in 22 persons given an injection of 0.5 mg intravenous arecoline (after peripheral blockade with 0.15 mg glycopyrrolate) timed to end at 25 minutes after the end of the first REM period. Arecoline was followed by wakening or the onset of the second REM period within 20 minutes in 9/22 subjects as compared with 1/14 subjects given no infusion or placebo ($p < 0.03$ by Fisher's exact test). At present 6/10 euthymic drug-free bipolar subjects demonstrate awakening or REM induction within 20 minutes, as opposed to 3/12 controls; increased sensitivity to cholinergic REM induction in bipolar subjects has been previously reported[1].

Arecoline effects in rat brain have been examined using the quantitative (^{14}C) 2-deoxy-D glucose method 3 minutes after intraperitoneal administration of 0.05 to 50 mg/kg arecoline. Rats were pretreated with methylatropine 4 mg/kg subcutaneously. Nine areas thought to be involved in REM generation were examined. After 0.05 mg/kg of arecoline, increases ($p < 0.05$) in cerebral glucose utilization were seen in the dorsal and median raphe nuclei and in the pons. After 0.5 mg/kg, increases were also seen in the mesencephalic and anterior pontine reticular formation and in the dorsal tegmental nucleus. Only after higher doses were significant effects seen in the locus coeruleus, the posterior pontine reticular formation and the lateral pontine tegmentum. Doses of 5 mg/kg and above cause an activation in most brainstem regions[2].

Cholinergic REM induction may be an effective tool for examining muscarinic receptor sensitivity in experimental animals and clinical studies. In another experimental paradigm, a correlation between the concentration of muscarinic receptors in the striatum of rats and their sensitivity to behavioral effects of arecoline has been demonstrated[3]

1. Sitaram et al., Science 208, 200, 1980. 2. Soncrant et al. Brain Research, in press. 3. Smith et al., CINP Abstracts, 1984, p. 518.

196.1 EFFECT OF EXTRACELLULAR IONIZED CALCIUM ON THE BIPHASIC GROWTH HORMONE RESPONSE TO RAT GROWTH HORMONE-RELEASING FACTOR. G.A. Ortolano and L.C. Terry. (SPON: K.L. Casey) Depts. of Neurol. & Physiol., Univ. of Michigan Med. School and Veterans Admin. Med. Center, Ann Arbor, MI 48105.

Thyrotropin-releasing hormone-induced growth hormone secretion by GH_4C_1 cells is biphasic (Aizawa, T. and Hinkle, P.M.: Endocrinol., 116:73,1985), as is glucose-induced insulin secretion (Curry, D.L. and Bennett, L.L.: Proc. Nat'l. Acad. Sci. 73:248,1976); however, similar effects in normal rat pituitary cells in response to rat growth hormone-releasing factor (rGRF) have not been reported. It is well established that Ca is required for the secretion of GH, but the sensitivity of pituicytes to small changes in extracellular ionized Ca, viewed as the physiologically important form of Ca, has not been determined. Using pituitary cell culture techniques (Brazeau, P. et al.,: Proc. Nat'l. Acad. Sci. 79:7909,1982) and a NOVA 2 Ionized Calcium Analyzer (Courtesy of NOVA Biomedical, Newton, MA), the GH secretory response to rGRF (100 pM) was shown to be temporally biphasic in the presence of 1.1 mM ionized Ca. The first phase reached a plateau within 1 hour and the second was evident at 3 hrs. Because most studies using Ca or Ca channel blockers utilized prolonged (> 2 hrs) incubation times, the 60 min incubation period was selected to study Ca-dependence associated with the first phase of the observed secretory response. A rGRF dose-response design was employed at 3 levels of ionized Ca (0.8, 1.1 and 1.4 mM). The EC(50) for rGRF (approx 4 pM) remained unchanged at all concentrations of extracellular ionized Ca, whereas, the maximal secretory responses were significantly different and positively correlated with ionized calcium (7, 8.5 and 12.5 ugm GH/0.5 X 10^6 cells for 0.8, 1.1 and 1.4 mM ionized Ca, respectively). Using a similar experimental design, a verapamil dose-response experiment was conducted in the presence of varying concentrations of extracellular ionized calcium and a fixed concentration of rGRF (100 pM). Increasing ionized calcium caused a shift to the right in the dose-response curve, and 100 uM verapamil inhibited only 60% of the GH secretory response to rGRF. From these data it is concluded that (a) temporally biphasic GH secretion from normal pituicytes occurs in response to rGRF at physiologic levels of extracellular ionized Ca, and (b) the GH secretory response to rGRF is only partially dependent upon extracellular ionized Ca because the maximal secretory response to rGRF increased with increasing Ca while verapamil was unable to totally inhibit the secretory response. One or both phases may be partially dependent upon extracellular ionized Ca.

196.2 SUBPOPULATIONS OF SOMATOTROPES REVEALED BY REVERSE HEMOLYTIC PLAQUE ASSAY ARE DIFFERENTIALLY INHIBITED BY SOMATOSTATIN. GY Nagy* and JD Neill* (Spon: J. Brown). Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama 35294

Two functionally distinct sub-populations of somatotropes are reported to exist in the rat pituitary gland. Using a reverse hemolytic plaque assay for measurement of GH release from individual cells, Frawley and Neill (Neuroendocrinology 39:484, 1984) obtained evidence for sub-populations of somatotropes, one of which is preferentially responsive to GHRH. The aim of this study was to determine the effect of somatostatin on the responses of sub-populations of GHRH treated somatotropes. Pituitary cells from proestrus rats were dispersed with trypsin and cultured for 24 h before the plaque assay for GH secretion was performed. Pituitary cells (plated at 6.5 x 10^4 cells/ml) were incubated for 2 h in the presence of anti-growth hormone antiserum (1:100) and guinea pig complement (1:50). We constructed dose response curves using GHRH or somatostatin at concentrations of 10^{-8} M- 10^{-11}M. We then used the dose of GHRH (10^{-8}M) which resulted in maximal plaque size (maximum GH secreted), for use in combination with different doses of somatostatin (from 10^{-8} to 10^{-11}M). All of the doses of somatostatin decreased the mean plaque size (mean amount of GH secreted per cell) but only the highest doses (10^{-8}M, 10^{-9}M) decreased the fraction of plaque forming cells (% secretory somatotropes). Frequency distributions of GH plaque areas in normal and somatostatin treated pituitary cells were unimmodal. A bimodal frequency distribution of plaque areas was observed when GHRH was applied. One mode of plaque areas was equal to the control group and a second larger mode appeared. The size of this mode was dose related. 10^{-8}M and 10^{-9}M GHRH increased both the mean plaque size and the % of plaque forming cells. When pituitary cells were treated with a combination of GHRH and different doses of somatostatin, the fraction of somatotropes forming large plaques after GHRH treatment was reduced in a dose dependent fashion with a corresponding increase in the number of smaller plaques; however the proportion of plaque forming cells did not decrease. These results confirm the previous findings that GHRH produces a bimodal frequency distribution suggestive of a preferentially responsive population of somatotropes. Furthermore, they suggest that somatostatin preferentially inhibits the population of cells forming large plaques which are preferentially stimulated by GHRH. (Supported by NIH Grant # HD18130 to J.D. Neill).

196.3 SOMATOTROPE AND LACTOTROPE RESPONSES TO cAMP: POPULATION ANALYSIS BY REVERSE HEMOLYTIC PLAQUE ASSAY. J.J. Mulchahey*, G. Nagy*, E. Luque* and J.D. Neill* (Spon: M. Friedlander). Dept. of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

The demonstration of heterogeneous responses of somatotropes to growth hormone releasing hormone (GHRH) and lactotropes to thyrotropin releasing hormone (TRH) suggests that the variability in cell response to these secretagogues may lie in differentially responsive subpopulations of cell types. We have applied the reverse hemolytic plaque assay to detect growth hormone (GH) and prolactin (PRL) secretion from individual somatotropes and lactotropes, respectively, to determine whether these secretagogue-responsive subpopulations remain during 8Br-cAMP stimulated hormone secretion. Monodispersed anterior pituitary cells were obtained by tryptic dispersion of anterior pitutiary tissue from estrus rats. Recently dispersed pituitary cells were incubated with Protein A-coated oRBC and anti-growth hormone antiserum (1:100) with medium, GHRH or 8Br-cAMP or anti-prolactin antiserum (1:40) with medium, TRH or 8Br-cAMP for 2h after which guinea pig complement (1:50 and 1:100, respectively) was added for 30min. Pituitary cells secreting hormone are visible in zones of hemolysis (plaques) proportional in size to the amount of hormone secreted. TRH consistently increased PRL secretion to 156±8.0 percent of control (mean±SEM; n=4) with a maximally effective dose of 10^{-7}M in this assay system. Frequency distribution analyses of plaque areas revealed unimodal, log-normal distributions in controls which were uniformly increased by TRH. 8Br-cAMP increased PRL secretion to 223±21 percent of control with a maximally effective dose of 10^{-3}M and slightly augmented frequency distributions as compared to TRH. GHRH increased GH secretion 18.6±5.0-fold compared to control (mean±SEM; n=5) with a maximally effective dose of 10^{-7}M Frequency distributions were unimodal, log-normal in control cells but bimodal in GHRH-treated cells. 8Br-cAMP increased GH secretion 11.9±3.4-fold compared to control with a maximally effective dose of $5x10^{-2}$M while the frequency distributions were similar to the GHRH treatment. Both GH increases were greater than those reported previously for cultured cells. Because the heterogeneity of cellular response to secretagogue persists when somatotropes or lactotropes are stimulated with 8Br-cAMP, an agent mimicking the intracellular messenger cAMP these data suggest that this heterogeneity in hormone secretion exists as an intrinsic, post-receptor occupancy, post-adenylate cyclase heterogeneity in cellular metabolism. (Supported by NIH grant HD-19314 to JDN).

196.4 EFFECT OF IN VIVO PRETREATMENT WITH ESTROGEN ON GROWTH-HORMONE AND PROLACTIN SECRETION IN A SUPERFUSED RAT PITUITARY CELL SYSTEM. J.Horvath*, M.Mason-Garcia*, E.Winsor*, L.Debeljuk* and A.V.Schally* (SPON:W.Macklin), Tulane Univ. Sch.of Med. and VA Med.Ctr., New Orleans, LA 70146.

Prolactin and growth-hormone (GH) secretion was investigated in a superfused rat pituitary cell system. The pituitaries were obtained from rats pretreated with estradiol (Progynon, 500 µg/kg, twice a week for three weeks prior to the experiment) and from rats in estrus. The basal prolactin secretion from pituitary cells of estrogen treated rats could be inhibited with infusion of somatostatin. Different concentrations of somatostatin ($2.5x10^{-9}$M - 10^{-7}M, for 15 min) produced the same inhibition during the infusion; however, larger doses resulted in a more prolonged inhibition. Pituitary cells of rats in estrus showed no response to somatostatin. Elevation of prolactin secretion above the baseline during 3 or 15 minute superfusion with TRH ($2.5 x 10^{-8}$M) was greater in estradiol pretreated cells. When the same concentration of TRH was infused together with somatostatin (10^{-9}M- $2.5x10^{-8}$M) prolactin secretion was more markedly inhibited in the pituitary cells from rats pretreated with estrogen, due to their increased sensitivity to somatostatin. Dopamine inhibited the TRH-induced prolactin secretion equally in both systems; however, on a molar basis somatostatin appeared to be a more potent inhibitor of TRH-induced prolactin secretion than dopamine. Estrogen pretreatment reduced $hpGH\text{-}RH_{44}$ (10^{-9}M, 3 or 15 min pulses) stimulated GH-release. When somatostatin (10^{-9}M- $2.5x10^{-8}$M) and $hpGH\text{-}RH_{44}$ (10^{-9}M) were infused simultaneously for 15 min, somatostatin completely prevented the release of GH during the infusion. However, after the infusion was ended, a marked release of GH could be observed which was much larger than the rebound in GH which occurs after the superfusion of pituitary cells with the same doses of somatostatin. TRH consistently induced some stimulation of GH-release in both cell types, but this release was much smaller than that observed with $hpGH\text{-}RH_{44}$. In conclusion, in vivo pretreatment with estradiol increases the sensitivity of lactotroph cells to somatostatin and TRH in a superfused pituitary cell system. The sensitivity of somatotroph cells from estradiol pretreated rats to $hpGH\text{-}RH_{44}$ is reduced. In the doses used in this study, somatostatin delays the effect of $hpGH\text{-}RH_{44}$ but does not prevent it.

196.5 RAPID DESENSITIZATION TO THE ACUTE STIMULATORY EFFECTS OF NICOTINE ON RAT PLASMA ADRENOCORTICOTROPIN AND PROLACTIN. B. Sharp* and S. Beyer* (SPON:S. Nicol). Dept. of Medicine, Hennepin Cty. Med. Ctr. and Univ. of Minnesota, Mpls., MN 55415.

Nicotine rapidly elevates plasma adrenocorticotropin (ACTH) and prolactin (Prl). Since tolerance to some of the acute depressant effects of nicotine has been shown after both acute and chronic dosing, we tested for acute desensitization to its stimulatory effects on ACTH and Prl.In each experiment, 200 g male Holtzmann rats received 2 ip injections (I) containing nicotine (N in mg free base/kg BW) and/or saline (S); decapitation was 7.5 min after the second I. Values for plasma Prl and ACTH measured by radioimmunoassay are mean ± SE. The interval (min) separating the 2 I is shown.

I_1	Min	I_2	ACTH(pg/ml)	Prl(ng/ml)
S	+60	S	54.6±7.4	3.2±0.9
S	+60	$N_{0.1}$	181.8±52.6	2.6±0.5
S	+60	$N_{0.25}$	807.2±86.5**	30.4±3.4*
S	+60	$N_{0.5}$	960.9±136.9**	21.4±4.6*
S	+60	$N_{1.0}$	1180.9±167.9**	31.8±3.3*
S	+60	$N_{2.0}$	953.5±66.0**	24.0±4.5*
$N_{0.5}$	+60	S	71.4±8.6	1.9±0.4
$N_{0.5}$	+60	$N_{0.25}$	146.8±32.7	1.8±0.4
$N_{0.5}$	+60	$N_{0.5}$	286.1±133.0	1.9±0.4
$N_{0.5}$	+60	$N_{1.0}$	526.3±113.8*	12.2±3.6

Comparisons by ANOVA are between S/S vs S/N or N/S vs N/N. *p<.025; **p<.01; ***p<.001. Since this experiment demonstrated significant desensitization following a single dose of $N_{0.5}$, a time course with increasing intervals between the 2 injections was done.

S	+60	$N_{0.25}$	538.6±70.0	29.7±3.3
$N_{0.5}$	+60	$N_{0.25}$	72.4±20.2***	5.9±1.2***
S	+120	$N_{0.25}$	588.9±23.0	23.2±3.9
$N_{0.5}$	+120	$N_{0.25}$	61.9±11.5***	3.9±0.7**
S	+240	$N_{0.25}$	858.8±111.0	21.8±3.7
$N_{0.5}$	+240	$N_{0.25}$	173.0±39.3***	6.1±0.5**
S	+360	$N_{0.25}$	842.0±121.7	22.5±5.0
$N_{0.5}$	+360	$N_{0.25}$	287.3±105.0**	10.5±2.9*

Asterisks are for unpaired t-tests between groups at the same time interval. ANOVA failed to show a significant difference in ACTH and Prl values between any of the groups that received the initial dose of $N_{0.5}$. The role of negative feedback by corticosterone in this desensitization was evaluated. All rats were bilaterally adrenalectomized 24H prior to receiving 2 injections separated by 2H. A partial ACTH response to the second N dosing was observed (S/S:284.5±31.9; $S/N_{0.25}$: 923.6±107.9; $N_{0.5}/N_{0.25}$:458.7±26.6; ANOVA showed S/S<N/N<S/N p<.01). Desensitization of the Prl response was unaffected.

In nicotine naive rats, a single dose of N elevated ACTH and Prl levels within 7.5 min. These responses desensitized to a second dose for at least 6H. Adrenalectomy did not affect the Prl desensitization; it minimally affected ACTH. (Supported by DA03977)

196.6 LITHIUM STIMULATES ADRENOCORTICOTROPIN (ACTH) RELEASE FROM CULTURED ANTERIOR PITUITARY CELLS. M. Zatz and T. Reisine. Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20205

Lithium (Li) is useful for investigating the role of phosphatidylinositol (PI) turnover in signal transduction because it inhibits the phosphatase that catalyzes the conversion of inositol phosphates (IPs) to inositol. In the presence of Li, the products of inositol lipid breakdown are not reutilized and hormones that increase PI turnover elevate the levels of IPs. To determine the role of PI turnover in the secretion of ACTH, Li was applied to a tumor cell line (AtT-20/D16-16) derived from mouse anterior pituitary, that consists of a homogenous population of corticotrophs. Cells were pretreated for 24 hours with ^{3}H-inositol and the effects of a number of agents on inositide metabolism and ACTH release were examined. Most ACTH secretagogues (CRF, isoproterenol, VIP, forskolin, 8-bromo-cAMP, K^+ and phorbol ester) did not change IPs levels either in the presence or absence of Li. Lithium itself did increase the levels of ^{3}H-inositol monophosphate (IP_1). Furthermore, Li itself stimulated ACTH release in a time and concentration dependent manner, which correlated with its effects on IP_1 levels. This effect was not unique to the tumor cell line; primary cultures of rat anterior pituitary cells also released ACTH in response to lithium. Pretreatment of AtT-20 cells with Li reduced the subsequent response to Li. Such pretreatment did not affect the response to CRF, isoproterenol, forskolin or K^+. Another class of drugs which stimulates ACTH release from these cells are the phorbol esters (PE). Active PE are thought to specifically stimulate protein kinase C. Pretreatment with Li did reduce ACTH release in response to subsequent treatment with PE, suggesting a link between lithium's effects and protein kinase C. Diacyl glycerol (DAG), another product of PI breakdown, is the putative intracellular regulator of protein kinase C. DAG itself did not stimulate ACTH release. However, pretreatment with DAG reduced lithium's effects on ACTH release and ^{3}H-IP_1 levels. DAG and IPs are formed from PI through the actions of phospholipase C. An activator of phospholipase C is calcium. Elevated extracellular calcium levels stimulated ACTH release and, in the presence of Li, markedly raise IP_1 levels. Pretreatment of AtT-20 cells with DAG also attenuates the cells' response to high extracellular calcium; both ACTH release and the increase in ^{3}H-IP_1 are blocked. The data suggests that Li and calcium stimulate ACTH release through mechanisms distinct from the other known ACTH secretagogues. These mechanisms may involve stimulation of phospholipases, PI turnover, and protein kinase C.

196.7 EFFECT OF AGENTS WHICH ALTER INTRACELLULAR SODIUM AND CALCIUM ON LUTEINIZING HORMONE RELEASE FROM CALF PITUICYTES. J.P. Kile, S. McConnell* and M.S. Amoss, Jr. Dept. Veterinary Physiology, Texas A&M University, College Station, TX 77843-4466.

While it is known that calcium (Ca^{++}) is required for release of hormones from both rat and bovine pituitary cells, little work has been done to study the role of calcium flux or intracellular mobilization in the release of luteinizing hormone (LH) from bovine gonadotrophs. Therefore, a study was done to compare the effects of agents which alter sodium (Na^+) and Ca^{++} entry into cells on LH release from calf pituicytes.

Calf pituitary cells from calves 4-10 months of age were dispersed using 0.2% collagenase for 1 hr; then plated at 3 x 10^5 cells/multiwell well for 5 days. On day 5, cells were washed and treated with 10 µl of ion agent alone or 10 µl ion agents plus 10 µl gonadotropin releasing hormone (GnRH, 10 µg/ml) in 1 ml of Hank's Balanced Salt Solution (HBSS). Cells were then incubated 6 hr at 37°C, the medium removed and stored at -20°C prior to LH determination by radioimmunoassay. Each treatment was repeated 2-3 times, n=3 or n=6 depending on the trial.

In cells pre-treated with the Ca^{++} channel blockers nifedipine or verapamil at concentrations ranging from 10^{-8} to 10^{-5} M, only nifedipine between 10^{-7} and 10^{-5} M produced a significant inhibition ($p<0.05$) of GnRH stimulated LH release with LH values falling from 7.6 ± 0.9 ng/ml to 4.6 ± 0.4 ng/ml respectively. These values compare to 5.6 ± 1.0 ng/ml for HBSS control and 28.1 ± 5.5 ng/ml for GnRH 100 ng/ml. In contrast, only verapamil reduced baseline LH values from 30-60% ($p<0.01$) at the concentrations tested. Mg^{++} (2-10 mM) produced no significant effects on either basal or stimulated LH release. The Ca^{++} ionophore A23187 over the range of 1-10 µM induced a linear increase in LH secretion, with significant release at 2.5 µM (23.2 ± 5.3 ng/ml) through 10 µM (79.7 ± 2.5 ng/ml). In comparison, the Na^+-K^+ ATPase inactivator, ouabain, at 10^{-5}-10^{-3} M also produced increases in basal LH ranging from 2.5 fold ($p<0.05$) up to 12 fold ($p<0.01$) respectively. Further challenge by GnRH produced no additional LH release over that by A23187 or ouabain alone. Finally, the Na^+ channel blocker tetrodotoxin (TTX) produced a dose-dependent, significant decrease in GnRH-induced LH release at concentrations between 10^{-9} and 10^{-7} M, while basal release was unaffected (9.4 ± 1.4 ng/ml at 10^{-9} M compared to GnRH (18.1 ± 2.7) and HBSS (9.2 ± 1.3 ng/ml) controls). The results of this study suggest that calcium influx and mobilization (whether or not subsequent to Na^+ fluxes) may play an important role in basal and GnRH-stimulated LH release from calf gonadotrophs. The findings are also consistent with the belief that nifedipine and verapamil have different sites of action.

196.8 1,2-DIDECANOLYGLYCEROL AND PHORBOL 12,13-DIBUTYRATE ENHANCE ANTERIOR PITUITARY HORMONE SECRETION IN VITRO. A. Negro-Vilar and E. G. Lapetina (SPON: P. Cuatrecasas). Reprod. Neuroendocrinology Sec., Lab. Reprod. Dev. Tox., NIEHS, NIH, Res. Tri. Pk., NC 27709, and Department of Molecular Biology, The Wellcome Research Labs.

Experiments were designed to evaluate the role of well-known activators of protein kinase C, such as 1,2-diacylglycerol and phorbol esters, on the release of all the anterior pituitary hormones in vitro. Dispersed rat anterior pituitary cells were incubated in the presence of 1,2-didecanoylglycerol (DiC_{10}), a synthetic diacylglycerol, or phorbol 12,13-dibutyrate (PDBu), a tumor-promoting phorbol ester, at different concentrations and for varying periods of time.

ACTH and β-endorphin (β-END) secretion were enhanced by DiC_{10} in a concentration-dependent manner, with a minimal effective concentration of 5 µM. PDBu at 5 nM produced a significant release of both ACTH and β-END. The effect of DiC_{10} and PDBu was time dependent, with maximal responses occurring at 15-30 min for DiC_{10} and 30-60 min for PDBu. Release of growth hormone (GH) was also enhanced significantly by DiC_{10} and PDBu, with minimal effective concentrations of 1 µM and 1 nM, respectively. Maximal release of GH was already attained within 15 min with DiC_{10} or 60 min with PDBu. In additional experiments, the effects of DiC_{10} and PDBu on secretion of LH, FSH, PRL and TSH were evaluated. The results indicate that 5-25 µM DiC_{10} produced a concentration-dependent release of each of those hormones, and that 5 µM was the minimal effective concentration in every case. Nearly maximal stimulation was achieved within 15 min for each hormone. PDBu (50 nM) significantly enhanced LH, FSH, PRL and TSH release within 30 min. Although qualitatively all hormones were similarly stimulated, both with respect to time and concentration, some quantitative differences were observed. ACTH and β-END release were enhanced 100% by DiC_{10} and 300% by PDBu, while the increase in other hormones was of a lesser magnitude.

The present study indicates that two specific stimulators of protein kinase C, diacylglycerol and phorbol ester, can enhance secretion of all anterior pituitary hormones in a concentration- and time-dependent manner. This suggests that formation of endogenous 1,2-diacylglycerol may represent a physiological intracellular messenger in the events leading to anterior pituitary peptide hormone release.

196.9 DEMONSTRATION OF DOPAMINERGIC BINDING SITES IN THE ANTERIOR PITUITARY OF THE MALE SYRIAN HAMSTER. D.M. Burns*, C.A. Leadem* and B. Benson (SPON:D.E. Blask). Dept. of Anatomy, Univ. of Arizona, Arizona Health Sciences Center, Tucson, AZ 85724.

The characterization of dopaminergic binding in the anterior pituitary has implications in the study of neuroendocrine regulatory mechanisms, particularly with regard to prolactin (PRL) secretion. Changes in the responsiveness of PRL cells to the inhibitory action of dopamine may play an important role in PRL physiology. Specific dopaminergic binding sites have been previously described in anterior pituitaries of several mammalian species. However, it has recently been reported (Steger et al., Biol. Reprod. 29:872) that dopamine is ineffective in inhibiting PRL secretion in long photoperiod exposed male hamsters. We therefore wished to investigate whether there are specific dopamine binding sites in the hamster anterior pituitary. We utilized the dopamine antagonist ^{3}H-spiroperidol to label binding sites in membrane preparations of adult male hamster (14:10 light:dark cycle) anterior pituitaries. Non-specific binding was determined in the presence of 10 µM (+)-butaclamol. The specific interaction of ^{3}H-spiroperidol with these membranes reached a steady-state level within 10 minutes at 37° C, and specific binding was directly proportional to the membrane concentration between 0.07 and 0.3 mg protein. Saturation curves were analyzed by an iterative least-squares nonlinear regression method (LIGAND). F-test comparison of goodness-of-fit shows that these curves fit a two-site model significantly better than a one-site model ($p<.001$). Dissociation constant (Kd) and total binding site concentration (Bmax) for the high affinity site were estimated to be 60 pM and 49 fmol/mg protein, respectively. Corresponding estimates for an apparent low affinity binding site were 6.6 nM and 105 fmol/mg protein. Additional investigations are currently under way to further define this low affinity binding. These data suggest that there are specific binding sites for dopamine in the anterior pituitary of the male hamster with physical parameters (Kd and Bmax) similar to those reported for other mammals. Therefore, the reported lack of effect of dopamine on PRL secretion in these animals is not due to an absence of specific dopaminergic binding sites.

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196.10 LITHIUM TREATMENT ENHANCES THE ACTIVITY OF TUBEROINFUNDIBULAR AND TUBEROHYPOPHYSEAL DOPAMINERGIC NEURONS. T. Koyama,* J.I. Koenig, H.Y. Meltzer and G.A. Gudelsky. Dept. of Psychiatry, Hokkaido Univ., Sapporo, Japan, Dept. of Psychiatry, Univ. of Chicago and Ill. State Psychiatric Institute, Chicago, IL.

Lithium has been shown to alter the dynamics of catecholaminergic and serotonergic neurotransmission within the CNS. Lithium also has been shown to alter the plasma concentrations of several hormones (e.g., PRL, corticosterone and beta-endorphin), as well as the serotonergic control of the secretion of these hormones. In the present study we have investigated the influence of chronic treatment of rats with lithium on the dopaminergic control of PRL secretion. The synthesis rates of dopamine within the terminal regions of tuberoinfundibular and tuberohypophyseal neurons (i.e., median eminence (ME) and neurointermediate lobe (NIL), respectively) were estimated from the in vivo accumulation of dihydroxyphenylalanine (DOPA) after the inhibition of the activity of amino acid decarboxylase with m-hydroxybenzylhydrazine (NSD 1015, 100 mg/kg). The accumulation of 5-hydroxytryptophan (5-HTP) also was measured as an index of 5-HT synthesis. Rats were killed 30 min after the administration of NSD 1015. DOPA was measured in acidic extracts of ME and NIL by HPLC with electrochemical detection. Dopamine concentrations also were measured in anterior pituitary gland tissue of rats not given NSD 1015. Maintenance of rats on a diet containing lithium carbonate (0.23%) for 3 or 21 days resulted in a 60-70% reduction of plasma PRL concentrations. There also was a significant increase in the accumulation of DOPA in the ME and NIL of animals given lithium for 3, 7 or 21 days when compared to control animals. The accumulation of 5-HTP in the ME and NIL was unaltered by lithium treatment. Dopamine concentrations in anterior pituitary glands of lithium-treated animals were more than twice those in tissues from control rats. The inclusion of lithium in the diet for 7 or 21 days also altered the secretion of PRL from anterior pituitary tissue in vitro and the effect of dopamine to inhibit PRL secretion. The secretion of PRL in 1 hr from pituitary tissue of control animals was 55 ± 9 ng/mg tissue, whereas that from tissue of rats treated with lithium for 21 days was only 22 ± 2 ng/mg tissue. Moreover, dopamine (10^{-6}M) effected a 70% inhibition of the release of PRL from pituitary tissue of control animals, whereas only a 45% dopamine-induced inhibition of PRL secretion was observed in tissue from lithium-treated rats. These results are suggestive that lithium treatment enhances the activity of tuberoinfundibular and tuberohypophyseal dopamine neurons. An increased secretion of dopamine from these hypothalamic neurons may account for the lithium-induced reduction of plasma PRL concentrations and the suppressed secretion of PRL in vitro from anterior lobe tissue.

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196.11 THE EFFECT OF NEONATAL PROLACTIN DEFICIENCY ON ADULT TUBEROINFUNDIBULAR AND TUBEROHYPOPHYSIAL DOPAMINERGIC NEURONAL ACTIVITY. S.W. Shyr*, W.R. Crowley, and C.E. Grosvenor. Dept. of Physiol. & Biophys., Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

In the adult, non-lactating rat, prolactin (PRL) regulates its own activity via stimulation of tuberoinfundibular dopaminergic neuronal activity (TIDA). We have shown previously that PRL from maternal milk crosses the gut into the circulation of the rat pup (J. Endocrinol. 79:191). In this study we investigated the effect of reducing the amount of PRL available to the pups during days 1-5 of lactation on the turnover of dopamine in median eminence (ME) and neurointermedial lobe (NIL) of the pituitary. Lactating Holtzman rats received daily sc injections of either saline or bromocriptine (0.5 mg/rat) for days 2-5 postpartum. Some rats were anesthetized on postpartum days 2, 3, or 5, at which time milk was collected and analyzed for PRL content by RIA. The remaining rats were kept with their litters until pups were weaned (day 21). The pups thereafter were fed and housed under standard conditions. When 33-35 days of age, the rats were injected sc with either saline or α-methyl-p-tyrosine(250 mg free base /kg); they were decapitated under light ether anesthesia 30 or 60 min later. ME and NIL were rapidly removed and analyzed for dopamine content, using radioenzymatic assay.

	Milk PRL (ng/ml)			Turnover rate of dopamine (pg/µg protein/hr)	
days	2	3	5	TIDA(ME)	THDA†(NIL)
saline	275.5 ±34.9	483.2 ±49.0	442.6 ±29.3	84.9 ±10.6	6.9 ±1.1
CB-154		137.1 ±24.0*	13.7 ±7.5*	29.0 ±6.6*	8.8 ±1.9

*$p < 0.05$ vs. saline control group.
†THDA: tuberohypophysial dopamine

Treatment of mothers with bromocriptine markedly reduced the PRL content of the maternal milk and decreased pup weight gain. However, by day 33-35, the pups' weight gain was comparable to the saline control group. In addition, such treatment resulted in a significantly decreased turnover of dopamine in the median eminence in the 33-35 day old offspring. Dopamine turnover in the NIL was unaffected in these animals. These results indicate that a deficiency in PRL delivered by maternal milk consequently decreased activity in TIDA neurons in the offspring. The data suggest that the normal PRL-TIDA feedback regulation may be impaired by such treatment. (Supported by HD 04358 to CEG and HD13703 and HD 00366 to WRC.)

196.12 QUANTITY OF TYROSINE HYDROXYLASE IN THE MEDIAN EMINENCE: EFFECT OF AGE, SEX, AND REPRODUCTIVE STATUS. John C. Porter. Cecil H. and Ida Green Center for Reproductive Biology Sciences, Departments of Obstetrics and Gynecology and Physiology, Southwestern Medical School, Dallas, TX 75235

In rats, secretion of dopamine from tuberoinfundibular dopaminergic neurons into hypophysial portal blood varies throughout the estrous cycle, is greater in females than in males, and is greater in young animals than in old. Since secretion of dopamine by tuberoinfundibular dopaminergic neurons is dependent on the rate of synthesis of L-dihydroxyphenylalanine from L-tyrosine, we investigated the relationship of these physiological states to the amount of tyrosine hydroxylase (TH) in the median eminence (ME), the site of release of dopamine into portal blood.

The ME (≅40 µg wet wt) was homogenized in Laemli buffer, heated 3 min in a boiling water bath, and subjected to acrylamide gel electrophoresis. Highly purified rat TH was used as the standard. After electrophoresis, protein on the gel was transferred to nitrocellulose paper. The paper was incubated overnight with rabbit antiserum (1:500 dilution) to rat TH, and then immersed in the color development solution. The developed nitrocellulose paper was dried, and the TH-containing band was analyzed by reflectance spectrometry. Between 0 and 100 ng of TH, the area enclosed by the reflectance envelope was a linear function of the quantity of TH.

In cycling female rats (12-16 wks old), the quantity of TH in the ME was as follows: estrus, 42 ± 2.9 ng (mean ± S.E.); diestrus 1, 61 ± 2.8 ng; diestrus 2, 75 ± 4.2 ng; and proestrus, 84 ± 3.3 ng. In ovariectomized rats (15-16 wks old), the ME contained 24 ± 1.6 ng TH. In pre-pubertal rats (5-5.5 wks old), the ME of females contained 25 ± 2.0 ng TH, and that of males 24 ± 2.1 ng. The amount of TH in the ME of mature males (15-16 wks old) was 34 ± 3.3 ng, whereas that of old males (17-20 mos old) was 37 ± 1.6 ng TH. In aged female rats (22-24 mos old), the ME of diestrus animals contained 75 ± 4.2 ng TH, and the ME of estrus animals contained 71 ± 2.8 ng TH.

In summary, TH in the ME (1) increases with sexual maturity, (2) is greater in mature females than in mature males, (3) and is greatest in cycling females when circulating estrogen levels are greatest (Butcher et al. ENDOCRINOLOGY 94:1704, 1974). The amount of TH in the ME of old female rats is much greater than that of old male rats. Prior to puberty, the amounts of TH in the ME of male rats is the same as that in female rats. Castration of mature female rats results in a reduction of TH in the ME to the level seen in pre-pubertal animals. It is suggested that estrogen causes an increase in the amount of TH in the ME. In the ME of aged rats, the existence of TH levels comparable to those in young, mature animals is inconsistent with the reduced secretion of dopamine seen in aged rats.

197.1 NERVE GROWTH FACTOR (NGF) REGULATES THE ACTION POTENTIAL DURATION OF MOUSE DORSAL ROOT GANGLION (DRG) NEURONS DURING MAINTENANCE IN LONG-TERM ORGANOTYPIC CULTURES. S.M.Crain, A.Chalazonitis and E.R.Peterson*. Dept. of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, N.Y. 10461.

NGF has been shown to regulate the levels of Substance P and somatostatin in mature mouse DRG neurons long after the developmental period when these cells require NGF for survival (Kessler & Black, PNAS '80,'81; Otten etal, Nature,'80). The present study investigates whether NGF may also regulate some of the bioelectric properties of DRG neurons. Intracellular recordings were made of the somatic action potentials (APs) of DRG neurons in cultures of fetal mouse spinal cord with attached DRGs (Chalazonitis & Crain, Soc. Neurosci.Abstr.'83).

Initial studies were carried out on DRG-cord explants exposed to the antimitotic alkaloid, taxol, for the first few days and then maintained for many weeks in high NGF (3ug/ml) media (see abstract by Peterson & Crain, this volume). Recordings from these DRG neurons (tested in 5mM Ca^{++}/5mM Ba^{++} BSS) revealed APs with Ca^{++} components that were about 3x shorter in duration than those from control cultures where NGF was routinely withdrawn after 1 wk in vitro. Proof that the shorter AP-Ca component was due not to prior taxol exposure, but to prolonged maintenance in NGF, was obtained by tests on drug-free cultures. The duration of the AP-Ca component (in 5mM Ca^{++}) was significantly shorter ($p<0.025$) for DRG neurons grown with NGF for 6 wks: 1.6±0.2 msec (n=31) vs 2.1±0.1 msec (n=52) in controls. This difference was demonstrated more sharply in tests made in Ca^{++}/Ba^{++} BSS: 5.4±0.7msec (n=24) in neurons on NGF for 6 wks vs 14.6±1.3 msec (n=62) in controls ($p<0.001$). Even after only 2 wks of exposure to NGF the AP-Ca component was much shorter than controls: 6.4±0.7 msec (n=17). Furthermore, when DRG-cord explants grown for weeks in high NGF were returned to control media (for 1-4 wks) the AP-Ca components again showed long durations: 12.8±0.7 msec (n=37). Neurons in isolated DRG explants maintained in high NGF for 6-8 wks also showed much shorter AP-Ca components: 6.9±0.8 msec (n=8) vs 18.5±1.5 msec (n=46) in controls, indicating that this NGF effect can occur in the absence of spinal cord tissue.

These data provide the first demonstration that NGF regulates a specific bioelectric property of DRG neurons--the duration of the Ca component of the somatic AP--at stages when the cells no longer require NGF for survival. Furthermore, these alterations in the AP duration occur within 1 wk after introduction or withdrawal of NGF. Further analyses are required to determine if NGF-regulation of the AP duration of DRG neurons is mediated by alterations in Ca^{++} and/or K^{+} channel functions. (Supported by research grants DA-01031 and BNS-821847 to S.M.C.)

197.2 DETECTION OF NERVE GROWTH FACTOR-BINDING PROTEINS SECRETED BY L-929 CELLS. K. Siminoski* and R.A. Murphy. (SPON:T.E. Phillips) Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115.

L-929 cells secrete a high molecular weight nerve growth factor (NGF) complex that differs from mouse salivary gland 7S NGF. The complex is known to have a molecular weight greater than 7S NGF by gel filtration and although it contains β NGF, it does not contain the α or γ NGF subunits (Biochem. 22:4264; C.R.Acad.Sc. Paris, Series III, 297:523). The other components of the complex have not been identified. In this study, we have detected proteins with NGF-binding activity in L-cell conditioned medium.

^{125}I 2.5S NGF was added to L-cell conditioned medium, the pH was briefly lowered (pH 2-3), then raised to neutrality. On Sephadex G-200, radiolabelled NGF eluted near the void volume, in a position indistinguishable from that of native immunoreactive NGF in L-cell conditioned medium. When the pH shift was carried out in the presence of excess unlabelled NGF, radioactivity near the void volume was diminished by >70%. Without a pH shift, radiolabel eluted with free NGF. Radioactivity also appeared near the void volume when L-cell conditioned medium was dissociated with 8 M urea, 6M guanidine HCl, 40 uM octyl glucoside or 2% SDS.

NGF-containing complexes were also examined by gel electrophoresis. ^{125}I NGF bound to L-cell proteins was cross-linked using dissucinimidyl suberate (50 uM), reduced with 2-mercaptoethanol, and electrophoresed on 7.5% SDS gels. Radioactivity migrated in 2 principle bands (160 KD and 60 KD) and in 7-8 fainter bands, all of which were competed by unlabelled NGF, and were not present in the absence of a pH shift. In unreduced samples labelling diminished in intensity and increased competible activity appeared near the top of the gel.

Efforts are now underway to characterize and isolate these NGF binding proteins.

197.3 LOW- AND HIGH-AFFINITY NGF RECEPTORS POSSESS NGF BINDING MOIETIES IDENTICAL IN MOLECULAR WEIGHT ON SDS-PAGE. S.H. Green and L.A. Greene*. Department of Pharmacology, New York University School of Medicine., 550 First Ave., New York, NY 10016.

We have reported the isolation of four mutant PC12 cell lines which neither respond to nor internalize NGF but are otherwise very similar to PC12 cells (Green et al., 1984, Soc. Neurosci. Abstr. 10, 367). Scatchard-type analysis of ^{125}I-NGF binding to these mutant $PC12^{nnr}$ lines reveals that they have low-, but not high-affinity NGF binding sites as opposed to PC12 cells which possess both forms. We have used EDC, a water-soluble cross-linking reagent, to affinity-label the NGF receptor with ^{125}I-NGF. Proteins were then separated and sized by SDS-PAGE. Crosslinking was done following conditions in which (1) only rapidly-dissociating ^{125}I-NGF should be washed off or (2) neither slowly nor rapidly dissociating ^{125}I-NGF should be washed off. Under the first type of condition, a 100 kD protein was labeled in PC12 cells and binding of ^{125}I-NGF to it was competed with by unlabeled NGF with an ID_{50}~0.2 nM. The labeled protein was not solubilized by 0.1 percent Triton X-100. No labeling under these conditions was seen in $PC12^{nnr5}$ cells. These experiments establish the high-affinity binding moiety as a 100 kD protein (including the covalently attached NGF). Under conditions in which both types of receptors should be labeled, a single 100 kD protein was observed and binding of ^{125}I-NGF to it was competed with by unlabeled NGF over a concentration range of 0.2-6 nM. In $PC12^{nnr5}$ cells, a labeled 100 kD protein was observed under these conditions and binding of ^{125}I-NGF to it was competed with by unlabeled NGF with an ID_{50} of 2-6 nM. Therefore the binding moieties of low- and high-affinity receptors have similar or identical molec. wts. and may be the same molecule. This is consistent with the reported interconvertability of low- and high-affinity forms (Block and Bothwell, J. Neurochem. 210, 1654). We are currently testing this by peptide-mapping. We have observed the 158 kD protein reported by Hosang and Shooter (JBC 260, 655, 1984) only if we use HSAB, a hydrophobic crosslinker, which, unlike EDC, can rapidly enter the cell. Therefore the 158 kD band may result from an association between the NGF binding moiety and a 58 kD protein not exposed on the cell surface. We are investigating the identity of this 58 kD protein and its possible role in regulating NGF receptor affinity or signal transduction. We have found one other difference between PC12 and $PC12^{nnr}$ cells: ^{32}P labeling reveals phosphorylation of 66 kD and 58 kD proteins in all $PC12^{nnr}$ lines, but not in PC12 cells, particularly after dibutyryl cAMP treatment. Possible relationships between these phosphorylation events and the NGF receptor are being investigated. Support was from NIH grant NS16036 to L.A.G. and from ACS Institutional grant IN-14-7 and a grant from Dysautonomia Foundation Inc. to S.H.G.

197.4 CELL FREE DETECTION OF AN NGF-ACTIVATED KINASE ACTIVITY IN PC12 CELLS. E.A.Rowland*,T.H.Müller*, M.Goldstein, L.A. Greene*, Depts. of Pharmacol. and Psychiat., NYU Med. Ctr., New York, NY, 10016.

Exposure of target cells to NGF causes a number of biochemical and morphological changes. Among these changes are differences in the phosphorylation of several proteins (Yu, Tolson and Guroff, J.Biol.Chem., 1980, 225, 10481; Halegoua and Patrick, Cell, 1980, 22, 571). To better understand the role of these phosphorylations in the mechanism of NGF action, we have begun to purify and characterize an NGF-activated protein kinase in PC12 cells. When the latter are exposed to NGF, they take on many of the properties of sympathetic-like neurons (Greene and Tischler, P.N.A.S. USA, 1976, 73, 2424). Tyrosine hydroxylase(TOH) has been shown to increase in phosphorylation and activity when PC12 cells are exposed to NGF. We therefore chose this enzyme as a substrate for measuring NGF-stimulated phosphorylating activity in a cell-free assay. In this assay, exogenous semi-purified TOH is incubated with [32P] ATP, EGTA and an extract of control or NGF-treated PC12 cells. TOH exposed to extracts of NGF-treated cells show a 2-3 fold faster rate of phosphorylation than controls. Characterization of the kinase has shown that: (1) activation does not occur in broken cell extracts, but only in intact cells; (2) maximal activation is at 4nM NGF and half maximal at 0.5 nM NGF; (3) activation occurs after 1-3 minutes of NGF treatment and is maximal by 10 minutes; (4) the kinase is more active in the presence of Mn^{++} than of Mg^{++}, does not require Ca^{++} and is not affected by the presence of cAMP; (5) the activity is found only in the soluble fraction of the cell; (6) material removed from extracts by gel filtration over G-10 Sephadex is not required for the activity; (7) DTT does not reduce the activity. In addition, inhibitors of Ca^{++}/calmodulin-dependant protein kinase, protein kinase C and casein kinase II do not inhibit the NGF-activated kinase. Peptide mapping by HPLC of TOH after phosphorylation by the NGF-activated kinase, shows a greatly enhanced radiolabeled peak relative to controls. This peak is distinct from that obtained when cAMP is added to the assay. Furthermore, the NGF-dependant site of phosphorylation appears resistant to mild alkali treatment. In a search for other substrates of the kinase, protamine, bovine serum albumin, casein, an amino acid polymer substrate for tyr phosphokinases [(Glu Na, Tyr) 4:1] and histones HV-S, HII-A, HI gave negative results. However, initial experiments indicate that the histone HIII may be a substrate. When EGF and FGF were tested under the same assay conditions, there was no increase in phosphorylation found with extracts from either the membrane or soluble portions of the cell. Additional experiments are in progress to further characterize and purify the NGF-activated kinase. Supported by NIH grant NS16036, NIH training grant GM07238 and NIMH grant 02717.

197.5 STRUCTURE AND PROCESSING OF THE NERVE GROWTH FACTOR PROHORMONE. D.O. Clegg and L.F. Reichardt. Division of Neurobiology, Dept. of Physiology, University of California, San Francisco, Ca. 94143.

The sequence of the beta - Nerve Growth Factor (NGF) cDNA clone from male mouse submaxillary gland predicts that the NGF peptide is synthesized as part of a larger precursor which is then processed in the submaxillary gland by cleavages at di- and tetrabasic amino acid residues to produce mature beta-NGF and several other peptides. However, the forms of NGF made in sites relevant to neuronal development, such as targets of sensory and sympathetic innervation, are not known. The structure of the gene makes it possible that the prohormone, alternately processed peptides containing the beta-NGF sequence, and other peptides from the prohormone are secreted and important for the developmental program.

The gene sequence suggests two possible initiation sites for translation. To determine the preferred initiation site, a synthetic mRNA, generated from the NGF cDNA fused to the SP-6 promoter, was translated in vitro. A protein of Mr 34,000 was the major product, and its N-terminal amino acid sequence indicates that the first possible initiator codon is utilized to make the larger of the two possible precursors (cf. Scott, J. et al.(1983), Nature, 303, 538-540. and Ullrich, A. et al. (1983), Nature, 303,821-825.)

To facilitate analysis of the prohormone and its derivative products, a synthetic peptide predicted to be a part of the precursor has been employed to raise antibodies specific for the prohormone. These antibodies precipitate the Mr 34,000 translation product made in vitro, and bind in sections to the same cells in the submaxillary gland that make beta-NGF in vivo. Furthermore, a cross-reacting antigen can be detected in saliva, which may correspond to the prohormone or a smaller product derived by proteolytic processing. The major antigen has been substantially purified. NGF gene products made in vivo and in vitro are both being used to examine processing in vitro. The antibodies are being used to examine processing of the NGF prohormone in sympathetic effector organs.

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197.6 DEVELOPMENTAL, REGIONAL AND POST-LESION EXPRESSION OF NERVE GROWTH FACTOR (NGF) AND NGF mRNA IN RAT BRAIN. S.R. Whittemore[1], T. Ebendal[2]*, L. Lärkfors[2]*, L. Olson[3]*, Å. Seiger[3]*, I. Strömberg[3]* and H. Persson[1]*. Departments of Medical Genetics [1] and Zoology[2], Uppsala University, Uppsala, Sweden and Department of Histology[3], Karolinska Institute, Stockholm, Sweden.

β-nerve growth factor (NGF) plays a major role in maintaining peripheral sympathetic and sensory ganglionic neurons. However, the existence, distribution and possible function in the central nervous system (CNS) remains elusive.

To assess the expression of NGF mRNA in the CNS, a cDNA probe complementary to mouse submaxillary gland NGF mRNA (Scott et al., Nature 302:538) was used both to probe poly A+ RNA from the rat brain and to isolate a rat genomic NGF clone from a Charon 4A phage library. This genomic clone was sub-cloned in phage M13 and used as a probe in S1 endonuclease analysis of NGF mRNA. Levels of endogenous NGF were determined using a two site enzyme immunoassay.

Northern blot and endonuclease S1 analyses showed wide variation in the levels of NGF mRNA in different regions of the adult rat brain. Maximal levels were observed in the cortex (CTX) and hippocampus (HC), at levels 500 fold less than those seen in the male mouse submaxillary gland. The olfactory tubercle, striatum, pons/medulla and thalamus were intermediate in NGF mRNA expression, while in the cerebellum, septum and hypothalamus NGF mRNA could not be detected. Control hybridization with a cDNA for actin showed little regional heterogeneity. Endogenous NGF levels were consistent with the distribution of NGF mRNA, with the CTX and HC having about 2.0 ng NGF/g tissue. In addition, NGF was found in the septum, a finding consistent with the reported retrograde transport of NGF from the HC to the septum (Schwab et al., Brain Res. 168:473).

NGF mRNA was first detectable in the later stages of embryonic development (E18) reaching adult levels by 3 weeks postnatal. At 1 week, NGF mRNA levels in the HC and CTX are 50% of those seen in the adult structures. Endogenous NGF was also detected prenatally.

Bilateral fimbria transections were made in 1 week old rat pups by a knife cut which also transected the overlying CTX. 3 days post-lesion, the levels of NGF mRNA in the lesioned HC had decreased 50%, while the NGF mRNA in the lesioned CTX had increased two-fold. Endogenous NGF levels were consistent with the alterations in NGF mRNA seen in the lesioned regions. Results of NGF and NGF mRNA expression in the HC following entorhinal lesions will also be discussed. Immunohistochemical studies are presently being undertaken to examine the cellular localization of the altered NGF expression.

197.7 NERVE GROWTH FACTOR (NGF) PROMOTES SURVIVAL OF SEPTAL CHOLINERGIC NEURONS AFTER INJURY. F. Hefti. Dept. of Neurology, University of Miami Medical School, Miami, FL 33101.

Several findings obtained in recent years suggest that NGF acts as specific neurotrophic factor for the group of ascending cholinergic neurons of the mammalian forebrain. NGF and the mRNA coding for NGF are present in target areas of these neurons, and forebrain cholinergic neurons have been shown to react to NGF with an increase in choline acetyltransferase (CAT) activity. I now present further support for a role of NGF in the function of forebrain cholinergic neurons by showing that NGF is able to promote survival of these cells after axonal injury in vivo and in vitro.

In vivo, NGF was given intraventricularly to adult rats with lesions of the cholinergic septo-hippocampal pathway. The pathway was cut unilaterally by transsecting the fimbria, and a cannula was chronically inserted into the lateral ventricle after lesioning. During 4 weeks, lesioned animals received intraventricular injections of mouse salivary gland 2.5S NGF (10μg twice weekly) or of equal amounts of a control protein. The animals were then taken for histochemical visualization of cholinergic cell bodies using acetylcholinesterase (AChE) histochemistry (after pretreatment with DFP). In control animals, the fimbrial transsections resulted in a pronounced reduction of the number of cholinergic cells in the medial septal nucleus and the vertical limb of the diagonal band of Broca on the side ipsilateral to the lesion. NGF-treament significantly attenuated this lesion-induced degeneration. In lesioned control animals, the total number of AChE-positive cells contained in medial septal nucleus and the vertical limb of the diagonal band on the lesioned side was reduced by 50% as compared to the number on the contralateral side. In lesioned animals treated with NGF, the number of cholinergic cells on the lesioned side was reduced by 12%.

To establish the dose-response relationship and the specificity of NGF's effect on survival of forebrain cholinergic neurons, further experiments were carried out in vitro. The septal area was dissected from brains of rats of postnatal day P2. At this developmental stage, cholinergic neurons had already started to invade hippocampal tissue and therefore lost their processes during the dissociation procedure. Dissociated cells were grown for 10 days in a modified L15 medium and were then taken for visualization of cholinergic neurons using CAT immunocytochemistry and AChE cytochemistry. NGF significantly elevated the number of cholinergic cells surviving in the cultures. The ED_{50} of NGF's effect was approximately 10ng/ml, and it was blocked by antibodies to NGF.

The findings of the present study indicate that NGF is able to rescue rat forebrain cholinergic neurons from degeneration after transsection of their processes in vivo and in vitro. Since forebrain cholinergic neurons degenerate selectively in human Alzheimer's disease, these findings might help to understand the cause and find an effective treatment for this disease.

197.8 NGF SPECIFICALLY ENCHANCES DEVELOPMENT OF BRAIN CHOLINERGIC NEURONS IN CULTURE. H.J. Martínez*, C.F. Dreyfus, G.M. Jonakait and I.B. Black. Cornell Univ. Med. Coll. New York, NY 10021.

Recent evidence suggests that the trophic protein nerve growth factor (NGF) affects brain cholinergic neurons. In the present study, we examined the effect of NGF on organotypic cultures of fetal rat neostriatum (NS) and basal forebrain (BF). The NS or BF from E19-20 (gestational day) rat fetuses were grown on collagen-coated coverslips and maintained in Maximov chambers as lying drop preparations. Treatment of NS or BF cultures with NGF for 10-12 days resulted in a 5- to 12-fold increase in the specific activity of the cholinergic enzyme, choline acetyltransferase (CAT), in a dose-dependent fashion. This effect was not elicited by insulin, ferritin or cytochrome C, proteins structurally or physicochemically similar to NGF, indicating specificity of the response to the trophic protein. Conversely, the effect of NGF was blocked by anti-NGF antiserum, a further indication of specificity. Immunocytochemical studies of the treated cultures, using a monoclonal antibody against CAT, identified the cholinergic population revealing a network of positively stained neurons, exhibiting long dendritic and axonal processes. NGF did not alter total protein content of NS or BF cultures, indicating selectivity of the effect. Moreover, levels of substance P, a peptide localized to non-cholinergic neurons in the NS, were not altered by NGF, while CAT activity increased 12-fold in sister cultures. Although the mechanisms of action of NGF on NS and BF cholinergic neurons remain to be determined, the marked, specific response of CAT suggests that this defined trophic protein plays a critical role in normal brain development. It may now be possible to determine whether NGF, its processing or its reception is defective in degenerative disorders that involve these brain cholinergic neurons. (Supported by NIH Grants NS 10259, NS 20788, HD 12108, NSF Grant BNS 8024081 and aided by a grant from the Hereditary Disease Foundation. HJM is a fellow from the Instituto de Investigaciones Clínicas, Universidad del Zulia, Maracaibo, Venezuela. CFD is the recipient of a Teacher-Scientist Award from the Andrew W. Mellon Fdn.).

197.9 NERVE GROWTH FACTOR EFFECTS ON DEVELOPING CENTRAL CHOLINERGIC NEURONS: TEMPORAL RESPONSE CHARACTERISTICS IN SEPTUM, HIPPOCAMPUS AND STRIATUM. M.V. Johnston, K. Buchanan*, J.L. Rutkowski and W.C. Mobley. Depts. of Ped. and Neurol., Univ. of Michigan, Ann Arbor, MI 48104; Div. N.P., Walter Reed Institute of Research, Washington, DC 20307; Dept. of Neurol., Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Nerve growth factor (NGF), injected intracerebroventricularly (ICV), stimulates choline acetyltransferase (ChAT) activity in regions of immature rat brain enriched in cholinergic perikarya (septum, nucleus basalis, striatum) and nerve terminals (neocortex, hippocampus). Previous work demonstrated a response on postnatal day (PD) 12 following injection of 4 doses of purified NGF (PD's 2, 4,6,8; Mobley et al, Neurosci. Abstr. 10,367,1984). Now we report results of experiments using simplified dose schedules to dissect regional and temporal features of the response. Single or multiple ICV injections (30 ugm) of mouse NGF were used (Biochem. 15,5543).

Three doses (PD's 2,4,6) elevated ChAT in the septum by 94% compared with littermate controls on PD12, and this effect could be duplicated by a single injection on PD8 (+90%). However, both protocols elicited less response than the complete 4 dose schedule, which raised ChAT by 290%. Injection on PD2 or PD2&4 produced no measurable increase on PD12. The results suggested that the neonatal doses (PD2&4) may have enhanced the response to NGF on PD6&8, but it was unclear if they stimulated ChAT independently.

To examine effects of a single early dose, groups of 5 neonatal rats were given single injections on PD2 and assayed along with littermate controls at intervals up to PD12. At 6, 12 and 24 hrs post-treatment, ChAT remained unchanged in septum, hippocampus and striatum, but at 48 hrs activity rose by 75% in septum and 152% in striatum. In contrast, hippocampal ChAT remained low for another 48 hrs (PD6) when it increased by 90%. By this time, septal and striatal ChAT activity had peaked (217% and 337% of control, respectively). At 8 days after injection (PD10), ChAT in septum and striatum returned to control levels but hippocampal activity remained high (+85%), falling to age appropriate levels 48 hrs later (PD12).

Immature cholinergic neurons respond strongly to a single dose of NGF on PD2, providing additional evidence that it is a potent signal for them during development. The initial delay in ChAT elevation and sequential responses in septum followed by hippocampus suggest that NGF may stimulate synthesis of new enzyme molecules which are transported outward from perikarya to nerve terminals. The temporal relationship between peaks of activity in septum and hippocampus is consistent with a transport velocity similar to slow axoplasmic flow. NGF may provide an important probe for studying the molecular events required for expression of ChAT enzyme activity in developing central cholinergic systems.

197.10 EFFECTS OF ANTIBODIES AGAINST NERVE GROWTH FACTOR ON DEVELOPING CHOLINERGIC FOREBRAIN NEURONS IN RAT. U.Otten, G. Weskamp*, M.Schlumpf, W.Lichtensteiger and W.C.Mobley. Dept. of Pharmacology, Biocenter of the University, Basel, Dept. of Pharmacology, University of Zürich, Switzerland and Div. N.P. Walter Reed Institute of Res.,Washington DC 20307.

A number of observations suggest that nerve growth factor (NGF) plays a physiological role for basal forebrain cholinergic neurons: messengerRNA encoding NGF as well as NGF immunoreactive material have been found in brain. Morphological studies showed that NGF is selectively transported retrogradely to cholinergic neurons of the basal forebrain. In addition, NGF increases the activity of choline acetyltransferase(ChAT) in rat forebrain cholinergic neurons both in vivo and in vitro. However, so far no effects of NGF-antibodies on central cholinergic neurons could be found.

In the present study, we have injected anti-NGF antibodies into rat fetuses in utero. Using this approach we have previously shown that NGF is necessary for the normal development of a significant population of prenatal rat dorsal root ganglion cells. We report that a single administration of anti-NGF antibodies to 15.5-day-old rat fetuses produced a significant reduction in ChAT-activity in the nucleus basalis region,septum and hippocampus (52%, 35% and 15% respectively, $P < 0,01$) in animals examined 7 weeks later.Concomitantly, reduction in binding of ^{3}[H]-N-methylscopolamine was observed in neocortex and hippocampus. In contrast, no changes in ChAT and muscarinic cholinergic receptor levels were observed in the striatum. Quantitative histological studies are in progress to determine whether these biochemical changes are a reflection of neuron destruction.

These results, however, indicate that endogenous NGF is a trophic agent for rat forebrain cholinergic neurons.

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197.11 NEURITE-PROMOTING FACTORS FOR SPINAL NEURONS. INHIBITORY SUBSTANCES IN MUSCLE OF PATIENTS WITH SPINAL MUSCULAR ATROPHY. C.E.Henderson*[1] T.Taguchi*[1], J.P.Changeux[1], S.L.Hauser*[2], P.A.Cazenave*[2], F. Hentati*[3] and M.Fardeau*[3]. Neurobiologie Moléculaire[1] and Immunochimie Analytique[2], Institut Pasteur, 25 rue du Dr.Roux, 75015 PARIS; INSERM U153[3], 17 rue du Fer-à-Moulin, 75005 PARIS, France.

Various lines of in vivo and in vitro evidence suggest that at different stages of their development, spinal motoneurons depend on muscle-derived factors for survival, axon outgrowth and acetylcholine synthesis. We have used serum-free cultures of embryonic (4.5 d in ovo) chicken spinal neurons to detect and study factors affecting neurite outgrowth. Early embryonic spinal cord was used with the aim of enriching motoneurons in the cultures, but the responsive cells have not been directly identified.

Neurite-promoting activity from two principal sources was studied: medium conditioned by embryonic myotubes in vitro ("embryonic" activity) and extracts of 4-day post-hatch leg muscle ("neonatal" activity). Levels of the latter were developmentally regulated.

In the course of the spinal muscular atrophies, there is a selective loss of motoneurons in the ventral horn. It has been hypothesised that this is due to a lack of muscle-derived trophic support. We tested the capacity of extracts of muscle from patients with spinal muscular atrophies (SMA) to inhibit the action in vitro of the neurite-promoting activities described above. In a series of soluble extracts prepared from fresh muscle biopsies, all SMA extracts tested (5/5) inhibited the "neonatal" activity, by up to 100%. Total muscle protein concentrations necessary for inhibition were <1 ug/ml; no cytotoxicity was observed. No extract (0/7) from neurological or morphologically normal control muscles inhibited to the same extent. In the same test using "embryonic" chick activity, there was no difference between SMA and control groups.A second series of biopsies conserved at -80° was tested in the same way. In this case, some SMA extracts (6/17) did not inhibit the "neonatal" activity, whereas one control (1/14) did. The difference between the groups was still highly significant ($p<.001$ by a χ^2 test). The nature of the inhibitory substances is under investigation.

Levels of neurite-promoting activity in chick muscle extracts were increased 15-fold three days after denervation. These muscle extracts were used as starting point for purification of the active factor(s). In the most highly purified fractions obtained to date (still containing several protein species), the specific activity was estimated to be 5×10^6 biological units/mg protein, which represents a 10^4-fold enrichment. The active species migrated with an apparent mol. wt. of 50 K upon gel filtration in non-denaturing conditions.

197.12 EFFECTS OF INSULIN AND INSULINLIKE GROWTH FACTOR-II ON TUBULIN mRNA LEVELS AND NEURITE FORMATION IN CULTURED HUMAN NEUROBLASTOMA CELLS. J.F. Mill*, M.V. Chao* and D.N. Ishii. Pharmacol. Dept., Columbia Univ., New York, NY 10032; and Dept. of Cell Biol. and Anat., Cornell Univ. Med. Sch., New York, NY 10021.

Tubulin is one of the major protein constituents of axons and dendrites. We have previously shown that insulin and insulinlike growth factor-II (IGF-II) can increase neurite formation in sensory, sympathetic and human neuroblastoma SH-SY5Y cells. Here, we demonstrate that insulin and IGF-II can increase both alpha- and beta-tubulin mRNA levels in SH-SY5Y cells, in a manner which correlates with their capacity to enhance neurite formation. SH-SY5Y cells can survive for longer than one week in serum-free media (SFM) without a loss in the number of cells. The quiescent cells resume growth on the reintroduction of serum. SH-SY5Y cells, grown for two days in SFM, were treated with and without 0.1 μM insulin for 24 hours, RNA was extracted, and equal quantities of RNA were analyzed by Northern blot with cDNA probes containing the coding regions for the alpha- and beta-tubulins. Densitometric scans of the resulting autoradiograms showed a 3-4 fold increase in both alpha- and beta-tubulin mRNAs relative to the controls. A similar result was seen on treatment with IGF-II. Cytoplasmic dot blot analysis showed that the observed increases were even greater on a per cell basis. There was a transient peak between 12 and 24 hours of exposure to insulin, followed by a decrease. Neurite outgrowth attains a maximum in about 1 day, but, in contrast, is sustained. These results show that elevated levels of tubulin mRNAs are associated with the extension of neurites, but may no longer be required once neurites have formed. The effects of 1 pM to 1 μM insulin were studied. Tubulin mRNA levels were increased half-maximally at 3 nM insulin. The dose-response curve for the increase in tubulin mRNA levels closely approximated that for the increase in neurite outgrowth in response to insulin. The broad dose-response curves for these functions may be explained by the cross occupancy of insulin and IGF-II receptors. Nerve growth factor (NGF) can induce neurite outgrowth in SH-SY5Y cells in either serum-containing media or in SFM containing low concentrations of insulin. In contrast to insulin and IGF-II, NGF did not appreciably increase tubulin message levels under either of these conditions. Because neurite outgrowth can be synergistically potentiated by the combination of insulin and NGF, we suggest these factors may cooperate to form neurites by two distinct mechanisms. Insulin and its homologs may act primarily to increase tubulin mRNA levels, whereas NGF may act largely to increase the fraction of the tubulin pool in the polymerized, microtubule state. (Supported in part by NIADDKD grant RO1 AM32841 to DNI, and NIH grant BRSG RR05396 to MVC.)

198.1 EVIDENCE FOR GABAERGIC INNERVATION OF MOUSE THYMUS AND SPLEEN. K. Bulloch, S.A. Cohen*, S.A. Robinson* and J.-Y. Wu (1), Dept. of Neurology, S.U.N.Y. Stony Brook, N.Y. 11794 and (1) Dept. of Physiol., Penn. State Univ. 17033

The thymus and the spleen are two major organs of the immune system. Both organs are innervated by fibers of the ANS. The thymus receives a rich cholinergic (AChE-positive) innervation from the vagus nerve which is distributed to the corticomedullary boundaries and from the phrenic and recurrent laryngeal nerves which is localized to the thymic subcapsular region. Catecholaminergic (CA) innervation derived from the superior cervical ganglia, stellate ganglia and other small ganglia of the sympathetic chain is primarily vascular in nature with dense plexuses evident at the corticomedullary boundaries. A more delicate distribution of nonvascular-associated CA fibers is evident in the subcapsular and cortical regions of the thymus. The spleen predominantly receives a rich CA innervation from fibers arising in the celiac ganglion. Most of these fibers are evident along the periarterial sheath and within the marginal zone of the lymphoid nodules. Anatomical studies coupled with pharmacological studies now show that these two neurotransmitters have a profound effect on the migration, differentiation and function of immune cells within these organs (Bulloch, K. Raven Press 111, 1985). In this study we have sought to determine the distribution of another neurotransmitter, gamma-aminobutyric acid (GABA), within the thymus and spleen of young adult C57BL/6J mice by means of standard immunocytochemical fluorescence techniques. We used as our GABA markers specific antibodies against GABA-T AND GABA. Our results show that the thymus contains a plexus of GABAergic-like nerve terminals which are densely distributed throughout the cortex. There is a close and rich association of these GABAergic-like nerves with the cortical autofluorescent cells. A less dense GABAergic innervation is evident within the spleen. These fibers are very fine and are distributed along the periarterial sheath and within the marginal zone of the lymphoid nodules. The density of GABAergic nerves within these two tissues correlates well with the biochemical analysis (J.G. Gerber and T.A. Hare, Diabetes 28:1073, 1979) which showed high levels of GABA within the thymus and low levels within the spleen. The presence of GABAergic-like nerves within the thymus and the T-cell zones of the spleen offers an exciting new probe into the multidimensional roles of neuroimmune interactions. Supported by NIH grant #18401

198.2 TRANSMITTER MODULATION OF IMMUNE CELLS. V.P. Calabrese and J. Yoshino*. Departments of Neurology and Biochemistry, Medical College of Virginia, Richmond, Virginia 23298-0001.

It is becoming increasingly evident that there is a functional relationship between the nervous system and the immune system. There are neurotransmitter receptor sites on the surface of lymphocytes which may have a functional role in the immune response.

In our study we have looked at the effect of neurotransmitters on the turnover rate of human mononuclear cells in culture as well as the immunoglobulin production. Epinepherine, at a concentration of 10^{-3}M and 10^{-5}M, had a suppressive effect on mononuclear cell DNA as measured by ^{3}H thymidine uptake. A similar suppression was seen with 5-HT at 10^{-4}M and 10^{-5}M. Epinepherine inhibited most of the stimulatory effect of pokeweed mitogen but had no effect on Concanavalin A stimulation. This would indicate a different effect of the neurotransmitters on various cell types.

The effects of neurotransmitters on mononuclear cells after the cells were stimulated by brain and viral antigens was also evaluated. The different effects on monocytes/macrophages (cells adherent to plastic) and lymphocytes (nonadherent cells) was also studied. Results of these studies will be presented.

198.3 NORADRENERGIC INNERVATION OF THE SPLEEN IN ADULT AND AGING FISCHER 344 RATS. L.L. Wheeler*, D.L. Bellinger, S.Y. Felten, P.D. Coleman, T.J. Collier, and D.L. Felten (SPON: J. Olschowka), Dept. of Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Noradrenergic (NE) innervation of the spleen was examined with fluorescence histochemistry in Fischer 344 rats of 8 months and 27 months of age. The 27 month old rats were divided into 2 groups, based upon behavioral testing for gustatory neophobia (enhanced fearfulness of novel stimuli), a condition previously associated with lesions of locus coeruleus in young rats and with noradrenergic diminution in aging rats (Collier et al., 1985). Spleens of 8 month old rats were innervated abundantly. Large plexuses of NE varicosities entered with the splenic artery, followed branches into the spleen, and formed a subcapsular plexus that continued alongside the trabeculae. These arterial and trabecular plexuses continued into the white pulp, associated mainly with the central artery and its branches. Only scattered varicosities were present in the red pulp, associated with vessels. Areas of white pulp were large and well defined. Linear chains and tortuous profiles of NE varicosities diverged from the vascular plexuses, extended into the parenchyma of the white pulp, and ended among lymphocytes in the periarterial sheath. In all of the 27 month old rats, NE innervation was decreased markedly. Capsular plexuses and parenchymal fibers, both linear and punctate in appearance, were reduced markedly. The varicosities associated with blood vessels, especially the central artery of the white pulp and its branches, were present but were diminished in density as well as in overall fluorescent intensity. The white pulp was diminished in size and was difficult to separate clearly from red pulp. In the neophobic 27 month old rats, the white pulp was reduced even further, and NE varicosities were even less abundant. The reduced innervation of the spleen in aging Fischer 344 rats may be related to the decline in immune function seen in the aging process. Since neophobia may be related to decreased central NE, the reduced NE innervation of the spleen may reflect a generalized reduction of NE in these rats. Supported by Grant N00014-84-K-0488 from the Office of Naval Research and by NIH Training Grant AG T32 107.

198.4 NORADRENERGIC INNERVATION OF MESENTERIC AND POPLITEAL LYMPH NODES IN YOUNG AND AGING MICE WITH AUTOIMMUNE DISORDERS. P. Yeh*, D.L. Bellinger, S. Livnat*, R. Ader*, S.Y. Felten, and D.L. Felten. Depts. of Anatomy, Psychiatry, and Microbiology (Immunol.), Univ. of Rochester Sch. of Med., Rochester, NY 14642.

Noradrenergic (NE) innervation of mesenteric and popliteal lymph nodes was examined with fluorescence histochemistry in several strains of New Zealand mice. NZW mice served as controls, and were examined at 4-6 weeks of age, 8 months, and 18-19 months. NZB mice, a strain that develops autoimmune hemolytic anemia, were examined at 4-6 weeks of age, and at 6 months of age, shortly before the lethal onset of the hemolytic anemia. NZB x NZW mice, a hybrid that demonstrates a lupus-like syndrome, were examined at 4-6 weeks of age, 8 months, and 18-19 months. All mice at 4-6 weeks of age showed similar distribution of NE fibers. Large plexuses of varicosities entered the nodes in the hilar region and continued with vessels through the medullary cords. Some fibers traveled just beneath the capsule in a subcapsular plexus; these varicosities also followed septa into the parenchyma. The fluorescent fibers from the medullary cords continued into the parenchyma and ended among lymphocytes in paracortical regions, while some fibers from the subcapsular plexus branched into the parenchyma to end among lymphocytes in cortical regions. No fibers were found in nodular regions. Mesenteric lymph nodes were innervated more densely than popliteal lymph nodes, particularly in the large plexuses. At 8 months of age, both the NZW and the NZB x NZW strains demonstrated a pattern of NE innervation whose density and distribution were similar to their young counterparts. In 6 month old NZB mice, there was a marked decrease in innervation of lymph nodes, and an infiltration of these nodes with metachromatic cells that resembled mast cells and possibly with macrophages. It is possible that this altered innervation may contribute to the autoimmune disorder in these mice, although it also may be secondary to other changes. In 18-19 month old mice, the innervation diminished in both extent and intensity of fluorescence, including the distribution in the paracortical and cortical regions in which T lymphocytes reside. However, this reduced innervation was more severe in NZB x NZW mice, again raising the possibility of an association of diminished NE innervation of lymph nodes with an autoimmune disorder. These findings suggest that the NE innervation of lymph nodes differed regionally (mesenteric more densely innervated than popliteal), diminished normally with age, and diminished further in certain strains of mice with autoimmune disorders. Supported by Grant N00014-84-K-0488 from the Office of Naval Research.

198.5 NORADRENERGIC INNERVATION AND ACETYLCHOLINESTERASE ACTIVITY OF LYMPH NODES IN YOUNG ADULT AND AGING MICE. D.L. Bellinger, S.Y. Felten, P.D. Coleman, P. Yeh and D.L. Felten. Dept. of Anat., Univ. of Rochester, Sch. of Med., Rochester, NY.

Popliteal and mesenteric lymph nodes in C57BL/6 mice of 7, 12, 21, 30 and 36 months of age, were examined with fluorescence histochemistry for catecholamines and with acetylcholinesterase (AChE) staining. In young adult mice, noradrenergic (NE) fibers entered the lymph node in the hilar region in a dense varicose plexus associated with the vasculature. These fibers continued with the vasculature into the medullary region, or formed a subcapsular plexus that extended around the lymph node and followed the septa between the nodules. Fine delicate strands of fibers exited the perivascular and subcapsular plexuses into the paracortical and cortical regions surrounding germinal centers, where they closely apposed, and ended among, lymphocytes. The paracortical and cortical regions also contained intensely yellow autofluorescent cells. No fibers entered the germinal centers. In lymph nodes of aging mice, there was a decrease in the number of intensely yellow autofluorescent cells, and the capsule appeared thicker. NE fibers in lymph nodes of aging mice were distributed similar to NE fibers seen in young mice; however, the density of fibers in the subcapsular and perivascular plexuses decreased. In addition, there was a loss of fluorescence intensity in the fibers. Neurochemical measurements currently are under way to confirm these findings.

A modified thiocholine method (El-Badawi and Schenk, 1967), specific for AChE, revealed positive-staining fibers that entered the lymph nodes in association with blood vessels in the hilar region. These fibers continued into the lymph node along with the vasculature. From these plexuses, AChE-positive fibers coursed into the paracortical and cortical regions surrounding the germinal centers, where they branched extensively among lymphocytes. Terminals of the AChE-positive fibers formed claw-shaped endings that surrounded individual lymphocytes. In popliteal lymph nodes, a large number of mast cells were present in clusters beneath the capsule, and to a lesser extent, near blood vessels. AChE-staining profiles also traveled in close proximity to these aggregates of mast cells. In aging mice, no differences in the distribution and appearance of AChE-positive fibers was noted. However, mast cells were more abundant in aging popliteal lymph nodes than in their younger counterparts. Since the distribution of NE fibers and AChE-positive fibers closely overlaps, it is possible that they coexist. It also is possible that lymph nodes are innervated by both NE and cholinergic fibers. Additional methods are being used to explore these possibilities. Supported by Grant N00014-84-K-0488 from the Office of Naval Research and NIH training grant AG T32 107.

198.6 IMMUNIZATION SELECTIVELY DECREASES NOREPINEPHRINE IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS AT THE TIME OF THE PEAK SPLENIC PLAQUE-FORMING CELL RESPONSE IN ADULT C3H MICE. S.L. Carlson, S.Y. Felten, S. Livnat* and D.L. Felten, Departments of Anatomy, Microbiology (Immunology) and Psychiatry, Univ. of Rochester Sch. of Medicine, Rochester, NY 14642.

Evidence from several lines of investigation reveals the existence of reciprocal communication between the nervous and immune systems, although the identification of specific sites of interaction in the CNS remains elusive. In this regard the hypothalamus has been examined as a potential major regulatory center for modulating immune responses. Hypothalamic norepinephrine (NE) was reported to decrease during the peak of a primary immune response in rats (Besedovsky, et al, 1983). The present study was undertaken to search for possible sites in the hypothalamus where specific alterations in NE may occur following immunization.

Adult male C3H mice were immunized intraperitoneally with the antigen sheep red blood cells or with vehicle (controls). The mice were sacrificed on days 2, 4 and 8 following immunization, corresponding to time points before, during, and after the peak immune response respectively, as measured by the splenic plaque-forming cell (PFC) response. Specific hypothalamic nuclei and brain stem regions were microdissected for subsequent measurement of monoamines using LCEC. The density, distribution, and appearance of catecholamine varicosities was examined with SPG histofluorescence. We report here our observations in paraventricular nucleus (PVN), supraoptic nucleus (SON), anterior hypothalamus (AH), and the A1 catecholamine cell group in the medulla.

NE was decreased in the PVN on day 4 only, but not in the SON, AH, or the A1 region at any time. In addition, no changes in dopamine were found in any of these nuclei at any time point. Serotonin was decreased only on day 4 in both PVN and SON, with no changes in the AH or A1 region. These results indicate that immunization results in a selective decrease in NE in the PVN during the peak PFC response. The lack of change in NE content in the A1 region, which contains NE cell bodies that project to PVN and SON, suggests that modulation of the NE system is likely to occur at the terminal fields in the PVN and not at the cell bodies. This may be important for the increased output in the CRF-ACTH-glucocorticoid axis reported to occur at the peak of an immune response, particularly in view of selective localization of CRF neurons in PVN but not SON, and the selective decrease in NE in PVN but not SON reported here. Direct projections from the PVN to preganglionic autonomic neurons may also be involved in the regulation of autonomic neurons that innervate lymphoid tissue. Therefore, PVN may be a specific anatomical link in the reciprocal communication between the nervous and immune systems. Supported by Grant N00014-84-K-0488 from the Office of Naval Research.

198.7 NEURAL MODULATION OF THE IMMUNE RESPONSE BY PHOTOPERIOD: SPLENIC CELL COUNTS AND IMMUNE RESPONSIVENESS. F.D. Lublin*, R.L. Knobler, P. L. Podolin*, G.C. Brainard . Department of Neurology, Jefferson Medical College, Philadelphia, Pa. 19107

To establish the effect of prolonged differences in photoperiod on the immune system, we studied Syrian hamsters which were exposed to either a long photoperiod (14 hours daylight/day) or a short photoperiod (10 hours daylight/day). After 12 weeks in either long or short photoperiod, half of each group (N=12) was immunized with an immunogenic random sequence amino acid polymer of glutamic acid, lysine, alanine and tyrosine (GLAT) The animals were bled by cardiac puncture and sacrificed after 14 weeks. Various organs were removed and weighed and immunologic assays performed. Splenic lymphocytes and macrophages were counted morphometrically by light microscope. Animals in a long photoperiod had means of 37×10^6 lymphocytes (nonimmunized group) or 64×10^6 lymphocytes (immunized group). In contrast, animals in short photoperiod had means of 100×10^6 lymphocytes (immunized group) or 165×10^6 lymphocytes (immunized) group). Data were analyzed by ANOVA and significance levels determined by a Neuman-Keuls test. Among the immunized animals, the short photoperiod animals had significantly more lymphocytes ($P<0.01$). Between the nonimmunized groups, the short photoperiod animals also had significantly higher lymphocyte counts ($P<0.05$). Similar analysis demonstrated that between immunized animals, the short photoperiod group had significantly more macrophages than the long photoperiod group ($P<0.01$). Among nonimmunized animals, the short photoperiod group had more than twice the number of splenic macrophages as long photoperiod animals, but this difference was not statistically significant. Preliminary data from cell proliferation assays demonstrate a trend towards increased splenic lymphocyte responsiveness to mitogens concanavalin-A and lipopolysaccharide in long photoperiod animals versus short photoperiod animals. Studies are underway to determine whether there are photoperiod induced differences in antibody production to GLAT. These data indicate an effect of photoperiod on certain components of immune tissue, and raise the possibility of specific light mediated neuroendocrine regulation of immune responsiveness. Supported by USPHS #RR-05414 and the Pfeiffer Research Foundation.

198.8 NEURAL MODULATION OF THE IMMUNE SYSTEM BY PHOTOPERIOD: IMMUNE ORGAN WEIGHTS AND CELL COUNTS. G.C. Brainard, F.D. Lublin,* R. L. Knobler, and P.L. Podolin*. Department of Neurology, Jefferson Medical College, Philadelphia, Pa. 19107

The neuroendocrine-reproductive axis of the Syrian hamter is physiologically regulated by the photoperiod in which animals are housed. The purpose of the following study was to examine the capacity of the environmental photoperiod to influence the immune organs and cells of adult male hamsters (Mesocricatus auratus) . Twenty-four age matched hamsters were divided randomly into two groups and housed under a daily light:dark cycle (LD) of either 14:10 or 10:14 (lights on in both groups 07:00 hours) for 14 weeks. After twelve weeks, 6 animals in each photoperiod were immunized with an immunogenic random sequence amino acid polymer of glutamic acid, lysine, alanine and tyrosine (GLAT). At 14 weeks, blood samples were collected by cardiac puncture, and testes, accessory sex organs, spleens, thymuses, Harderian glands, adrenal glands and brown fat pads were weighed. Data were analyzed by ANOVA and significance levels determined by a Newman-Keuls test. Animals in LD 14:10 had significantly higher reproductive organ weights than animals in LD 10:14 ($P<0.001$). Immunization did not significantly alter the effect of photoperiod on testicular weights; however, immunized animals in LD 10:14 had lower accessory sex organ weights than nonimmunized animals in the same photoperiod ($P<0.05$). Neither photoperiod nor immunization had any influence on thymus weight. In contrast, spleen weights were significantly reduced by long photoperiods compared to short photoperiods ($P<0.01$). Furthermore, immunized animals had significantly higher spleen weights than nonimmunized animals in short photoperiods ($P<0.05$). The spleen weight differences observed between groups were paralleled by differences in spleen lymphocyte and macrophage cell counts. Specifically, higher spleen weights were matched by increased numbers of lymphocytes and macrophages while lower weight spleens had fewer lymphocytes and macrophages. Thus, photoperiod induced changes in total spleen weight as well as in the immunologically functional cells of the spleen. These data demonstrate that the immune system is responsive to ambient photoperiod and may be regulated by neuroendocrine control systems. Supported by USPHS #RR-05414 and the Pfeiffer Research Foundation.

198.9 CHRONIC ADMINISTRATION OF PROPANOLOL DELAYS DEVELOPMENT OF A MURINE PLASMACYTOMA TUMOR. C.G. Frondoza*, R. Grzanna, G.L. Hung*, and A. Sastre. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Tumor incidence and growth rates have been suspected to be influenced by the activity of the sympathetic nervous system. We have previously shown that LPC-1 plasmacytoma tumors develop significantly slower in sympathectomized mice than in untreated animals. This study was conducted to determine the effect of prolonged adrenergic receptor blockade on the development and growth of subcutaneous LPC-1 plasmacytoma tumors.

Mice were implanted with Alzet osmotic mini-pumps for continuous delivery over a 15 day period of either D,L-propranolol, D-propranolol, L-propranolol, or saline. Tumor development was monitored by electrophoretic quantitation of the tumor specific protein IgG2a$^{\kappa}$ in plasma and by caliper measurements of the tumor. Administration of D,L-propranolol at concentrations of 0.6, 6 and 60 mg/kg/day produced a dose-dependent, highly significant delay in the appearance of LPC-1 tumors. The propranolol effect was stereospecific in that the L-isomer produced the same effect as the racemic mixture while the D-isomer had no effect. The tumor growth curves in mice injected with $1 \cdot 10^6$ LPC-1 tumor cells and treated with 60 mg/kg/day were similar to those seen in untreated mice after an inoculum of $1 \cdot 10^5$ LPC-1 cells suggesting an apparent 10-fold reduction in the initial tumor load.

The findings extend our earlier observation of a significantly delayed appearance of LPC-1 tumors in sympathectomized mice and provide the first direct evidence for an involvement of β-adrenergic receptors in the regulation of tumor development by the sympathetic nervous system. (Support: NS 16654 and CA 31499)

198.10 DEPRESSION AND IMMUNITY: ROLE OF AGE, SEX, AND SEVERITY. S.J.Schleifer*,S.E.Keller*,J.Cohen*,M.Stein, Mount Sinai School of Medicine, CUNY, New York,N.Y. 10029.

We have previously reported that mitogen induced lymphocyte stimulation and the number of peripheral blood lymphocytes (PBLs) were lower in 18 apparently healthy, drug-free hospitalized patients with major depressive disorder (MDD) compared with matched controls. In contrast, 15 ambulatory patients with MDD had decreased PBLs but did not differ from their controls in mitogen responses. The sample was expanded to include an additional 20 inpatients and 22 outpatients to investigate the role of severity of depression, inpatient vs ambulatory status, age, and sex. All patients met Research Diagnostic Criteria for unipolar MDD. Severity of depression was assessed using the Hamilton Depression Scale. Subjects were free of medical disorders, were not taking medications known to affect immunity, and had not received antidepressant treatment for at least 3 months prior to study. Patient-control pairs were age and sex matched and were each studied on the same day during the morning hours.

Heirarchical regressions (forced stepwise) on responses to the mitogens PHA, ConA, and PWM, on PBLs, and on numbers of T and of B cells were performed on the total sample of 75 patient-control pairs for age, sex, severity, hospitalization status, first vs second patient series, and interactions among the independent variables. Differences between patients and controls for each of the mitogens were found to be related to the subject's age and to severity but not to sex. Older and more severely depressed patients had significantly lower mitogen responses than matched controls, while responses of younger patients were similar or higher than those of their controls. Neither hospitalization status nor first vs second patient series was related to the findings when age and severity of depression were statistically controlled. Lymphopenia in the depressed patients was related to increased age but was not associated with severity suggesting that different mechanisms may be involved in the quantitative and functional lymphocyte changes found in depression.

The findings demonstrate a complex relationship between MDD and immunity. Specific patterns of neuroendocrine dysregulation in older and more severely depressed patients may account for the role of age and severity in the altered immunity found in MDD.

198.11 STRESS-INDUCED CHROMOSOMAL ALTERATIONS: SISTER CHROMATID EXCHANGES AND UNSCHEDULED DNA SYNTHESIS. Dennis D. Kelly (1), Ronald W. Pero* (2) and Harlow K. Fischman* (1). (1) New York State Psychiatric Institute & Dept. of Psychiatry, Columbia University, New York, NY 10032 and (2) University of Lund, Sweden, & Preventive Medicine Institute/Strang Clinic, New York, NY 10016.

We have established that exposure of an intact organism to a variety of behavioral stressors can trigger within 24 hrs an increase in exchanges between sister chromatids (SCEs) in dividing bone marrow cells. Within 2 hrs of exposure to stress there is also an increase in DNA repair in circulating leukocytes. Genotoxicity is a property shared by a diverse range of physical and psychogenic stressors and appears to be graded with respect to the severity of the stressor.

Exp 1: 24 rats were exposed to a forced 3.5-min swim in either cold or warm water, or to no stress. Two hrs later a bromodeoxyuridine pellet was implanted subcutaneously. Sacrifice occurred 25 hrs post-stress and bone marrow analyzed for SCEs. Both warm and cold swims significantly elevated SCEs and lengthened the cell cycle.

Exp 2: The generality of the phenomenon was tested in 20 rats exposed to one of 5 conditions: cold swim, intense white noise, intermittent inescapable footshock (IFS), continuous IFS or no stress. All four experimental stressors increased SCEs, although to differing degrees. Hence, neither exercise nor physical pain appears to be a prerequisite for SCE induction.

Exp 3: 16 rats were exposed either to intermittent IFS or to no stress. Trunk blood was collected 2 hrs later and unscheduled DNA synthesis (UDS) measured in leukocytes. This method utilizes 3H-thymidine to measure the incorporation of DNA precursor in non-dividing cells as evidence of DNA damage and repair. The level of UDS in stressed rats was twice that of the controls.

Exp 4: 20 rats received either 480, 240 or 120 shocks during 20 mins, or were placed in the same chamber where other rats had been shocked, or were left in their homecages. SCEs were related in a graded manner to the severity of IFS. Rats exposed only to the physical environment in which conspecifics had been stressed showed significant intermediate elevations. UDS measurements produced similar, graded results.

A hypothesis supported by these results is that stress may trigger pathology by directly altering the molecular instructions that guide cellular behavior. [PHS Grants R03 MH39420 (HKF) and R01 NS 18822 (DDK)]

198.12 MORPHINE-INDUCED SUPPRESSION OF NATURAL KILLER (NK) CELLS: EVIDENCE FOR CENTRAL MEDIATION VIA OPIOID RECEPTORS. Y. Shavit, A. Depaulis, G.W. Terman, F.C. Martin, C.J. Zane*, R.P. Gale* and J.C. Liebeskind. Departments of Psychology and Medicine, University of California, Los Angeles, CA 90024.

Much evidence suggests that the immune system is partially regulated by neural and hormonal influences. Studying the role of opioids in modulating NK cell activity, we have recently found that both systemic morphine and stress-released opioid peptides suppress NK cell cytotoxicity in rats. A single systemic injection of relatively high doses of morphine (>20 mg/kg, s.c.) induced transient NK suppression evident 3 h after the injection and returning to normal by 24 h. This suppression is blocked by the opiate antagonist, naltrexone, and develops tolerance with repeated morphine injections. Furthermore, systemic administration of a quaternary analogue of morphine (N-methyl morphine) that does not cross the blood brain barrier, had no effect on NK activity, indicating central mediation of the morphine effect. In the present study, we examined the effect on NK activity of morphine adiministered intracerebroventricularly (ICV).

Male Fischer 344 rats were implanted with cannulae in the right lateral ventricle. Two weeks after surgery, groups of rats were given ICV injections of 10, 20 or 40 µg of morphine or vehicle. Other rats were treated with naltrexone (10 mg/kg, s.c.) 10 min before ICV morphine. Three hours after injections, spleens were removed and dissociated into single cell suspensions. Spleen cells were co-cultured with chromium labeled YAC-1 target cells, and NK cytotoxicity was determined in a 4 hr chromium release assay.

ICV morphine at doses of 20 and 40 µg significantly suppressed NK activity to a similar degree as 20 or 40 mk/kg of morphine given systemically. ICV morphine at a dose of 10 µg did not affect NK activity. The NK suppression when induced by ICV morphine (40 µg) was blocked by systemic naltrexone (at a dose as low as 4 µg/kg, s.c.), and when induced by systemic morphine (30 mg/kg) was blocked by ICV naltrexone (two injections of a dose as low as 0.5 µg, 1.5 h apart). These low doses of naltrexone did not antagonize morphine's cataleptic effect. These results indicate that morphine-induced NK suppression is mediated by central opioid receptors. These results also complement other reports showing the involvement of the central nervous system in mediating NK activity. (Supported by NIH grant NS-07628, Fondation Fyssen, and a gift from the Brotman Foundation).

199.1 COMPARATIVE DISTRIBUTION OF [^{3}H]PIRENZEPINE AND [^{3}H]HEMICHOLINIUM-3 BINDING SITES IN RAT BRAIN. C. Pilapil and R. Quirion. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill Univ., Verdun, Quebec H4H 1R3 Canada.

Recent data have suggested that [^{3}H]pirenzepine labels the muscarinic$_1$ (M_1) cholinergic receptor sub-type in brain. Moreover, it appears that most of the M_1 receptors are post-synaptically located at the level of the cholinergic terminals (Potter et al, TIPS, Jan. 1984, p.22). As putative presynaptic markers, hemicholinium-3 appears to be one of the best candidates. Here, we report on the comparative autoradiographic distribution of [^{3}H] hemicholinium-3 and [^{3}H]pirenzepine binding sites in rat brain using them as marker of pre- and post-synaptic cholinergic nerve terminals, respectively. Rat brain sections were prepared as described before. For [^{3}H]hemicholinium-3, sections were incubated for 60 min at 4°C in 50 mM Tris.HCl, pH 7.4 plus 300 mM NaCl and 20 nM [^{3}H]hemicholinium-3 in presence or absence of 10 µM hemicholinium-3 to determine the amount of specifically bound ligand. For [^{3}H]pirenzepine, sections were preincubated for 15 min in Krebs buffer followed by a 60 min incubation at 22°C in Krebs buffer with 10 nM [^{3}H]pirenzepine in presence or absence of 1 µM atropine to determine the amount of specifically bound ligand. At the end of the incubation period, slides were washed for six min ([^{3}H]hemicholinium-3) or 12 min ([^{3}H]pirenzepine) in cold incubation buffer. Slides were then juxtaposed against tritium-sensitive film for appropriate exposure. In many brain regions, [^{3}H]pirenzepine and [^{3}H]hemicholinium-3 binding sites are similarly distributed. High densities of sites are present in areas such as the striatum, nucleus accumbens, olfactory tubercule and superficial layers of the cortex. Low to moderate densities of sites are seen in the thalamus, hypothalamus and reticular portion of the brainstem. However, important differences are also observed. For example, high densities of [^{3}H]hemicholinium-3 binding sites are found in the habenula, superior colliculus and cerebellum, three regions devoid of [^{3}H]pirenzepine binding sites. In the hippocampus, the laminar distribution of [^{3}H]hemicholinium-3 binding sites correlates well with the localization of cholinergic terminals. [^{3}H]pirenzepine binding sites are not as discretely distributed in the hippocampus. These data demonstrate that [^{3}H]hemicholinium-3 and [^{3}H]pirenzepine could be used as respective pre- and post-synaptic markers of cholinergic nerve terminals in various brain regions but not in others.

199.2 EFFECT OF CONCANAVALIN A ON ACETYLCHOLINE RECEPTOR REDISTRIBUTION DURING NEUROMUSCULAR JUNCTION FORMATION IN CULTURE. Y.Kidokoro, B.Brass*, and H.Kuromi*
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During neuromuscular junction formation acetylcholine receptors(AChR) accumulate at the nerve contact region. It has been shown that this is, at least partly,due to lateral migration of existing receptors in the membrane (Anderson,Cohen and Zorychta,1977). Randomly diffusing AChR molecules in the membrane may be trapped at the nerve-contact region to form a high receptor density area. If this were the major mechanism, cross-linking AChRs by tetravalent concanavalin A (Con A) should immobilize receptors and prevent nerve-induced receptor accumulation.

We examined the effect of Con A on nerve-induced accumulation and on the mobility of AChRs in cultured Xenopus muscle cells. ACh receptors were stained with rhodamine conjugated α-bungarotoxin. The cells were then briefly treated with Con A and neural tube cells were added to these cultures. The mobility of AChRs was measured by the fluorescence photobleaching recovery method. The Con A treatment prevented rapid diffusion of AChRs as well as nerve-induced receptor accumulation.In contrast, divalent succinyl Con A did not affect the mobility of AChRs nor prevented nerve-induced AChR accumulation. When the Con A concentration was varied the blocking effect on the nerve-induced receptor accumulation was inversely related with the mobile fraction of receptors. Newly inserted AChRs after the Con A treatment were found mobile and to accumulate to the nerve-contact region.In these cultures at some spots along the nerve contact, new receptors were accumulated surrounding old, immobilized ones. This observation suggests that new receptors were inserted elsewhere and migrated to the nerve-contact region surrounding immobilized old ones.

In addition to accumulation of receptors,the nerve disperses pre-existing receptor clusters prior to induction of high density regions along the contact area and at this early stage denervation disperses nerve-induced receptor clusters in Xenopus cultures. When cultures were treated with Con A both of these events did not occur, suggesting that these are also diffusion mediated.

Anderson,Cohen and Zorychta (1977) J.Physiol.268,731.

199.3 ATYPICAL ANTIPSYCHOTICS PROFILED BY DOPAMINE RELEASE SHOW PREFERENTIAL S_2 AND MESOLIMBIC D_2 POTENCIES WITH [3H]SPIPERONE AUTORADIOGRAPHY. C. A. Altar, P. L. Wood and A. M. Wasley*. Neurosci. Res., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

We determined effects of atypical and typical antipsychotics on neostriatal dopamine (DA) release inferred from 3-methoxytyramine (3-MT) levels and turnover (DOPAC and HVA levels) and on the binding of [3H]spiroperidol to serotonin (S_2) and dopamine (D_2) sites throughout the cortex and basal ganglia.

Mice were injected with the ID_{50} dose for apomorphine-induced climbing (S. Gerhardt and J. Liebman, Pharmacologist, 25:129, 1983) and killed 80 min later. DA, DOPAC, HVA and 3-MT were determined by GC/MF with single ion monitoring (P. L. Wood, Biomed. Mass Spec. 9:302, 1982). N=6-9/group, * $p < 0.05$, ** $p < 0.01$:

	CAUDATE-PUTAMEN CONC. (% control)			
Neuroleptic (Climbing ID_{50})	3-MT	DOPAC	HVA	DA
Clozapine (8)	94	143	142	105
Thioridazine (5)	115	280*	174*	92
Chlorpromazine (2)	128*	180*	170*	85
Metoclopramide (2)	144**	373**	218**	94
Haloperidol (.12)	137*	368**	245**	55**

The atypical antipsychotics clozapine and thioridazine promoted the smallest, and haloperidol and metoclopramide (typicals), the largest increases in DA release (3-MT) or turnover (DOPAC, HVA). The potencies of these antipsychotics at S_2 sites in claustrum or layer V of motor cortex or D_2 sites in the caudate-putamen, nucleus accumbens, or olfactory tubercle were determined (C. A. Altar et al, J. Pharm. Exp. Ther. 233:1985).

The atypical antipsychotics were 2- or 3-fold more potent at D_2 sites in nucleus accumbens or olfactory tubercle than in caudate-putamen whereas the typicals were equipotent in these regions. The typicals were equal or more potent at D_2 than S_2 sites whereas the atypicals were 4- to 30-fold more potent at S_2 sites.

These data indicate that at behaviorally effective doses, atypical antipsychotics increase DA release and turnover less than typical antipsychotics. The considerable preference of clozapine and thioridazine for cortical S_2 sites and their small potency advantage at mesolimbic versus mesostriatal D_2 sites indicates that their atypical behavioral and neurochemical profiles may result from interactions with S_2 binding sites.

199.4 AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-METHYLPHENDIATE BINDING SITES IN THE RAT BRAIN. A.S. Unis, T.M. Dawson, D.R. Gehlert* and J.K. Wamsley. Depts. of Psych. and Pharm., University of Utah Medical Center, Salt Lake City, UT 84132.

Methylphenidate (MPH), a mild central nervous system stimulant used primarily in the treatment of attention deficit disorder (ADD), appears to exert its therapeutic effect by blocking the reuptake of dopamine by the presynaptic neuron. As such ^{3}H-MPH could be a marker for dopaminergic nerve terminals. Using the techniques of in vitro receptor autoradiography we have localized specific ^{3}H-MPH binding sites within the rat brain.

Ten micron-thick brain sections were incubated for 40 min. at 4°C in 25 nM ^{3}H-MPH (spec. act.=87Ci/mmol). Alternate sections were incubated in medium to which unlabeled MPH was added to a final concentration of 25µM. Autoradiograms were generated by apposing the slide-mounted tissue sections to tritium-sensitive film along with tritium-containing standards in X-ray cassettes and these were analyzed using computer-assisted microdensitometry.

Autoradiographic grains, representing specifically bound ^{3}H-MPH, were localized to several discrete brain areas. The highest specific binding was observed in the caudate-putamen, the olfactory tubercle, the median eminence, the bed nucleus stria terminalis and the nucleus accumbens. Low density, diffuse specific binding was also demonstrated in the frontal cortex and the dentate gyrus. The biochemical parameters of ^{3}H-MPH binding were similar to those obtained using tissue homogenates. Optimal specific binding occurred at 40 minutes and half of the specifically bound ^{3}H-MPH dissociated after 10 seconds. ^{3}H-MPH was not only displaced by unlabeled MPH but also by 25µM nomifensine which has also been shown to be a dopamine-reuptake blocker.

^{3}H-MPH appears to bind to areas in which there are high concentrations of dopamine nerve terminals and our results suggest that MPH is interacting primarily with dopaminergic pathways. Autoradiography can be used to characterize the ligand-binding site interactions and thus improve our understanding of the mechanisms of action of the psychostimulants.

199.5 **β_2-Adrenergic Receptors Are Specifically Associated with the "Whisker Barrels" in the Somatosensory Cortex of the Rat.** P. Vos*[1], D. Kaufman[2], E. Mansfield*[3] B.B. Wolfe[1] P.J. Hand[3], U. of Penn., Sch. of Med., [1]Dept. Pharmacol., [2]Inst. Neurol. Sci., [3] Sch. of Vet. Med., Dept. Animal Bio.

The primary neocortical representation of the mystacial vibrissae of the rat is located in the posteromedial barrel subfield of the somatosensory cortex (PMBSF). In lamina IV of the PMBSF this representation shows a one-to-one correspondence with the pattern of "whisker barrels" as seen in succinate dehydrogenase (SDH) stained tangential cortical sections. We now report that this same "whisker barrel" pattern is also observed for β_2-adrenergic receptors using the quantitative autoradiographic techniques developed by Rainbow et al. (PNAS, 1984)

Serial tangential sections (32 μ) were cut from the frozen brains of adult rats. Sections were thaw-mounted and prepared for autoradiography or SDH staining. The "whisker barrels" were located by staining every fifth section for SDH activity. Intervening sections were labelled for either: Total β- adrenergic receptor binding, β_1-adrenergic receptor binding, β_2-adrenergic receptor binding, or nonspecific binding.

Tangential cortical sections labelled for total β-adrenergic receptor binding revealed a diffuse pattern of binding with regions of slightly increased labelling in the PMBSF which seemed to correlate with SDH stained "whisker barrels" seen in adjacent sections. Cortical sections labelled for β_1-adrenergic receptor binding also revealed a diffuse pattern of receptor distribution. However, this pattern did not appear to correspond to the pattern of "whisker barrels" seen in SDH stained adjacent sections. Alternatively, cortical sections labelled for β_2-adrenergic receptors showed a discrete distribution of receptors in the PMBSF which closely correlated with the SDH stained "whisker barrels" seen in adjacent sections.

Deafferentation experiments were done to determine if the β_2-adrenergic receptor distribution was developmentally modifiable. In order to deafferent the "whisker barrels" unilaterally all mystacial vibrissae and underlying follicles were surgically removed from neonatal rats before postnatal day three. No significant changes were seen in the distribution of β_1-adrenergic receptors. A dramatic change, however, was observed in the distribution of β_2-adrenergic receptors in the PMBSF. Specifically, the altered SDH staining patterns of the "whisker barrels" observed after peripheral neonatal lesions corresponded to similarly deranged patterns of β_2-adrenergic receptors from adjacent sections. For example, total unilateral mystacial vibrissae follicle removal produced a five banded pattern in SDH stained sections. The same banded pattern was observed in adjacent sections labelled for β_2-adrenergic receptors. No discrete SDH stained or β_2-adrenergic receptor labelled "whisker barrels" were observed.

We conclude that a specific receptor subtype, the β_2-adrenergic receptor, is specifically associated with the vibrissae follicle representation in the PMBSF of the rat. It is suggested that the β_2-adrenergic receptors may be involved in the development of this cortical representation. Studies are currently under way to test this hypothesis.

199.6 IS KETANSERIN A SELECTIVE 5-HT_2 LIGAND? E.D. Mitchell*, D.R. Gehlert, J.K. Wamsley and T.M. Dawson (Spon: W. Stevens). Depts. of Psychiatry and Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah 84132.

[^{3}H]-ketanserin is believed to be a ligand which is highly selective for the serotonin type 2 (5-HT_2) receptor. Recent homogenate binding studies have shown that 28% of [^{3}H]-ketanserin binding to the striatum, as defined with 10^{-6}M methysergide, is due to binding to the 5-HT_2 receptor, 10% of the binding is due to adrenergic alpha-1 receptors, and a negligible amount to histaminergic H-1 receptors (Leysen et al., Mol. Pharmacol. 21:301, 1982). Therefore, approximately 60% of the total binding cannot be accounted for by an interaction with 5-HT_2, alpha-1 or H-1 receptors.

[^{3}H]-Ketanserin was evaluated for its selectivity as a 5-HT_2 ligand in several discrete rat brain structures by combining the technique of quantitative autoradiography with classic saturation and competition experiments. Serial tissue sections from areas corresponding to the prefrontal cortex, substantia nigra, dorsal raphe and striatum were incubated with 2 nM [^{3}H]-ketanserin with varying concentrations of several 5-HT antagonists and uptake blockers, SCH 23390, prazosin and verapamil. In addition, several major classes of compounds were added at a 10^{-6}M concentration to the incubation media. Autoradiograms were produced by apposition of the labeled sections along with tritium standards to LKB Ultrofilm. Binding was quantified by using computer-assisted microdensitometry.

The binding of [^{3}H]-ketanserin to several brain regions was saturable and of high affinity. In the areas with the highest amount of [^{3}H]-ketanserin binding sites, such as the striatum, substantia nigra compacta and dorsal raphe, approximately 20% to 30% of the binding was due to an interaction with 5-HT_2 receptors. However, a majority of the binding was only displaceable by unlabeled ketanserin and demonstrated the presence of a "ketanserin recognition site." Imipramine, fluoxetine, SCH 23390, verapamil, and prazosin had K_i's in the micromolar range. Of the other compounds studied, an insignificant displacement was observed.

Selective lesioning of the catecholamine and serotonin containing neuronal systems using 6-hydroxydopamine (6-OHDA) and 5,7-dihydroxytryptamine (5,7-DHT) indicates that the "ketanserin recognition site" in several brain structures is presynaptic on catecholamine neurons (Dawson et al., in preparation). This observation coupled with the data presented here suggest that a majority of [^{3}H]-ketanserin binding is not interacting with the 5-HT system. Therefore, both in vivo and in vitro studies utilizing ketanserin as a selective 5-HT_2 ligand must be interpreted with caution, especially in experimental designs utilizing striatal preparations.

199.7 QUANTITATIVE AUTORADIOGRAPHY OF GUANINE NUCLEOTIDE BINDING SITES: COMPARISON WITH THE DISTRIBUTION OF ^{3}H-FORSKOLIN BINDING SITES. D.R. Gehlert, T.M. Dawson, H.I. Yamamura and J.K. Wamsley (Spon: B.I. Grosser). Depts. Psychiatry and Pharmacology, Univ. Utah Sch. Med., Salt Lake City, UT 84132 and Dept. Pharmacology, Univ. Arizona, Tucson, AZ 85724.

Second mesenger generation is an important component of central neurotransmission. Adenylate cyclase has often been implicated as a principal second mesenger for a number of neurotransmitter systems. The activity of adenylate cyclase is modulated by both neurotransmitters acting via specific receptors and guanine nucleotides acting via a nucleotide binding protein (N). Recently, we reported the distribution of high affinity binding sites for ^{3}H-forskolin, a potent stimulator of adenylate cyclase activity. This compound is believed to interact with a site on the catalytic subunit of adenylate cyclase. In order to determine the distribution of the N protein, we have labeled slide-mounted tissue sections with a radiolabeled guanine nucleotide analog to detect the presence of the N protein by quantitative autoradiography.

Ten micron tissue sections were labeled with 400nM ^{3}H-Gpp(NH)p. Nonspecific binding was assessed by adding a 100 micromolar concentration of unlabeled GTP to the incubation media. Labeling with ^{3}H-forskolin was accomplished according to a previously described protocol (Gehlert et al., Eur. J. Pharmacol. 106:223, 1984: Brain Res., in press). Dry, labeled tissue sections along with tritium standards were apposed to LKB Ultrofilm (Rockville, MD). Binding was quantitated using a DADS-560 (Stahl Res., Rochester, N.Y.) microdensitometry system.

Initial biochemical studies indicated that ^{3}H-Gpp(NH)p binding rapidly reached equilibrium at 25°C and slowly dissociated at 4°C. By optimizing these binding conditions we were able to obtain 99% specific binding to slide-mounted tissue sections. As previously reported, high affinity ^{3}H-forskolin binding sites were found primarily in structures in the basal gangila such as the caudate-putamen, nucleus accumbens, olfactory tubercle and substantia nigra. Binding was also detected in discrete regions of the hippocampal formation and in the molecular layer of the cerebellum. In contrast, ^{3}H-Gpp(NH)p bound to a variety of brain structures not only in the basal gangila, but also in brain regions such as the cerebral cortex, thalamus, hypothalamus, brainstem and olfactory bulb; regions where ^{3}H-forskolin binding was relatively low. Therefore, high densities of ^{3}H-forskolin binding sites were present in only a few of the areas which contain the N protein. These results indicate that the N protein is present in brain regions which do not contain high densities of "forskolin-identified" adenylate cyclase.

200.1 NEUROTRANSMITTER PLASTICITY IN CHOLINERGIC NEURONS: ACCUMULATION OF CATECHOLAMINES BY TRANSPLANTED CILIARY NEURONS. J.N. Coulombe and M. Bronner-Fraser, Depts. of Devel. and Cell Biology, and Physiology and Biophysics, Univ. of California, Irvine, CA. 92717

Under appropriate conditions, post-mitotic sympathetic neurons can acquire cholinergic traits. Here, we examine whether the reciprocal transition can occur; i.e. can cholinergic neurons acquire adrenergic properties? We have developed techniques for selectively labeling cholinergic ciliary neurons, using retrogradely transported fluorescent latex microspheres. These cells are then microinjected into regions of the avian embryo where neural crest cells normally give to rise to sympathetic ganglia and adrenomedullary cells.

Fluorescent latex microspheres (0.05 to 0.2 microns) were injected into the iris and ciliary body of eyes from 6½ day quail embryos. The microspheres were retrogradely transported from these target sites to a subpopulation of cholinergic neurons (approximately 20%) in the ciliary ganglion. Only ganglion cells with large cell bodies (characteristic of neurons) were labeled by the latex microspheres. Those cells containing latex microspheres also stained with antisera recognizing neurofilament proteins, indicating that the labeled cells are, in fact, neurons. The important advantage of this technique is that only differentiated neurons which have sent axons toward their target tissues are labeled with the retrograde marker.

Microsphere labeled ganglia were dissociated into a suspension of single cells and microinjected into the trunk region of 2½ day chicken embryos. Embryos were allowed to develop for an additional 4 days during which neural crest cells had coalesced and differentiated into adrenergic derivatives. They were then freeze-dried, processed for catecholamine histofluorescence, and serially sectioned. Those neurons labeled with latex microspheres were easily recognizable by their intense, punctate, reddish-yellow fluorescence.

In these embryos, cholinergic ciliary neurons survived and translocated to neural crest-derived structures. Heavily labeled cells were primarily found in the region surrounding the dorsal aorta, where sympathetic ganglia, adrenal gland, and aortic plexuses form. As a control, embryos were injected with unlabeled ciliary ganglion cells together with cell debris containing latex microspheres. No cells labeled with multiple microspheres were found in these controls. Thus, the the microsphere label is not acquired by previously unlabeled cells during either transplantation or incubation in the host.

To date, 13 cells in 5 different embryos have been identified that contain both fluorescent latex microspheres and catecholamine histofluorescence. Thus, the previously cholinergic ciliary neurons have acquired catecholamine stores. Our results suggest that some cholinergic neurons are able to acquire adrenergic neurotransmitters in the proper embryonic environment. (NIH HD15527-04 and Basic Research Grant 1-896 from the March of Dimes)

200.2 DEVELOPMENT OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN NEURONS OF THE PARASYMPATHETIC SPHENOPALATINE GANGLION IN THE RAT G.G. Leblanc* and S.C. Landis (SPON: J. Dodd). Dept. of Neurobiol., Harvard Medical School, Boston, MA 02115.

Some populations of parasympathetic neurons express one or more adrenergic traits. For instance, tyrosine hydroxylase immunoreactivity (TH-IR) is expressed in subpopulations of neurons in the ciliary ganglia of adult rats (Landis et al., Soc. Abs. 9, 937) and embryonic chicks (Teitelman et al., J. Neurosci. 5, 29). Most of the neurons in the submandibular ganglion of adult rats exhibit dopamine-beta-hydroxylase-IR (Grznna and Coyle, Brain Res. 151, 206).

As a first step toward understanding the factors which cause the expression of adrenergic traits in parasympathetic neurons, we examined the time of onset of these traits in the rat sphenopalatine ganglion (SPG). The SPG is a cranial parasympathetic ganglion which innervates the nasal mucosa, lacrimal glands, and palate. The SPG seemed particularly well suited to developmental studies because, unlike most rat parasympathetic ganglia, it is large and relatively discrete.

TH immunoreactivity is present in a small proportion (less than 5%) of principal neurons in the adult SPG. The TH-IR neurons resemble other SPG neurons in size and morphology, and are clearly distinct from SIF cells, which are also present in the SPG. In order to determine when TH-IR first appears in SPG neurons, the ganglion was located in sections of embryonic rat heads using either acetylcholinesterase (AChE) staining or an antiserum which recognizes neurofilament protein. No TH-IR neurons were seen in the SPG of E16.5 embryos, although ganglionic condensation appeared nearly complete at this stage, and AChE staining revealed bundles of processes issuing from the ganglion. By E18.5, a few TH-IR neurons were present near the rostral pole of the ganglion. The number of TH-IR neurons in the SPG reached a peak around postnatal day 1. In fact, preliminary cell counts suggest that the proportion of TH-IR neurons in the newborn SPG is at least twice that observed in the adult. SIF cells, on the other hand, were far less numerous in the newborn than the adult SPG.

The expression of adrenergic traits in sympathetic neurons is thought to depend on signals received by the precursors of these neurons during their migration from the neural crest. Thus, sympathoblasts express TH-IR as soon as they begin to condense into ganglia. In contrast, TH-IR does not appear in parasympathetic SPG neurons until several days after ganglionic condensation has begun, and at least two days after the initiation of process formation. Interestingly, TH-IR neurons are not evenly distributed in the neonatal SPG, but rather appear to be concentrated in the ventral half of the ganglion. We are therefore examining the possibility that the TH-IR neurons share a common target structure.

200.3 THE EXPRESSION OF CATECHOLAMINE ENZYMES BY NEOCORTICAL NEURONS. L.M. SMITH, T.H. JOH, AND F.F. EBNER. Center for Neural Science, Brown University, Providence, R.I. and Department of Neurology, Cornell University Medical College, New York, N.Y.

Tyrosine hydroxylase (TH) is the initial and rate limiting enzyme in catecholamine synthesis and is first expressed on embryonic day 12 (E12) in the developing rat brain. A few cell processes are labeled transiently with TH immunocytochemistry in the ventricular zone of the telencephalic vesicle from E12 to E16. In addition, a few cell bodies become TH-positive near the olfactory bulb for 1 or 2 days around E18. However, with this exception neocortex never contains TH-positive neurons either in the developing or in the adult brain.

In contrast to normal development, we have found that when neocortex is removed from the anlage of mouse sensory-motor cortex and placed in a comparable area of adult mouse neocortex, it develops cells that express TH for months and presumably for the lifetime of the adult host. Over half of the implanted cells develop TH-immunoreactivity if the embryos are very young (E12-13). Embryos that are older at the time of surgery give rise to progressively fewer labeled cells, until by the age of E19 no cells are induced to express TH. The TH-positive neocortical cells never show catecholamine fluorescence. Embryonic hippocampal cells do not express TH under these conditions.

We have now examined other catecholamine enzymes to determine why the TH-positive cells do not show catecholamine fluorescence. Only a small number of transplanted cells ever develop immunoreactivity to aromatic-L-amino acid decarboxylase, and none has been immunocytochemically labeled at any donor age with antisera to dopamine-B-hydroxylase or phenethanolamine-N-methyltransferase.

This is the first demonstration of a set of conditions that induces the expression of TH in CNS cells which normally never synthesize this enzyme. Inducing TH enzyme synthesis depends upon the state of differentiation of the embryonic neocortical cells when they are placed in the adult neocortex. TH is the only one of four main catecholamine enzymes that is induced under these conditions. The enzyme induction initiated by embryonic cells growing in adult neocortex appears to be specific for neocortical cells, since hippocampal cells are not similarly affected when they are required to differentiate in adult neocortex. (supported by grant #NS-13031)

200.4 EMBRYONIC RAT CEREBRAL CORTICAL CELLS EXPRESS TYROSINE HYDROXYLASE IN TISSUE CULTURE. L. Iacovitti, J. Lee*, T.H. Joh and D.J. Reis. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

The neurotransmitter phenotype expressed by peripheral autonomic neurons remains labile during development in vivo and in vitro. In the present study, we sought to determine whether neurotransmitter phenotypic plasticity is also characteristic of neurons of the central nervous system. Although intrinsic catecholaminergic (CA) cells have not been observed in rat cerebral cortex in vivo (Specht et al., J. Comp. Neurol. 199:255-276, 1981), cultures of chick cortex develop activity for the CA enzyme, tyrosine hydroxylase (TH) (Arnold and Vernadakis, Dev. Neurosci. 2:46-50, 1979; Pettmann et al., Nature 2:46-50, 1979) and transplants of rat cortex contain numerous TH-immunoreactive cells (Ebner et al., submitted for publication, 1985). We further examined whether the central neurons of the cerebral cortex could be influenced by environmental factors in culture to express other CA phenotypic traits.

Whole brains removed from embryonic day (E) 14, 16 or 18 rat were fixed, sectioned and processed for the immunocytochemical localization to TH according to the PAP method. In agreement with previous reports, sections of cortex did not contain intrinsic TH-immunoreactive cells at any of the times examined. However, when cortices from E14 rat were dissociated into single cells, plated on collagen-coated dishes at a density of 2 cortices/dish and maintained on feed supplemented with 10% fetal calf serum, we found that a population of approximately 500 cells/dish were TH immunoreactive after only 3 days in vitro. These cells had a darkly stained rim of cytoplasm surrounding a large nucleus and, in many cases, extended long varicose stained processes. Cultures of rat cortex isolated from older embryos (E16 or E18) contained only an occasional TH immunoreactive cell. To test whether contact with older brain or substances released by it prevent the expression of TH in cortical cells, co-cultures were established consisting of both E14 and E18 cerebral cortex. After 5 days in vitro, co-cultures contained the same number (500/dish) of TH immunoreactive cells as was seen in cultures comprised of E14 cortex only.

In contrast to rat, cultures established from E8 chick cortex did not contain any TH immunoreactive cells even at 7 and 14 days in vitro, suggesting that either by E8 chick cortex is developmentally too advanced or that chick cortical cells are incapable of TH expression.

The results of these studies indicate that a) a subpopulation of early embryonic rat cortical cells can express a trait which is characteristic of a CA phenotype but that this ability is lost in older embryos and b) neither contact with older cortex nor substances released by it inhibit the expression of TH in young cortical cells in culture. Since TH immunoreactivity is not observed in the rat cortex in vivo, our findings further suggest that certain classes of central cells, like peripheral cells, can respond to environmental cues in culture and express traits fo a novel neurotransmitter phenotype. (Supported by NSF Grant PCM-8303019.)

200.5 TARGET INFLUENCES ON TRANSMITTER CHOICE BY SYMPATHETIC NEURONS DEVELOPING IN OCULO. L.M. Stevens* and S.C. Landis. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

The sympathetic fibers which innervate rat sweat glands (SGs) are initially noradrenergic and undergo a transition in which they acquire cholinergic properties as their noradrenergic traits become diminished. Experiments on cultured sympathetic neurons have shown that a similar switch in transmitter status can be induced by factors secreted by non-neuronal cells. To examine the possibility that the SGs promote the development of cholinergic properties in those neurons which innervate them, we caused the glands to become innervated by sympathetic neurons which would normally develop noradrenergically. SGs and superior cervical ganglia (SCG) from one day-old rat pups were grown in the anterior eye chamber of 6 week-old host rats using the transplantation technique devised by Olson and Malmfors (Acta physiol. scand. Suppl. 348, 1970).

Glands developed in footpad tissue which was transplanted without an accompanying ganglion, but they did not become innervated by the nerve supply of the host iris. Ten to 12 days after ganglia and footpads were co-transplanted, the developing glands had become innervated by fibers which exhibited both acetylcholinesterase (AChE) activity and catecholamine (CA) fluorescence, a dual phenotype also seen during the normal development of the gland innervation in situ. By 28 days after co-transplantation, the AChE-positive fiber plexus surrounding the glands no longer demonstrated CA fluorescence. The development of AChE activity and the loss of CA fluorescence in the transplanted glands parallels the development of the innervation in situ. Biochemical and immunocytochemical studies are in progress to characterize further the transmitter status of the transplant innervation. The small percentage of transplanted neurons surviving at 28 days exhibited strong AChE activity and did not contain endogenous CAs. We believe that these neurons are the source of the gland innervation; to verify this we are currently sympathetically and parasympathetically denervating the host iris prior to assay.

To determine whether this change in phenotype is specific to the SGs, we are growing SCG in the anterior chamber with targets which receive only noradrenergic sympathetic innervation. In preliminary experiments in which SCG were grown with pineal gland for 28 days, both the gland innervation and the neurons exhibited prominent CA fluorescence. These results suggest that the change in phenotype seen in the innervation of the transplanted SGs is due to a specific influence of the glands and is not a result of growth in the anterior chamber. This raises the possibility that during the normal development of the SGs, the noradrenergic neurons which innervate the glands are induced by the target to differentiate cholinergically.

200.6 PURIFICATION OF A GLYCOPROTEIN WHICH CONTROLS TRANSMITTER CHOICE. Keiko Fukada. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Individual neurons can be plastic with respect to transmitter phenotype (Patterson, P.H., Ann. Rev. Neurosci. 1:1, 1978). The existence of a diffusible factor which controls the choice of transmitter in developing sympathetic neurons, without affecting neuronal survival or growth, has been demonstrated in culture studies. Moreover, in vivo studies have shown that the transition from the noradrenergic to the cholinergic phenotype demonstrated in culture actually occurs in vivo as part of the normal development of sympathetic cholinergic neurons (Landis, S., Fed. Proc. 49:1633, 1983). In order to understand the molecular mechanism of such plasticity and the role of the cholinergic factor in development, it is essential to purify the molecule.

The cholinergic factor is obtained from conditioned medium (CM) prepared by incubation of serum-free, hormone-supplemented medium on cultures of rat heart cells (Fukada, K., Nature 287:555, 1980). Using column chromatography and SDS-PAGE, $>10^5$-fold purification in specific activity has been achieved with a reasonable recovery. The most purified fraction is active at <10 ng/ml and retains the ability to both inhibit the development of noradrenergic characteristics as well as to induce cholinergic differentiation. The activity has the same apparent molecular weight of 45 Kd under denaturing and native conditions, suggesting that the factor is a single subunit. The 45 Kd area where activity resides on the SDS gel is only faintly stained by silver methods, and it is not labeled by the Chloramine T, Iodogen or lactoperoxidase methods. However, the Bolton-Hunter (B-H) reagent does label this area. That the labeled 45 Kd protein is the cholinergic factor is strongly suggested by the following recent results. (i) The activity and the iodinated 45 Kd protein are both sensitive to trypsin but not to chymotrypsin and the 45 Kd protein is only labeled by B-H iodination which labels lysine residues. (ii) When the iodinated Sephadex fraction is analyzed by 2-D gel electrophoresis, five discrete labeled spots of different charge are seen in the 45 Kd region. Each of these ^{125}I-labeled spots precisely lines up with the five activity peaks in the second dimension. (iii) Cleavage of the labeled 45 Kd protein with endoglycosidase F (Endo F) gives six discrete bands of different MWs on 1-D gels. When Endo F is allowed to exhaustively digest the labeled 45 Kd protein, only the lowest MW band of the six is found, and the cholinergic activity comigrates with this 22 Kd protein. Thus, the cholinergic factor is a slightly basic molecule whose core protein is composed of a single polypeptide of 22 Kd, and the biological activity resides in the protein moiety. (Supported by grants to P.H. Patterson from the NINCDS and the McKnight Foundation)

200.7 DIFFERENTIAL REGULATION OF CHOLINERGIC AND PEPTIDERGIC DEVELOPMENT IN THE RAT STRIATUM IN CULTURE. J.A.Kessler. Lab. of Developmental Neurobiology, Albert Einstein College of Medicine, Bronx, New York 10461.

The development of substance P, somatostatin, and choline acetyltransferase activity was examined in embryonic rat striatum in vivo and in culture. Choline acetyltransferase was present in striatum before gestational day 13.5 (E13.5), and enzyme levels increased continually between E13.5 and birth. By contrast, substance P and somatostatin did not develop in vivo until E15, and peptide levels fluctuated between E15 and birth. However cultured striatal neurons from E13.5 embryos expressed substance P and somatostatin de novo after several days in culture, and peptide levels and choline acetyltransferase activity increased significantly in vitro. Insulin was required for substance P expression in culture but not for development of somatostatin or choline acetyltransferase activity. Treatment with potassium (35mEq) to depolarize neuronal membranes significantly increased choline acetyltransferase activity, but significantly decreased levels of substance P and somatostatin. Co-culture of striatal neurons with ventral mesencephalon (substantia nigra) significantly increased choline acetyltransferase activity and substance P levels but had no significant effect on levels of somatostatin. Co-culture of striatum and substantia nigra also stimulated development of tyrosine hydroxylase in nigral neurons. Finally, medium conditioned by exposure to one of a variety of cell types had widely differing effects on development of substance P, somatostatin, and choline acetyltransferase in cultured striatal neurons. Moreover, treatment with a purified factor found in human, bovine, and rat tissues elevated choline acetyltransferase activity and substance P but not somatostatin in cultured striatum. Our observations suggest that different populations of neurons in the striatum are regulated by different mechanisms, so that alterations in the environment may produce strikingly different responses in the develoment of different phenotypic traits within the same structure.

200.8 PEPTIDE REGULATION OF CATECHOLAMINERGIC TRANSMITTER METABOLISM IN PRIMARY SENSORY NEURONS IN VITRO. D.M. Katz, J.E. Adler, and I.B. Black. Cornell Med. Coll. New York, NY 10021.

Catecholaminergic (CA) phenotypic expression in the mature peripheral nervous system is now known to extend beyond the sympathoadrenal axis. As we have previously shown, primary sensory neurons in the nodose and petrosal (NP) cranial nerve ganglia also express functional CA traits, including catalytically active tyrosine hydroxylase (TH), formaldehyde induced CA fluorescence, and sensitivity of CA levels to monoamine oxidase inhibition (Katz, et al., PNAS, '83). It is not known, however, whether sensory and sympathetic CA neurons, differing markedly in function, morphology and presumptive embryonic origin, express similar mechanisms of CA phenotypic regulation.

In sympathetic neurons, TH activity is regulated by specific trans-synaptic stimuli, including cholinergic and peptidergic depolarizing agents (Kessler, et al., '83; Ip, et al., '83). To define the potential role of depolarizing stimuli in sensory CA regulation, initial experiments examined TH activity in NP explant cultures grown in the presence or absence of depolarizing concentrations of potassium (K+). After one week in 40mM K+, TH activity was increased 2-3 fold over controls. Total protein was unchanged, indicating that enzyme specific activity rose, and suggesting that the increase was not due to increased cell survival. Thus, these data suggest that depolarizing stimuli may normally play a role in sensory CA regulation.

To determine whether agents known to activate NP neurons in vivo also increase sensory TH activity, we examined the effects of the stable Substance P (SP) agonist, Sar-SP. The peptide dramatically increases impulse activity in petrosal afferents in vivo (Prabhakar, et al, '84), and is immunocytochemically detectable in glomus cells, presynaptic to NP neurons (present study). Exposure to 10^{-6}M Sar-SP for one week increased TH activity in NP cultures, mimicking the effect of high extracellular K+. These data suggest that peptide ligands regulate sensory, as well as sympathetic, CA metabolism. Consequently, catecholaminergic sensory neurons may be subject to orthograde trans-synaptic regulation by peptides of peripheral target origin. (Supported by American Heart Assoc. and Dysautonomia Fndtn., Inc.).

200.9 ENVIRONMENTAL REGULATION OF NEUROTRANSMITTER GENE TRANSCRIPTION. K. Spiegel and J.A. Kessler (SPON: G.S.F.Ling). Laboratory of Developmental Neurobiology, Albert Einstein College of Medicine, Bronx, New York 10461.

The role of the environment in regulating gene transcription of neurotransmitter traits was examined in pure neuronal cultures of the neonatal rat sympathetic superior cervical ganglion (SCG). Previous studies have shown that the expression of tranmitter phenotype is influenced both qualitatively and quantitatively by the environment of the neuron. However, it is not known whether this phenotypic plasticity reflects transcriptional, translational, or post-translational events. To define the mechanisms regulating transmitter expression, somatostatin (SS) and tyrosine hydroxylase (TH) levels and levels of their messenger RNA's (mRNA's) were examined in SCG neurons cultured in different environments.

Sympathetic neurons cultured for two weeks in control medium ($F_{12}FCS_{10}$) developed substantial levels of both somatostatin (2.9 fg per neuron) and tyrosine hydroxylase activity (2.7 fmole product per neuron minute). However, cultures in the presence of elevated potassium (35 meq) to depolarize neuronal membranes reduced somatostatin to negligible levels (0.1) but increased TH activity (4.4). Northern blot analysis of mRNA levels demonstrated that potassium treatment greatly reduced levels of SS mRNA, in parallel with the decrease in neuronal SS levels. Conversely, the potassium-induced increase in TH activity was accompanied by an increase in TH mRNA, suggesting that membrane depolarization regulated gene transcription of both SS and TH.

In contrast to the stimulatory effects of potassium treatment on noradrenergic traits, exposure of sympathetic neurons to medium conditioned by non-neuronal cells decreased noradrenergic development while enhancing cholinergic expression. To determine whether conditioned medium also altered neurotransmitter gene transciption, TH mRNA levels were examined in sympathetic neurons cultured in medium conditioned by human fibroblasts (FCM). Exposure to FCM increased cholinergic expression (choline acetyltransferase activity) but decreased TH activity by 45%. Northern blot analysis demonstrated a corresponding decrease in TH mRNA in cultures exposed to conditioned medium. Our results suggest that the environment of a neuron may influence neurotransmitter phenotypic expression by regulating gene transcription.

200.10 DEPOLARIZATION REGULATES THE LEVEL OF PREPROTACHYKININ MESSENGER RNA IN THE CULTURED SUPERIOR CERVICAL GANGLION. A.H. Roach*, J.E. Adler, J. Krause and I.B. Black (SPON: E. DiCicco-Bloom). Cornell Med. Coll., New York, NY 10021, and Dept. of Anatomy and Neurobiology, Washington Univ., St. Louis, MO 63110.

Multiple neurotransmitters have been demonstrated within single neurons of many classes. These findings raise questions concerning the patterns and mechanisms of regulation of the individual transmitter types both during development and in maturity. The rat superior cervical ganglion (SCG) is a simple model system in which single neurons produce both substance P (SP) and norepinephrine (NE). Decentralization in vivo, or explantation to culture (with consequent denervation) elicit an increase of up to 40-fold in ganglion SP levels. In contrast, tyrosine hydroxylase, the rate-limiting enzyme in NE biosynthesis, does not increase. The recent cloning of cDNA copies of the messenger RNAs (mRNAs) for the preprotachykinins, the precursors of SP (Krause, this volume), now allows study of underlying mRNA synthetic and processing mechanisms.

Previous work has shown that explanted ganglia exhibit small increases in SP content after 12 hours in culture, but achieve a 40-fold rise by the fourth day. In the present studies, we report that PPT mRNA increased significantly by RNA blot analysis after only six hours in culture, well before an increase in SP was detectable. PPT mRNA levels reached a maximum after 24 hours and declined thereafter to lower steady-state values. Camptothecin and actinomycin D, inhibitors of RNA synthesis previously shown to prevent SP elevation, also prevented the increase of PPT mRNA in the present experiments. Moreover, veratridine, which depolarizes neurons in culture by increasing sodium channel conductance, prevented the rise in SP, and also prevented the increase in PPT mRNA in the current studies. Finally, tetrodotoxin, which blocks the same sodium channels, simultaneously prevented the effects of veratridine on both peptide and mRNA levels.

The sequential increases in PPT mRNA and SP, in conjunction with the effects of RNA synthesis inhibitors, suggest that the elevation of SP reflects altered expression of the PPT gene. Our observations further suggest that depolarization changes the pattern of PPT gene expression in sympathetic neurons. We are presently exploring underlying mechanisms. (Supported by NIH Grants NS 10259 and HD 12108. A.R. is supported by a postdoctoral fellowship from the Medical Research Council of Canada).

BEHAVIORAL PHARMACOLOGY: AMINERGIC SYSTEMS

201.1 QUANTITATIVE AND QUALITATIVE DIFFERENCES BETWEEN THE DOPAMINE SYSTEMS INVOLVED IN YAWNING AND FORWARD LOCOMOTION IN THE RAT. J.M. van Rooyen and J. Offermeier. Dept. of Pharmacology and MRC Research Unit for the Design of Catecholaminergic Drugs, Potchefstroom University, Potchefstroom 2520, South Africa

Piribedil and apomorphine dose-dependently increase yawning and forward locomotion in rats. The maximal increases of yawning occur at intraperitoneal (ip) doses of 5mg/Kg piribedil and 0,5mg/Kg apomorphine respectively while the maximal increases for locomotion occur at ip doses of 50mg/Kg piribedil and 5mg/Kg apomorphine. The maximal increases of yawning and of locomotion produced by piribedil is greater than the maximal increases produced by apomorphine.

Metoclopramide (1,6 - 3,2mg/Kg) antagonizes piribedil- and apomorphine-induced yawning. Metoclopramide (1,6mg/Kg) potentiates apomorphine- but not piribedil-induced locomotion. Metoclopramide (16mg/Kg) antagonizes both piribedil- and apomorphine-induced locomotion.

Reserpine pretreatment attenuates piribedil-induced yawning, apomorphine-induced yawning and piribedil-induced locomotion but potentiates apomorphine-induced locomotion.

Atropine pretreatment (0,625 - 10mg/Kg) dose-dependently antagonizes piribedil- and apomorphine-induced yawning. Atropine pretreatment (10mg/Kg) does not alter apomorphine-induced locomotion but potentiates piribedil-induced locomotion.

These data suggest a partial agonistic action of apomorphine on the dopamine receptors involved in yawning and in locomotion. The dopamine system involved in piribedil-induced locomotion appears to differ from the system responsible for apomorphine-induced locomotion.

201.2 QUANTITATIVE BEHAVIORAL ANALYSIS OF DOPAMINERGIC SUPER- AND SUB-SENSITIVITY: METHODOLOGY AND COMPUTER SIMULATION FOR CONTINUOUS AND CATEGORICAL RESPONSE VARIABLES. P.K. Randall, Dept. of Physiology and Biophysics, USC Sch. of Med. and Andrus Gerontology Ctr. U.S.C., Los Angeles, CA 90089

Quantitative analyses of a DA agonist- and antagonist-induced behavior in a number of preparations involving alterations in DA receptor number are presented. In general the degree to which variation in receptor number leads to predictable consequences in behavioral response is highly dependent upon the model system used. Changes in apomorphine-induced stereotypic behavior in the mouse resulting from chronic haloperidol treatment for example, are highly consistent with the moderate increases in DA receptor number usually reported (a 1.5 -fold shift in a family of logistic dose response curves representing individual components of the response). Selective depletion of striatal DA, in the striatal output lesion model has similar consequences. However, chronic agonist treatment, strain differences in receptor number, and selective striatal depletion produce very different alterations in agonist dose-response curves for stereotypic behavior which are difficult at present to resolve with measurements of DA receptor number.

Computer simulations of both continuous and categorical response variables based on receptor binding equations and classical "null" pharmacological methodology suggest that experimental design and data analysis procedures must be highly optimized to discriminate between different models and to test quantitative predictions made from them. Analysis of variance techniques, for example, are much less powerful than weighted least-squares, non-linear curve-fitting in detecting the behavioral consequences of moderate alterations in receptor number when a full agonist is used as a test substance. Multivariate extensions of "null" methods for agonist affinity, and competitive and functional antagonists are suggested as the appropriate models for experiments of this type.

201.3 SPECIFIC [^{3}H] MAZINDOL AND [^{3}H] P-CHLOROAMPHETAMINE BINDING SITES IN THE HYPOTHALAMUS: CORRELATION WITH ANORECTIC PROPERTIES OF PHENYLETHYLAMINES I. Angel*, M.D. Luu*, R. Hauger*, B. Giblin*, P. Skolnick, and S.M. Paul* (SPON: M. Goldman). Sections on Molecular Pharmacology and Preclinical Studies, Clinical Neuroscience Branch, National Institute of Mental Health, Bldg. 10, Room 4N214, 9000 Rockville Pike, Bethesda, Maryland 20205 USA

Specific and saturable binding sites for the anorectic drug mazindol were demonstrated in crude synaptosomal membranes from rat hypothalamus. The kinetic analysis of specific [^{3}H] mazindol binding revealed a single class of binding sites with an apparent affinity constant (Kd) of 9.3 ± 1.4 μM and maximum number of binding sites (B_{max}) of 433 ± 88 pmol/mg protein (n = 12). The association and dissociation of [^{3}H] mazindol to synaptosomal membranes were very rapid. Specific binding reached equilibrium in approximately 15 min. and dissociation was complete by approximately 2 min. Specific binding was temperature sensitive, labile to preincubation with proteolytic enzymes, and inhibited by physiological concentrations of NaCl. Subcellular fractionation of crude brain homogenates revealed that specific binding was highly enriched in the synaptosomal fraction with very low levels of binding detected in nuclei or mitochrondria. Specific [^{3}H] mazindol binding is also unevenly distributed in brain with the highest binding observed in the hypothalamus and brain stem. In most peripheral organs, such as the liver and kidney, only low levels of binding were detected whereas the adrenal gland had relatively high levels of specific binding. The characteristics of [^{3}H] mazindol binding were very similar to the previously described [^{3}H] amphetamine binding site and the relative potencies of a series of drugs in inhibiting both ligands were highly correlated. Moreover, structure activity studies revealed a good correlation ($r = .84$, $p < 0.01$) between the potencies of a series (n = 15) of phenylethylamine-like drugs in inhibiting specific [^{3}H] mazindol binding and reducing food intake (anorectic potencies) but not with their motor stimulatory effects nor their potencies in inhibiting drinking behavior. In related experiments saturable and low affinity (Kd 4μM) binding sites for [^{3}H] p-chloroamphetamine were also demonstrated in rat hypothalamic membranes. These binding sites had virtually identical structure-activity characteristics as the [^{3}H] mazindol binding sites and a good correlation existed between the inhibitory potencies of a series of compounds at displacing both ligands. Our results suggest the presence of novel recognition sites for mazindol and p chloroamphetamine in brain that appear to mediate the anorectic actions of mazindol and various phenylethylamine derivatives. Preliminary data suggests that these low affinity (high capacity) binding sites are associated with a membrane-bound enzyme.

201.4 EFFECTS OF CLONIDINE ON THE MOTOR SYNDROME OF THE GENETICALLY DYSTONIC RAT. J. Lutes, J. F. Lorden, and G. A. Oltmans, Dept. Psych., Univ. Alabama at Birmingham, AL 35294; and Dept. Pharmacol., Chicago Med. Sch., No. Chicago, IL 60064.

The dystonic (dt) rat displays an inherited neurological disorder characterized by sustained twisting movements, frequent falls to the side, hyperflexion of the trunk, poor limb placement during locomotion, and self-clasping the hindlimbs and forelimbs. Steady state levels of norepinephrine (NE) are elevated in the cerebellum of the dt rat around the time that clinical signs begin to appear. Forebrain terminal fields of the locus coeruleus are unaffected; however, recent studies on this mutant suggest that NE levels are also increased in the spinal cord. Therefore, we have attempted to modify the motor syndrome of the dystonic rat with adrenergic drugs.

Sixteen to 20 day old dystonic rats were observed in a open field for three 3-min periods: prior to injection and 20 and 40 min postinjection. During each period, the number of axial twisting movements, falls, and paw clasps was counted. Three doses (.05, .1, and .3 mg/kg, i.p.) of the alpha 2 adrenergic agonist clonidine were administered to separate groups of rats (N = 8/group). Clonidine decreased the characteristic movements and postures of the mutant rats. At the two highest doses, all dystonic movements were affected. However, the tendency of the dystonic rats to fall to the side and hold their limbs rigidly extended was significantly reduced even at the lowest dose. Two additional groups of mutants were tested in an open field for 3 min prior to drug injection. Immediately after, they were given 2 or 4 mg/kg, i.p., of rauwolscine, the alpha 2 receptor antagonist. Twenty min later, the rats were again examined in the open field and injected with .05 mg/kg of clonidine. The final observation period took place 20 min later. Rauwolscine alone had no effect on the movements of the mutant rats, but blocked the clonidine effect.

The effect of clonidine on gross locomotor activity was also quantified. After a 30 min habituation period to the photocell chambers, normal and dystonic rats were given injections of saline or clonidine. Although the .3 mg/kg dose produced a general suppression of activity in the 40 min following the injection, the lowest dose increased activity. Thus, the effects of clonidine on the falls and hindlimb extension of the dystonic rats cannot be attributed simply to a general suppression of movement. The effects on clonidine in conjunction with the alterations in NE levels in the cerebellum and spinal cord of the dt rat suggest that central NE mechanisms may be involved in the motor syndrome of the mutant. This is interesting in light of reports of altered NE metabolism in humans with inherited dystonia. (Supported by NS 18062 and the Alabama Consumer Research Fund.)

201.5 CHLORPROMAZINE: EFFECTS ON THE DETECTION OF NON-REWARDING BRAIN STIMULATION. J.E.G. Williams and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118

Previous experiments in our laboratory demonstrated that chlorpromazine (CPZ) raises the threshold for rewarding brain stimulation to the medial forebrain bundle-lateral hypothalamic (MFB-LH) (Esposito, R.U. et al, Pharmacol. Biochem. Behav. 15(6):903-905, 1981). In order to determine whether or not these threshold changes are due to actions on a motivational system rather than alterations in attentional or perceptual processes the effects of CPZ on the detection of stimulation to this same brain site were determined. In addition to thresholds, intertrial responding and latency of response as a measure of the psychomotor effects of CPZ were recorded. The threshold level of stimulation for detection as employed in this procedure is by itself neither positively nor negatively reinforcing, however, this level of stimulation can be used as a discriminative stimulus in a simple instrumental task.

Bipolar stainless steel electrodes were stereotaxically implanted in male albino rats (CDF-Charles River Laboratories). Electrodes were aimed bilaterally at the MFB-LH. Following surgery, the animals were trained in a rate-independent procedure to make an instrumental response to a non-reinforcing 0.5 sec MFB-LH stimulation cue (S1). Responding to the cue within 5 sec was maintained by the delivery of a positively reinforcing stimulus (S2) to the contralateral MFB-LH area. Absolute detection thresholds were determined by varying the current intensity of the brain stimulation cue (S1) according to a modification of the psychophysical method of constant stimuli. The reinforcing stimulus (S2) remained at a fixed highly rewarding intensity level. Preliminary results indicate that chlorpromazine caused a raising of the threshold for detection of stimulation at doses that had no effect on the threshold for rewarding stimulation. These results suggest that the threshold raising effect of CPZ on rewarding brain stimulation is, at least in part, due to an alteration in attentional or perceptual processes. Also, since these doses of CPZ did not alter latency of response or intertrial responding it is unlikely that the observed changes in the detection threshold are due to a drug effect on motor behavior. (Supported in part by NIDA grant DA 02326 and NIDA Research Scientist Award (CK) KO5 DA 00099).

201.6 MAGNESIUM ALTERS CATECHOLAMINE FUNCTION: IMPLICATIONS FOR ABUSE POTENTIAL OF DRUGS. K.M. Kantak and L.K. Adlerstein*. Laboratory of Behavioral Neuroscience, Dept. Psychology, Boston Univ., Boston, MA 02215.

Previous research from my laboratory has demonstrated that limiting dietary magnesium (Mg^{2+}) to 15% or 25% of the daily requirement leads to reductions in offensive threat and attack behaviors in male mice. These reductions began 3 weeks after initiation of these diets and continued through 8 weeks. We have also examined the effect of Mg^{2+} excesses on offensive behavior. At doses of 15 mg/kg and 30 mg/kg $MgCl_2$, threat and attack behaviors on mice were significantly elevated. At higher doses, 125 mg/kg and 250 mg/kg, these behaviors were significantly reduced. These data on excesses and deficiencies suggest an inverted U-shape function to magnesium's influence on behavior. This is highly suggestive that Mg^{2+} is influencing catecholamine function in vivo, because catecholamine stimulation typically displays this type of relationship to behavior, including aggression. Consequently, apomorphine-induced sniffing, which is an index of dopamine function, is reduced by Mg^{2+} deficiency as is l-amphetamine-induced locomotion which is an index of norepinephrine activity. In the present experiments, we determined if aggression altering doses of $MgCl_2$ were capable of increasing catecholamine function as assessed by these drug-induced behaviors in male mice.

Pretreatments consisted of either 0.9% NaCl, 30 mg/kg $MgCl_2$ or 125 mg/kg $MgCl_2$ and groups of mice were injected with 1 of 5 doses of apomorphine or l-amphetamine. $MgCl_2$ caused a dose-dependent shift to the left for the dose response to apomorphine-induced sniffing and l-amphetamine-induced locomotion. The ED_{50} values for half maximal stimulation of sniffing were 0.34, 0.19 and 0.14 mg/kg for increasing doses of $MgCl_2$. The ED_{50} values for half maximal inhibition of locomotion were 3.0, 0.12 and 0.0 mg/kg for increasing doses of $MgCl_2$. This indicates an enhancement of apomorphine action and dopamine function as well as an enhancement of l-amphetamine action and norepinephrine function with increasing doses of $MgCl_2$. These data indicate that behaviorally active doses of $MgCl_2$ can increase the efficacy of drugs that stimulate catecholamine systems. This may have bearing on the abuse potential of drugs such as cocaine and amphetamine, in that Mg^{2+} status can either contribute to or prevent tolerance and physical dependence to these agents or alter their effectiveness.

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201.7 A NEW ANIMAL MODEL OF DEPRESSION THAT PRODUCES LONG-LASTING EFFECTS. L. A. Hoffman* and J. M. Weiss, Rockefeller University, New York, NY, 10021.

A rodent model of depression in which the depressive syndrome endures for long periods of time has yet to be developed. Whereas certain procedures generate a sufficient number of symptoms to approach reproducing an adequate animal model of depression, most of these symptoms are short-lived. We report here a procedure which, following treatment, generates depression of active behavior that endures for at least two months. Also, this behavioral depression, once produced, can be reversed by the chronic, but not acute, treatment with the tricyclic antidepressant desipramine.

In these experiments, depression of active behavior was measured by placing animals in a tank of water and measuring the time that animals struggled vigorously and also floated. Time spent floating is subtracted from time spent struggling to obtain an Activity Score (AS). This index has proved to be particularly convenient because untreated animals normally show approximately the same amount of struggling and floating in the 15-min swim test, thus achieving an AS of zero. A negative AS thereby indicates depression of active behavior. This test has been previously shown to reflect depression of active behavior produced by exposure to stressful conditions (e.g., Weiss, Goodman, Losito, Corrigan, Charry, and Bailey, Brain Res. Rev., [1981] 167-205).

Behavioral depression was produced by administering clonidine (CLON) to animals for 14 days and then withdrawing the drug. In the first study, drug was administered in two ways: (1) by daily injection (0.1 mg/kg) together with drug in the animal's drinking water (3 ug/ml) (CLON Group A), and (2) drug in drinking water only (CLON Group B). After 14 days, drug was withdrawn. When tested in the swim test 10 days after CLON withdrawal, CLON-withdrawn animals showed significant behavioral depression (mean AS for CLON A = -273.7 ± 136.9; for CLON B = -258.7 ± 71.1; for no-drug controls = -16.7 ± 43.2). When tested repeatedly up to 65 days after drug withdrawal, CLON-withdrawn animals continued to show behavioral depression in the swim test.

In two subsequent experiments, it was shown that this behavioral depression following CLON withdrawal could be reversed by chronic treatment with desipramine (DMI). Following 14 days of CLON treatment (Procedure of CLON A), CLON was withdrawn, which resulted, as expected, in behavioral depression in the swim test 7 days later. CLON-withdrawn (i.e., behaviorally depressed) animals were then divided into two groups, one treated daily with DMI (4mg/kg) and one treated with vehicle. After 8 days of DMI, CLON-withdrawn animals showed significant attenuation of behavioral depression, the Activity Scores for this group averaging only 53.5 ± 51.1 lower than the mean AS of no-drug controls, whereas CLON-withdrawn animals not given DMI showed Activity Scores averaging 171.1 ± 65.0 lower than mean of the control group. By the 36th day of DMI treatment, CLON-treated animals were virtually the same as non-treated animals (AS averaged 12.9 ± 37.4 lower than mean AS of control group) whereas the CLON-treated animals not given DMI remained depressed (AS averaged 233.7 ± 86.7 lower than mean AS of control). A third experiment replicated these findings with testing on the 8th day of DMI treatment but also showed that DMI had no significant beneficial effect on the first day of its administration.

In summary, when clonidine was administered for 14 days and then withdrawn, a long-lasting depression of active behavior in a swim test was seen. This procedure therefore generated persisting behavioral depression in the absence of any further or ongoing behavioral or pharmacological treatment. Behavioral depression produced in this manner was reversible by the tricyclic antidepressant desipramine. Whether the procedure described above produces other symptoms of depression in addition to reduction of motor activity remains to be determined.

201.8 OBLIGATORY D1-D2 RECEPTOR COACTIVATION AND THE GENERATION OF DOPAMINERGIC BEHAVIORS. A.R. Braun* and T.N. Chase. (SPON: T. Lanthorn) Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

Dopamine agonists produce a variety of behaviors in laboratory animals. We have sought to distinguish between further characterize these behaviors on a pharmacologic basis, utilizing agents which are selective for the D1 and D2 subclasses of dopamine receptors.

Two hundred thirty male Sprague-Dawley rats were utilized. Behaviors were observed for 3 to 10 hours, and were scored using a behavioral check list which allowed independent quantification of locomotion, endogenous behaviors, movements of the head, mouth and limbs. Responses to a novel object, a noxious stimulus and behavior in an open field were also quantified.

The D1 agonist SK&F 38393, administered in 6 doses from 2 to 32 mg/kg i.p., produced a dose dependent increase in grooming behavior. No stereotypic behavior was observed. The D2 agonist LY 171555 was administered in 8 doses from .05 to 24 mg/kg s.c. The lowest dose produced cataleptoid hypomotility; the higher doses produced dose dependant increases in locomotor activity, grooming, nonstereotypic sniffing and responsiveness to environmental stimuli. Stereotypies characteristic of high doses of mixed agonists such as apomorphine or amphetamine were not seen.

A dose response matrix was constructed utilizing 12 combinations of the D1 and D2 agonists. As levels of both agonists were increased, locomotor activity, other endogenous behaviors, and general sensory responsiveness decreased and all classes of well recognized stereotypies appeared. These ranged from licking, biting or gnawing syndromes seen with mid-range doses of both drugs, to more complex head and limb stereotypies and self-mutilatory behavior seen at higher abolute doses and higher ratios of D1 to D2 stimulation.

Animals in whom endogenous catecholamines had been depleted by acute pretreatment with reserpine and AMPT displayed no recognizable agonist-induced behaviors when challenged with either agonist alone. The full range of behaviors, from locomotion to complex stereotypy, could be restored in these animals, however, by concurrent administration of D1 and D2 agonists in the same doses and ratios which elicited these behaviors in intact animals. Finally, in the intact animal, all behaviors elicited by either agonist alone or in any combination were completely inhibited by either the specific D1 antagonist SCH 23390 or the specific D2 antagonist RO 22-1319.

These results suggest that all well-recognized dopamine induced behaviors depend upon concurrent activation of D1 and D2 receptors. The particular category of behavior expressed - from goal directed exploratory behaviors to fragmented stereotypies - depends upon the absolute levels of D1 and D2 activity and the ratio between these levels. D1 receptor stimulation appears to be critical in the transition from locomotion to stereotypy.

201.9 CONTINUOUS AMPHETAMINE INFUSION PRODUCES A BIPHASIC LOMOTOR AND EXPLORATION RESPONSE IN THE RAT. P.F. Gately*, D.S. Segal, and M.A. Geyer, Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA 92093.

Alzet minipumps were implanted subcutaneously into male rats under ether anesthesia, enabling the delivery of either saline or amphetamine sulfate at a rate of 0.65 mg/kg/hr. Commencing 24 hours post-implantation, and continuing on a daily basis for nine days, rats were tested in 40-minute sessions in a behavioral pattern monitor, which provided computer-generated graphic representation of the spatio-temporal patterns of locomotion, rearings, and holepokes. Animals were also systematically observed and rated for the occurrence of stereotyped behaviors. For the first four days of infusion, amphetamine-treated rats displayed highly focussed stereotypy, weight loss, and normal levels of locomotor and exploratory activity. By day six, and extending through the end of the experiement, fewer and less intense episodes of stereotypy were seen; the anorexogenic effect was lost, and progressively higher levels of locomotor and exploratory activity were evident. The biphasic locomotor and exploratory responses have previously not been reported and may be manifestations of the "end stage" behaviors associated with chronic exposures to amphetamine.

201.10 DIALYSIS PERFUSION STUDIES OF THE EFFECTS OF AMPHETAMINE ON THE STRIATUM. R.W. Keller, C.L. Hucke*, M.J. Zigmond and E.M. Stricker. Dept. of Biological Sciences and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260

Many effects of amphetamine (AMPH) have been proposed to result from its ability to release catecholamines from terminals and block their reuptake into neurons. The use of in vivo voltammetry to monitor dopaminergic activity in the rat striatum has led to confusion regarding the amount of dopamine (DA), versus other electroactive species, that actually is released after AMPH treatment. Some electrochemical studies indicate that a significant amount of DA is released, whereas other studies indicate that virtually no DA is released and the signal observed is almost solely due to ascorbic acid (AA). We have studied the source of the rise in the electrochemical signal after AMPH by examining the extracellular fluid using a hollow fiber dialysis perfusion system.

After AMPH (5 mg/kg, ip) treatment there was a 20-fold increase in extracellular DA, from 0.015 uM to 0.30 uM; however, even this elevated value was a fraction of the extracellular concentration of AA (13 uM), dihydroxyphenylacetic acid (DOPAC, 2.8 uM), homovanillic acid (HVA, 2.6 uM) and 5-hydroxyindoleacetic acid (5HIAA, 1.6 uM). Large amounts of AA (peak value of 23 uM) were released by AMPH and probably accounted for most of the electrochemical signal at carbon electrodes. The concentration of the DA metabolites, DOPAC and HVA, in the extracellular fluid decreased after AMPH administration and hence it cannot have played a role in the observed increase in electrochemical signal. Similarly, the dose of AMPH used in these studies did not produce significant changes in extracellular serotonin or its major metabolite, 5HIAA.

In contrast to the effects of systemic AMPH, the administration of AMPH via the perfusion medium produced no significant increase in AA; however, it did result in a marked increase in DA release in the striatum, whereas extracellular concentrations of DOPAC and HVA were reduced significantly. This finding indicates that AMPH can cause the release of DA without AA, and therefore suggests that AA and DA may be released from different populations of neurons when AMPH is administered systemically.

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201.11 EFFECTS OF d-AMPHETAMINE ON THE DETECTION THRESHOLD FOR BRAIN SELF-STIMULATION AND ON LOCOMOTOR ACTIVITY. Gerald J. Schaefer and Richard P. Michael. Dept. of Psychiatry, Emory Univ., School of Medicine and the Ga. Mental Health Inst., Atlanta, GA 30306

Rats were implanted with stimulating electrodes aimed at the medial forebrain bundle-lateral hypothalamus (MFB-LH), and trained in a discrete trial procedure to make a differential response (right or left lever press) in the presence or absence of brain stimulation (ICSS). For each trial, the animal was required first to press an "initiating" lever which gave either suprathreshold ICSS or no ICSS in a random sequence. When the first press of the initiating lever produced stimulation, half of the animals were required to press the right choice lever for further stimulation; when the first press did not produce stimulation, these animals were required to press the left choice lever for stimulation. The opposite conditions were in effect for the remaining animals. A wrong choice terminated the trial without brain stimulation reward. When animals reached 95% accuracy in this discrimination, testing with d-amphetamine (0.03, 0.1, 0.3, 1.0 mg/kg) and saline began. During test sessions, the initiating lever produced current intensities of from 0 to 100% of the training current. The intermediate intensities were presented in random order. During test sessions, but not in training sessions, a response on either choice lever produced the reinforcing stimulus and terminated the trial. We measured the number of trials completed on the stimulation-appropriate choice lever as a function of the current intensity on the initiating lever; 20 trials occurred at each intensity. For saline and each dose of drug, the current intensity was plotted against the number of trials completed on the stimulation appropriate lever. In addition, the time to complete the test session and the number of lever presses between trials were recorded. Three animals lowered the detection threshold as evidenced by a shift to the left in the current intensity necessary to produce 50% responding on the stimulation-appropriate lever; the two remaining animals produced opposite effects, resulting in higher detection thresholds. The overall result was not significant. However, a significant increase in both time to complete the test session ($P < 0.025$) and in inter-trial presses on the initiating lever ($P < 0.025$) occurred. These animals were also tested with d-amphetamine and saline in an Omnitech Digiscan^{R} activity monitor. A reliable increase occurred in both total horizontal ($P < 0.001$) and in locomotor activity ($P < 0.005$) scores. These data aid in differentiating the effects of d-amphetamine on motor performance from the discriminative stimulus properties of ICSS.
(Work supported by Georgia Department of Human Resources.)

BLOOD-BRAIN BARRIER I

202.1 AVENUES FOR ENTRY OF EXOGENOUS PROTEIN TO THE CNS. B.J. Balin* and R.D. Broadwell (SPON: M. Salcman). U of MD, Balt., MD 21201.

Pathways for the extracellular diffusion into the brain of peripherally administered protein tracers were investigated by light and electron microscopy in adult mice. Native horseradish peroxidase (HRP) was injected intravenously, into the cerebral ventricles, or intranasally; wheat germ agglutinin (WGA)-HRP, which binds to the cell surface, was delivered intraventricularly or intranasally. All animals were fixed by vascular perfusion 5 mins-24 hrs post-injection. Blood-borne HRP entered the brain through fenestrated capillaries in circumventricular organs. When the circulating titer of blood-borne HRP was high (5 mins), peroxidase reaction product was associated with the ventricular and pial surfaces, the Virchow-Robin space of large vessels, and with pericytes and deep microvasculature. Reaction product was seen frequently on the luminal surface and less so on the abluminal surface of cerebral capillaries. Numerous endocytic vesicles and tubules derived from the endothelial luminal surface were labeled with blood-borne HRP and directed to endosomes/dense bodies. Extravasations of HRP around arterioles/venules, but not capillaries, were scattered throughout the brain; tight junctions between the cells of these leaky vessels were open and filled with HRP reaction product, perhaps the result of the perfusion-fixation process. Similar extravasations were not evident when the titer of blood-borne peroxidase was low (30 mins-2 hrs). Intraventricular administration of HRP and WGA-HRP revealed that the endocytic activity of the abluminal surface of the cerebral endothelium was virtually non-existent. This observation indicates that: a polarity exists regarding the internalization of the endothelial plasmalemma from the luminal vs. abluminal side; bidirectional vesicular transport across the cell does not occur; and HRP is not toxic to and does not stimulate endocytosis in the endothelium. When HRP and WGA-HRP were delivered intranasally, only native HRP diffused extracellularly to the pial surface of the olfactory bulbs (45 mins). The results suggest that pathways for the extracellular diffusion of large molecular weight substances into the brain exist from the blood and nose. Blood-borne HRP enters the cerebral ventricles through circumventricular organs, distributes in the CSF over the pial surface of the brain, and enters the perivascular clefts of large penetrating vessels for diffusion to pericytes and deep microvasculature. HRP delivered intranasally diffuses into the brain, presumably between nasal epithelial cells. Patent avenues through the circumventricular organs and the nasal epithelium offer potential routes for the introduction of blood-borne and air-borne toxic, carcinogenic and infectious agents to the CNS.
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202.2 VASCULARIZATION OF BRAIN TRANSPLANTED INTO THE THIRD CEREBRAL VENTRICLE. R.D. Broadwell, H.M. Charlton*, and W.F. Ganong. Univ. MD, Balto., MD 21201, Univ. Oxford, Oxford, England, Univ. CA, San Fran., CA 94143.

The potential for fenestrated capillaries of the median eminence in the hypothalamus to invade grafts containing neurosecretory cells from areas in the CNS devoid of such vessels was investigated by light/electron microscopy. Grafts of the medial preoptic area harboring neurons that secrete gonadotrophic hormone releasing hormone (GnRH) and of the arcuate nucleus from late fetal/early neonatal AKR mice were transplanted into the third cerebral ventricle of adult female, hypogonadal(HPG) mice incapable of synthesizing GnRH. The grafts were allowed to develop within the host brains for 1 day - 3 mos. Vasculature of the host and grafted brain parenchyma was analyzed with diaminobenzidine cytochemistry in two ways: (1) areas possessing "leaky" vessels were identified with HRP injected intravenously 2-5 mins prior to perfusion-fixation; (2) the pattern of vascularization was outlined in sections of brains fixed by immersion with incubation of the sections for endogenous HRP activity in red blood cells retained in the vessels. Fenestrated capillaries within the median eminence of HPG mice not receiving grafts were restricted largely to the outer plexiform layer with loops extending into the subependymal zone. Grafts delivered into the brains of HPG mice frequently filled the third ventricle. The graft parenchyma and ependymal layer of the host median eminence at one month were well-demarcated and organized; however, at 3 mos. the ependymal layer appeared in disarray with neuronal processes and glial cells from the graft invading the median eminence proper. Vasculature linking the host brain and graft was identified at the dorsal, lateral, and ventral margins of the graft. Occasionally, a vessel linking the graft and median eminence was continuous with that in the host arcuate nucleus. Blood-borne HRP leaked from fenestrated vessels in the host median eminence and diffused into the graft; no leak of HRP was discerned from vasculature supplying the graft. Fenestrated endothelial cells, readily identified within the median eminence, were not observed in the graft. Contiguous endothelial cells within the graft exhibited tight junctions typical of those that contribute to the formation of a blood-brain barrier (BBB); the host median eminence contained similar endothelial cells, perhaps derived from the graft. The results suggest that fetal/neonatal CNS tissue destined to develop and possess a BBB does so when grafted into the third ventricle and placed in contact with the median eminence. If fenestrated capillaries of the host median eminence contribute to the vascularization of grafted tissue, this contribution may be minimal.
NINCDS Grant #NS18030.

202.3 NORADRENERGIC INNERVATION OF BRAIN MICROVESSELS FROM THE NUCLEUS LOCUS CERULEUS MODULATES THE TRANSPORT OF SODIUM AND POTASSIUM ACROSS THE BLOOD-BRAIN BARRIER. S.I. Harik. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

The functions of the putative noradrenergic innervation of brain microvessels from the nucleus locus ceruleus (LC) remain controversial. We recently presented evidence that the LC is important for preserving the integrity of the blood-brain barrier (BBB) to macromolecules during hypertension and seizures (Harik & McGunigal, Ann. Neurol., 15:568, 1984). We now present further evidence suggesting that this noradrenergic innervation regulates Na^+ and K^+ transport across the BBB. Since brain microvessels possess specific ouabain binding sites (Harik et al., J. Cereb. Blood Flow Metabol., 5:156, 1985) that probably represent molecules of Na^+,K^+-ATPase, a major transport system of the BBB, we measured specific ouabain binding to microvessels isolated from the cerebral cortex, ipsilateral and contralateral to unilateral LC lesion.

Male Wistar rats (200-250 g) had unilateral LC lesion by the stereotaxic injection of 6-hydroxydopamine or sham operation. Two weeks later, the rats were decapitated and the cerebral mantles dissected and used to obtain cerebral microvessels. Samples from each cerebral cortex were taken for norepinephrine (NE) assay, to assess the efficacy of LC lesion, and for ouabain binding assays to membranes of the whole cerebral cortex. Microvessels from 4-10 ipsilateral or contralateral cerebral cortices were pooled. Particulate fractions of brain microvessels and of cerebral cortex were assayed for specific [^{3}H]ouabain binding as described by Harik et al. (J. Cereb. Blood Flow Metabol. 5:156, 1985).

LC lesion depleted NE by about 90% in the ipsilateral cortex without affecting the contralateral cortex. Specific ouabain binding to membranes of cerebral cortex and cerebral microvessels was saturable. Maximal binding was reduced by about 40% in microvessels of the ipsilateral, NE-depleted, cortex (8.9 $\pm$ 1.1 and 5.5 $\pm$ 0.9 pmol/mg protein in contralateral and ipsilateral microvessels, $p < 0.01$, paired Student t-test, 2-tailed). Ouabain binding to preparations of the whole cerebral cortex was not affected by LC lesion. Also, LC lesion did not affect the dissociation constants of ouabain binding in any of the preparations.

These results indicate that LC lesion decreases the density of ouabain binding sites in brain microvessels although it has no effect on the density of ouabain binding sites in the NE-depleted cortex. This suggests that intrinsic NE innervation of cerebral microvessels modulates their density of Na^+,K^+-ATPase and, consequently, the transport of Na^+ and K^+ across the BBB. The data provide further evidence for the functional importance of the neural control of the cerebral circulation.

202.4 ADENOSINE TRANSPORT IN CEREBRAL MICROVESSELS AND CHOROID PLEXUS. Rajesh N. Kalaria and Sami I. Harik. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

Evidence derived from in vivo and in vitro experiments shows that adenosine and other nucleosides are transported across the blood-brain barrier (BBB) via a saturable carrier-mediated mechanism. Adenosine also appears to modulate neural and cerebrovascular functions by interacting with specific receptors. This action of adenosine may be terminated by a rapid uptake or transport process in cell membranes. Nitrobenzylthioinosine (NBI) has been used as a probe to investigate the adenosine transporter of erythrocytes and neural tissue. In this study, we used [^{3}H]NBI as a ligand to investigate the adenosine transporter of isolated cerebral microvessels and the choroid plexus of the rat and pig.

Rat and pig cerebral microvessels were obtained by bulk isolation as described previously (Harik et al., J. Cereb. Blood Flow Metabol. 5:156, 1985). Microvessels, choroid plexus and cerebral cortical samples were homogenized using a polytron and their particulate fractions washed and resuspended in 50 mM Tris buffer, pH 7.4. Total [^{3}H]NBI binding was performed by incubation at 22^{o}C for 30 min. Nonspecific binding was determined in the presence of 10 uM of nitrobenzylthioguanosine (NBG). Specific binding was estimated by substracting nonspecific binding from total binding.

Our results indicate that specific [^{3}H]NBI binding to the various preparations was saturable. Scatchard analysis showed a single class of binding sites (Kd about 0.5 nM). Maximal binding density (Bmax) was up to 6-fold higher in microvessels and choroid plexus than in particulate fractions of the cerebral cortex. The results suggest that adenosine transporters of the BBB are important for the supply of adenosine, not only to the endothelial cells of cerebral capillaries but to the larger mass of surrounding brain. There were no diffrences in [^{3}H]NBI binding between brain microvessels obtained from perfused and unperfused rats, thus ruling out the possibility that erythrocytes trapped within microvessels contribute to the [^{3}H]NBI binding. In further experiments, we studied the ability of unlabeled adenosine, other nucleosides and adenosine analogues, to displace specific [^{3}H]NBI binding to microvessels. NBI, NBG and dipyridamole were most effective in displacing [^{3}H]NBI from specific binding sites (IC_{50} about 1 nM). In contrast, adenosine, uridine, inosine, papaverine and cyclohexyladenosine were 4 to 5 orders of magnitude less active in displacing [^{3}H]NBI from its specific binding sites. Our results indicate the existence of a high density of adenosine transporters in brain microvessels and choroid plexus.

202.5 BLOOD-BRAIN BARRIER PROTEIN PHOSPHORYLATION AND DEPHOSPHORYLATION. W.M. Pardridge, J. Yang*, and J. Eisenberg*. Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Capillaries in vertebrate brain have unique permeability properties which make up the blood-brain barrier (BBB). Although it is known that capillaries are innervated by both aminergic and peptidergic nerve endings of intracerebral origin and that brain capillary function is likely acutely regulated by neuronal inputs, the possible mechanisms of neuronal regulation of capillary function are at present unknown. One possible mode of regulation is via the phosphorylation of brain capillary proteins. The present studies characterize, for the first time, the major phosphoproteins in the bovine brain capillary using both intact bovine cortical brain capillaries and plasma membrane fractions from bovine brain capillaries. Phosphorylation studies using ^{32}P-γ-ATP labeling of intact capillaries is possible since brain microvessels obtained with a mechanical homogenization technique are permeable to small molecules. The patterns of endogenous phosphorylation of capillary proteins are compared to similar patterns obtained with synaptosomal (P_2) fractions from bovine brain cortex. Based on measurements of γ-glutamyl transpeptidase activity, a BBB-specific enzyme, the contamination of P_2 proteins by BBB proteins was only 2.4%. The major findings of this study are: (a) the activity of protein phosphorylation in brain capillaries is localized almost exclusively to the capillary plasma membrane, and is nearly comparable to the activity of protein phosphorylation in synaptosomal membranes; (b) a major phosphoprotein doublet in the capillary fraction comigrates on an SDS gel with a major phosphoprotein doublet of approximate molecular weight of 80K in the synaptomal fraction, and the latter is presumed to be synapsin I; in dephosphorylation assays the synaptosomal 80K phosphoprotein doublet is not subject to measurable dephosphorylation whereas the capillary 80K doublet is subject to rapid dephosphorylation, and is essentially completely dephosphorylated within 5 seconds at 0^{o}C; (c) a prominent triplet of phosphoproteins with molecular weight of 50K-55K is present in the capillary fraction, and is not present in the synaptosomal fraction; thus, this 50K-55K triplet of phosphoproteins appears specific for brain capillaries. In summary, these studies provide the basis for future investigations of a protein phosphorylation paradigm in regard to the rapid control of brain capillary function by brain.

202.6 ISCHEMIC BRAIN EDEMA AND ALTERED BLOOD-BRAIN BARRIER FOLLOWING PHOTOCHEMICALLY INDUCED CORTICAL INFARCTION . R. Busto*, W. D. Dietrich, M. D. Ginsberg and B. D. Watson* (Spon: M. Rosenthal). Cerebral Vascular Disease Research Center, Dept. of Neurology, Univ. Miami School of Medicine, Miami, FL 33101

Brain edema is known to play an important role in the pathophysiology of stroke. In this study, we monitored the early development of ischemic brain edema following photochemically induce cortical infarction in the rat. This stroke model results in small vessel occlusion initiated by severe platelet aggregation. Alterations in brain water content were correlated with blood-brain barrier alterations to serum proteins as assessed with Evans blue . Cortical infarction was induced by irradiating the intact skull for 20 minutes with green light following the systemic injection of Rose Bengal.

As early as 30 minutes following irradiation, water content in the irradiated zone increased from control levels of 79.6% to 80.4%. By 8 hours, water content was significantly elevated within the irradiated zone (88.5%), and in ipsilateral cortical regions not destined to undergo irreversible cell damage (81.0%). Significant increases in water content were not demonstrated within the contralateral hemisphere. Widespread cortical permeation of Evans blue with prominent pial-surface staining was observed at 30 minutes and 8 hours when Evans blue was given immediately post-irradiation. In contrast, when Evans blue was infused 30 minutes prior to sacrifice, peri-infarct permeation was slight at 8 hours. These findings suggest early vasogenic edema in this experimental model of cortical infarction. Cerebral blood flow studies in this model have indicated that the central ischemic zone continues to increase in size after the period of irradiation has ceased. It is therefore hypothesized that this increase is due to progressive microvascular compression secondary to brain edema. Ultrastructural investigations are needed to answer this question.

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202.7 DIETHYLDITHIOCARBAMIC ACID FACILITATES ENTRY OF CADMIUM INTO BRAIN BUT PREVENTS CADMIUM-INDUCED NEUROTOXICITY. J.P. O'Callaghan and D.B. Miller. Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 and Northrop Services, Inc., Research Triangle Park, NC 27709.

Although cadmium is toxic to the central nervous system (CNS) of the developing rat, the adult CNS is not affected, presumably because cadmium does not cross the blood-brain barrier. Substituted dithiocarbamates have metal-binding properties that have proven effective in the treatment of cadmium intoxication. These compounds form a lipophilic complex with cadmium that readily penetrates the blood-brain barrier, resulting in elevated cadmium concentration in the brain, which might be expected to increase the potential for neurotoxicity. To investigate this possibility we assessed the effects of diethyldithiocarbamic acid (DDC) on cadmium-induced neurotoxicity in the developing and adult rat. Indices of neurotoxicity included: brain weight and histology, and radioimmunoassays of the neuron-specific phosphoprotein, synapsin I and the astrocyte-specific protein, glial fibrillary acidic protein (GFAP). Distribution of cadmium to brain, liver, kidney and teste also was determined.

Cadmium (2.75-3.75 mg/kg,s.c.) alone or in combination with DDC (100 mg/kg,i.p.) was administered to Long-Evans rats on postnatal day (PND) 5. All measurements were obtained on PND 22. Deposition of cadmium was reduced in liver and elevated in brain and kidney as a result of co-administration of DDC. Cadmium administration alone caused large dose-related decreases in brain weight. DDC did not affect brain weight and attenuated the cadmium-induced decreases in brain weight. Histological examination of the brain revealed necrosis of the neostriatum and overlying cortex following exposure to 3.0-3.75 mg/kg cadmium; these effects were blocked by co-administration of DDC. Synapsin I was decreased 39% and GFAP was increased 29% in neostriata obtained from rats that received 2.75 mg/kg of cadmium, a dosage that did not result in overt cytopathology. When expressed as per mg neostriatal protein, synapsin I was decreased by 19% and GFAP was increased by 80%. These effects, which are indicative of cadmium-induced loss of neurons and reactive gliosis, were blocked by concommitant administration of DDC.

Cadmium (1.0 mg/kg/day, i.p. x 4 days) alone or in combination with DDC (500 mg/kg/day, s.c. x 4 days) also was administered to adult Long-Evans rats. Co-administration of DDC caused a significant accumulation of cadmium in brain which persisted for at least 2 weeks. Measurements of brain weight and regional amounts of synapsin I and GFAP did not show any significant changes as result of any of the treatments. Collectively, these data suggest that the increased brain concentration of cadmium resulting from co-administration of DDC does not increase the potential for cadmium-induced neurotoxicity.

202.8 PHARMACOLOGICAL CHARACTERIZATION OF ADRENERGIC INFLUENCE ON BRAIN EXTRACELLULAR SPACE. T.A. Kent*, A. Oke, B. Moghaddam and R.N. Adams. Dept. of Neurology, Univ. of Texas Medical Branch, Galveston, TX and Dept. of Chemistry, Univ. of Kansas, Lawrence, KS.

Local brain neuronal activity alters the extracellular space (ECS) volume in the brain, an effect thought to be due to changes in membrane ion permeability and resultant osmotic shifts. We have been interested in remote neuronal influences on ECS. Our studies have focused on the possible role of the central noradrenergic system, because of reports that this biogenic amine innervates the cerebral microvasculature. We have previously found that increases in inspired CO_2--a stimulus to the firing rate of the main noradrenergic cell body, the locus coeruleus--increase ECS volume in the rat thalamus as measured by ion-selective microelectrodees sensitive to the ECS marker, alpha-naphthalene sulfonate (ANS). This effect of CO_2 was potentiated by the amine reuptake inhibitor, amitriptyline, and reversibly attenuated by injection of lidocaine into the region of the locus coeruleus (Kent et al. Brain Res., in press).

In this study, we investigated whether adrenergic receptors mediate this effect. Rodents were anesthetized (chloral hydrate, 400 mg/kg i.p.), intubated, and passively ventilated with N_2 (55-70%), O_2 (30%) and CO_2 (0-15%). A double-barrel micropipette was implanted into the thalamus. One side was filled with 0.9% NaCl and used to follow field potential changes. The pipette used to follow ANS concentration changes was filled with a PVC-based ion exchanger mixture containing Aliquat (Aldrich)/Na-ANS as active material. Previous studies have demonstrated that the electrode is not sensitive to changes in chloride, glutamate, ascorbate, or pH. A pipette filled with 0.01 M ANS was placed 1-3 mm away and ANS was injected via a nanoliter pump. We confirmed that CO_2 produced a decrease in ANS concentration, indicating a 15-20% increase in ECS volume. Prazosin, an alpha-1 adrenergic antagonist (0.05-1 mg/kg i.v.) reduced the effect of CO_2 by 60-70%. Propranolol, a beta-adrenergic antagonist, had no effect in doses from 0.005 mg/kg i.v. to 6 mg/kg i.p. Yohimbine, an alpha-2 adrenergic antagonist with some alpha-1 effects, only minimally reduced (20%) the effect of CO_2 in doses from 1-2 mg/kg i.v.

These results indicate that the effect of CO_2 on increasing ECS volume is mediated primarily by alpha-1 adrenergic receptors. These receptors may be located on cerebral vessels, or other structures, such as glia, which may influence the local concentration of compounds in the brain.

202.9 STRUCTURAL ENDOTHELIAL CHANGES THAT UNDERLIE THE MATURATION OF THE BLOOD-BRAIN BARRIER. P.A. Stewart, E.M. Hayakawa* and K. Hayakawa* Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The blood-brain barrier (bbb) is a selectively permeable interface between blood and brain that embodies both structural and functional specializations. The formation of a structurally "tight" layer of endothelium, that is essential for maintaining concentration gradients across the bbb, develops in tandem with the functional specializations. In highly permeable (non-barrier) vessels, blood-borne molecules cross the capillary wall via 3 routes: a) between adjacent endothelial cells, b) through tubulovesicular channels, or chains of vesicles in the endothelial cytoplasm, and c) through fenestrae, when these are present. In mature bbb vessels continuous bands of tight junctions seal the paracellular route, few vesicles exist and there is a virtual absence of fenestrae.

The non-specific permeability of brain capillaries is high in embryonic life, but drops towards adult levels around the time of birth in most species. We have investigated the structural basis of this developmental "tightening" in the mouse by quantitating the density of endothelial vesicular structures, and the number of gaps within the junctional areas. Recent evidence indicates that gaps, or spaces, seen between successive tight junctional strands are profiles of anastomosing channels through the junctional regions.

Cerebral hemisphere and cerebellum taken from perfusion-fixed mice were processed for routine electron microscopy. We examined vessels in brain at various ages from fetal stages to two weeks after birth. Vessels sectioned transversely were photographed and the negatives projected onto a digitized bit pad for computer-assisted image analysis.

We found that fenestrae are absent in fetal as well as mature bbb vessels. Vesicular density dropped only slightly during development. The incidence of enlarged junctional clefts, a frequent finding in fetal endothelium decreased dramatically by two weeks after birth. Changes in the cerebellum occurred slightly later than those in the cerebrum. These results indicate that a major factor in the developmental "tightening" of the bbb is a change in the ultrastructure of the junctional areas.

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202.10 **NEOSYNEPHRINE INDUCED CHANGES IN CEREBRAL BLOOD FLOW IN FREEZE LESIONED RATS.** W.D.Johnson*, D.Dow-Edwards, T.H.Milhorat, (SPON: M.Friedlander). Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Downstate Medical Center, Brooklyn, NY 11203.

The freeze lesion is a time-honored model of vasogenic cerebral edema first introduced by Klatzo who demonstrated the qualitative relationship between hypertension and the extent of edema spread (Klatzo et al., Brain Edema, p. 554, 1967). Blasberg et al. reported a profound depression in local cerebral blood flow (LCBF) acutely in freeze lesioned areas (Adv. Neurol., Vol. 28:255, 1980). In order to determine the effects of increased systemic blood pressure on LCBF in the traumatized brain, we applied the ^{14}C-antipyrine method (Sakurada et al., Am. J. Physiology 234(1):H59-H66, 1978) to four groups of animals: 1) normotensive control, 2) hypertensive control, 3) normotensive freeze lesion, 4) hypertensive freeze lesion.

Sprague-Dawley rats (150-225g) of either sex were anesthetized with halothane/O_2/N_2O mixture and subjected to either freeze lesion by application of a metal rod at -75°C to the exposed cranium midway between the coronal and lambdoid sutures, just to the right of midline for one minute, or to a sham operation. A solution of 2% Evans Blue (1.0cc/kg) was injected intravenously as a marker of blood-brain barrier breakdown. Thirty minutes following operation, either 0.1% Neosynephrine or normal saline was infused for sixty minutes; the Neosynephrine was titrated to maintain a mean blood pressure 20-30% above baseline. LCBF was determined during the last minute of drug or saline infusion.

The current study demonstrates that moderate hypertension increases both the amount of vasogenic cerebral edema and its rate of spread which was significantly reduced in the Neosynephrine treated animals compared to controls. The decrease in LCBF appeared to be congruent with the field of edema.

202.11 PERFORATION OF CNS BASAL LAMINA AND MARGINAL GLIA BY EMBRYONIC LEPTOMENINGEAL CAPILLARIES: EM STUDY. M. Marin-Padilla. Department Pathology, Dartmouth Medical School, Hanover, N.H. 03755.

At first, the embryonic CNS is deprived of its own vasculature. It is surrounded by a prominent perineural vascular plexus, from which its vasculature will progressively evolve. Leptomeningeal capillaries start to perforate the CNS basal lamina and marginal glia of the cerebral cortex, around the 9th - 10th day gestation in the hamster. The mechanism of the vascular perforation of the CNS by leptomeningeal capillaries remain poorly understood. Transmission electron microscopic preparations of the developing cerebral cortex of 9,10 and 11 day hamster embryos were made. Each preparation represents a perpendicular section including the leptomeninx primitiva and the upper portion of the cerebral cortex. Perforating leptomeningeal capillaries showing different degrees of penetration were identified in semi-thin sections and ultra-thin sections of them were subsequently prepared. Four distinct phases were identified in the vascular perforation of the CNS. First. The leptomeningeal capillary aproaches and establishes direct contact with the CNS surface. Its endothelium becomes aligned parallel to the marginal glia and contacts between the vascular and the CNS basal laminae are established. Second. Endothelial cell filopodia from this glia-touching capillary perforate both the vascular and the CNS basal laminae and penetrate into the neural tissue through an opening formed between two contiguous marginal glial endfeet. The glial endfeet facing the perforating filopodium undergo hydropic changes and focal disintegration. Third. The original opening enlarges thus allowing the entire endothelial cell (or cells) to penetrate into the neural tissue. The glial endfeet in front of the penetrating endothelium undergo disintegration and fragmentation. Fourth. Proliferation of the penetrating endothelial cells and their subsequent canalization result in the in situ formation of a new cortical capillary bed. New glial processes, which seem to replace degenerating ones, start to surround the newly formed cortical capillary establishing a new vascular-glial barrier around it. Also, a shallow pial-funnel can be recognized at this time around the entrance of the capillary. This represents an early embryonic stage in the formation of the Virchow-Robin space. Each phase of the active vascular perforation of the embryonic mammalian cerebral cortex will be illustrated and discussed.

202.12 REGIONAL CEREBRAL GLUCOSE UTILIZATION DURING INSULIN INDUCED HYPOGLYCEMIA IN UNANESTHETIZED RATS. R M Bryan, K A Keefer*, and C MacNeill* Depts. of Surgery (Neurosurgery) and Physiology, M S Hershey Medical Center, Hershey, PA 17033.

Regional cerebral glucose utilization (rCMRgl) was measured during insulin induced hypoglycemia in unanesthetized rats in order to answer the following questions: (1) Can some brain regions extract glucose from the blood more effectively than other brain regions and thus tolerate more severe hypoglycemia without compromising glucose utilization? (2) Can the existing models for glucose transport from blood to brain predict when the supply of glucose becomes limiting during progressive hypoglycemia causing rCMRgl to decrease? Rats were anesthetized with halothane (1.5%) and nitrous oxide (70%) in a balance of O_2 and surgically prepared with catheters in the left femoral artery and vein. A plaster restraining girdle was fitted around the lower quarter of each rat and anesthesia was discontinued. Each rat was allowed 5 hours to recover from the anesthesia before rCMRgl was measured. The rats were divided into 3 groups consisting of: (1) a normoglycemic group which received only saline, (2) hypoglycemia A which was given 15 units of insulin IV per kg body weight 30 min before rCMRgl was measured, and (3) hypoglycemia B which was given 15 units of insulin IV per kg body weight 2 hours before rCMRgl was measured and 5 additional units per kg body weight 1 hour before rCMRgl was measured. rCMRgl was measured using [6-^{14}C]glucose. Mean arterial blood pressure and PaO_2 were not different in the three groups, while $PaCO_2$ and pHa decreased in the hypoglycemic group B. Plasma glucose was 7.03 um/ml in the normoglycemic group, 1.96 um/ml in the hypoglycemic group A, and 1.40 um/ml in the hypoglycemic group B. rCMRgl in the hypoglycemic group A decreased 8-12% in seventeen brain regions measured of which 9 were statistically significant. rCMRgl in the hypoglycemic group B decreased significantly in all of the brain regions measured. The decrease ranged from a 15 % decrease in the pyramidal tract to 36% in the motor and auditory cortices. rCMRgl in every brain region began to decrease when plasma glucose fell below 1.5-2.5 um/ml demonstrating all brain regions were affected by glucose limitation at about the same plasma glucose concentration. Thus no brain region was more effective than other brain regions in maintaining rCMRgl in the face of decreasing plasma glucose. Theoretical models of glucose influx (Hawkins et al., J. Neurochem. 40:1013, 1983; LaManna and Harik Br. Res. 326:299, 1985) accurately predicted the plasma glucose concentration below which rCMRgl was limited by the supply of glucose and the degree that rCMRgl decreased for a given plasma glucose. (AHA Grant-in-Aid with funds contributed from the Palm Beach County Chapter, FL and PHS Grant NS19341)

STRUCTURE AND FUNCTION: CORTICAL AND SUBCORTICAL ORGANIZATION III

203.1 "COUNTER-CURRENT FLOW" OF CORTICO-CORTICAL INFORMATION PROCESSING THROUGH THE LAMINAR SEGREGATION OF RECIPROCAL CONNECTIONS. T. W. Deacon. Biological Anthropology, Harvard University, Cambridge MA 02138. (SPON: W. B. Forbes)

A variety of hydrodynamic exchange systems found in organisms (e.g. gills) and man-made devices (e.g. heat-exchangers) employ a pattern of "counter-current flow" to maintain an optimal exchange gradient between two fluid media. It is argued that an analogous principle may be employed for information processing in both sensory and motor cortico-cortical circuits.

HRP-WGA tracer experiments were employed to investigate laminar patterns of cortico-cortical connections in macaque brains. Reciprocal connections between neocortical areas in all regions of cortex analysed exhibited similar, distinctly asymmetric laminar patterns of origins and terminations, previously described by other investigators for different cortical regions. The two major patterns can be described as "upstream" and "downstream" projections respectively, where "downstream" characterizes the pattern exhibited by major sensory input and motor output directions. "Downstream" projections are typified by connections originating from pyramidal cells in deep supragranular layers (especially IIIc) and terminating in granular (IV) and deep supragranular layers (IIIc; sensory-prefrontal projections also include a projection to layer II). The reciprocal projections constituting the "upstream" flow are typified by connections which originate from cells predominantly in infragranular layers as well as some in middle supragranular layers (more prominant in eulaminate areas) and terminate predominantly in superficial layer I and infragranular layers, but not in granular layers. In general, posterior limbic cortex is downstream from eulaminate sensory cortex, is downstream from koniocortex; motor cortex is downstream from prefrontal cortex, is downstream from anterior limbic cortex; and finally prefrontal-motor areas are downstream from sensory areas. Upstream projections follow the reverse pattern.

Although the simple analogy must be modified to incorporate variations in quantities of connections, lamination, cross-modal relations, as well as noncortical connections at each stage, these do not obscure the prominence of these two major countervailing directions of information flow which are superimposed in neocortex. The countercurrent analogy suggests that each cortical area embodies a particular intermediate "gradient" of information transfer at an interface between neural pathways carrying information in opposite directions, from opposite origins: endogenous limbic activity at one extreme and extrinsically patterned and constrained sensory or motor information at the other. Increasing the number of intermediate stages decreases the "gradient" of difference at each stage.

203.2 THE ORGANIZATION OF INTRACORTICAL NEURONS WITHIN THE PRE- AND POSTSUBICULUM. J.M. Wyss, B. Sripanidkulchai and K. Sripanidkulchai. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, AL 35294.

In past studies, the organization of diencephalic and entorhinal projections from the subiculum cortex have been examined. To extend these findings, the present study investigated the laminar organization of neuronal cell bodies within the pre-and postsubicular cortices in relation to their cortical and/or thalamic projections. In each of 38 rats, a single 10-50nl injection of 4% fast blue (FB) was placed into a small region (~ 0.5mm^3) of the retrosplenial cortex, or the anterior thalamic nuclei. Following appropriate survival time the animals were perfused, and the brains were removed, sectioned and inspected using a Leitz A filter system.

The results demonstrate that the pre- and postsubicular neurons projecting to the posterior cingulate cortex, have cell bodies which reside in the deep portion of layers V and VI. The layer VIb neurons are continuous with the associational projecting neurons located in layer VIb of posterior cingulate cortex, and the cell bodies of layer VIb neurons in both regions are morphologically similar. The layer Vb cell bodies which have associational projections lie in a thin, AChE rich strip of cortex which has the same position and staining pattern as deep layer V of the posterior cingulate cortex. Again these neurons are morphologically similar to those in layer Vb of the posterior cingulate cortex; however, both the layer V and VI associational cell bodies are smaller than their entorhinal counterparts. Further experiments demonstrated that the neurons which divide layer Vb from VIb, project to the anterior thalamic nuclei. A few layer VIb, pre- and postsubicular neurons were labeled by thalamic injections, but double labeling studies demonstrate no collateralization between the thalamic and cortically projecting populations.

Injections of the pre-and postsubicular cortices demonstrate that the homotopic projections arise from neuronal cell bodies in layer II, III, V and VIb of the ipsilateral cortex. The homotypic projections arise in the contralateral layer III and to a lesser extent in layer II and V neurons.

Thus, the cell bodies of the pre- and postsubicular cortices are laminated in a fashion quite similar to that seen in the posterior cingulate cortex.

203.3 LAYER VI OF CINGULATE CORTEX: ASSOCIATIONAL PROJECTIONS. K. Sripanidkulchai and J.M. Wyss. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, AL 35294.

Layer VI of the cortical mantle has usually been considered to be populated by neuronal cell bodies with axons that project to the thalamic nuclei. Catsman-Berrevoets and Kuypers (Brain Res 154:359-365 [1978]) suggested that these cells could be classified as superficial neurons that project to the corresponding principal thalamic nuclei and deeper neurons that project to non specific thalamic nuclei. In the present study, we have examined both the thalamic and cortical projections of the layer VI neurons of the rat cingulate cortex using the fluorescence dye, retrograde labeling technique. Each of thirty albino rats received an injection of 10-50nl of 2-4% fast blue (FB) via a 500nl Hamilton syringe placed into a restricted region (approximately 0.5mm effective injection site) of the posterior cingulate cortex. In 15 of these rats 2-5 days after the FB injection, a 10-50nl injection of 2-4% nuclear yellow (NY) was placed either into a second region of the ipsilateral or contralateral posterior cingulate cortex, or into the anterior thalamic nuclei. All rats were sacrified 4-7 days after the FB injection; the fixed brains were sectioned at 30μ, and these sections were inspected under fluorescent illumination (Leitz A cube). Alternate sections were stained with methylene blue (1 in 3 series) and thiocholine (1 in 3 series for ACHE).

Injections into the retrosplenial cortex resulted in the labeling of a large number of neuronal cell bodies in layers II, III, V and VIb of the ipsilateral homotopic cortex near (within 2mm) the injection site. In regions of the posterior cingulate cortex more distant from the injection site, a considerable number of neuronal cell bodies in deep layers V and VI were labeled, but few cell bodies were labeled in layers II, III and superficial V. Within the anterior cingulate cortex deep layer V and VI neurons were labeled continuously to the rostral pole of the cortex. Very seldom was a layer II, III or superficial layer V or VI neuron labeled in the anterior cingulate cortex following a posterior cingulate cortex injection. The double labeling studies demonstrate that very few of the deep layer V and VI neurons innervate the contralateral, homotopic cortex. Further, the thalamic injections indicate that the posterior cingulate cortico-thalamic neurons are located primarily in the superficial part of layer VI. The few thalamic projecting cell bodies that are located in deep layer VI do not appear to have associational projections to the homotopic cortex.

203.4 TEMPOROPOLAR CORTEX OF THE MACAQUE: M.A. Moran,* E.J. Mufson, M-M. Mesulam, Harvard Med. Sch., Boston, MA.

The temporopolar cortex (TP) is one of the major paralimbic (mesocortical) components of the primate brain. The agranular-periallocortical sector of TP is directly contiguous with the temporal limb of prepiriform olfactory cortex (POC). This periallocortical core of TP is surrounded by a dysgranular zone which is, in turn, flanked by granular cortex. Dorsally, the cortical transition in TP proceeds from the POC towards the auditory association cortex of the anterior superior temporal gyrus. Ventrally, a similar transition proceeds towards the granular cortex of inferotemporal visual association areas. The two lines of differentiation converge within the multimodal association cortex of the superior temporal sulcus. The present study was undertaken to determine some cortico-cortical connections of TP cortex in the rhesus monkey.

Injections of HRP in subsectors of TP resulted in neuronal labeling within the amygdala, the uncal hippocampus, POC, anterior cingular cortex (including the parolfactory region FL), the caudal orbitofrontal and anterior insular regions, prorhinal cortex, entorhinal cortex, parahippocampal areas TF-TH, the granular isocortex of rostral orbitofrontal cortex (areas 10, 14, 12 and 13), the banks of the superior temporal sulcus, the auditory association area TA and the visual association area TE. It is important to note that TP is one of the very few cortical areas to have direct connectivity with the hippocampus, with area FL and with the entorhinal region.

Individual sectors of TP showed differences in their connectivity. For example, the nonisocortical parts of TP (the agranular and dysgranular sectors) received the majority of their inputs from limbic areas and from the nonisocortical sectors of paralimbic regions. In contrast, the granular part of TP received much less input from entorhinal cortex, the amygdala, POC and the hippocampus. The dorsal part of TP receives most of its modality-specific input from auditory association cortex whereas an analogous visual input dominates the modality-specific afferents of the ventral TP. Most of the inputs into TP from nonisocortical areas tended to come from infragranular layers whereas those from isocortical cortex tended to originate mostly within supragranular layers.

These observations, together with those previously obtained in the insula, show that the connectivity of subsectors within paralimbic zones is organized along cytoarchitectonic lines of demarcation. Furthermore, area TP, together with other components of the paralimbic brain, provides a major site for spatially organized sensory limbic interactions. For example, olfactory-visual and olfactory-auditory interactions occur in medial TP. Furthermore, dorsal TP provides a site for auditory-limbic intractions whereas analogous visuo-limbic interactions occur in ventral TP. Supported in part by NS 20285, NS 09211, AG 05134, the ADRDA, the McKnight Foundation, IPB 8402034 and Fogarty Fellowship FO5 TW03624.

203.5 CORTICO-CORTICO AND LIMBIC-CORTICO PROJECTIONS TO RODENT PREFRONTAL CORTEX. B.L. Wilhite* and T.J. Teyler, Northeastern Ohio Universities College of Medicine, Neurobiology Dept., Rootstown, Ohio 44272.

The afferent projections to the rodent prefrontal cortex were examined using the horseradish proxidase (Sigma VI) localization technique in an attempt to elucidate possible cortico-hippocampal pathways. Injection sites were localized to the Prc_m (prefrontal) and/or adjacent Prc^I (premotor) areas of Zilles et al. (Anat. Embryol. 159:335, 1980). Neither the pipette nor the reaction product involved the medially adjacent cingulate cortex, or the underlying white matter (corpus callosum). The tissue was processed by the TMB procedure.

Analysis of the tissues revealed two previously uncharacterized projections to prefrontal cortex. A sparce projection from the hippocampus was discovered and although these cells were primarily ipsilateral to the injection sites, a few contralateral cells were filled as well. Label was principally limited to non-pyramidal cells, although in some instances pyramidal cells of CA1 were filled. The cells appeared most generally in the stratum oriens and were found throughout the septo-temporal extent of the hippocampus.

The other, and much more predominant projection, was from the somatosensory cortex (Sm 1 of Zilles). The labelled cortical cells which projected to prefrontal cortex were found primarily in the middle layers of the neocortex and appear to be layer III pyramidal cells. The pattern of labeled cells exibited a columnar organization, and in three-dimensional perspective, appear as linear bands within the confines of the somatosensory cortex. A small number of cells were also indentified in temporal and entorhinal corticies but appeared to be few and random in their arrangement. Previously documented projections to prefrontal cortex from medial dorsal thalamus and perirhinal cortex were also reconfirmed.

203.6 INTERHEMISPHERIC CONNECTIONS OF THE NEOCORTEX OF THE BAT, ANTROZOUS PALLIDUS. G.O. Ivy, L. Beasley*, M. Leon and G. Lynch. CNLM and Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

The insectivorous Microchiroptera are generally considered to have poorly developed parietal, temporal and occipital neocortical areas and to lack entirely a frontal region (Henson, 1970). These views are based on a combination of cytoarchitectonic and physiological data; to date, studies demonstrating neocortical connectivity have not been carried out. Since patterns of interhemispheric connections are thought to reflect the relative development of a cortical region, we decided to examine these patterns in the bat.

Four bats were anesthetized, and the skull covering one hemisphere surgically removed. Multiple injections of HRP were placed at regular intervals throughout the neocortex. The following day the animals were perfused and the brains were processed using TMB and BDHC histochemical procedures on alternate sections.

Retrogradely labeled neurons were found continuously throughout the tangential extent of neocortex contralateral to the injection site. In addition labeled neurons were found in every lamina of the cortex. However, the laminar distribution of labeled cells was found to vary substantially among cortical regions. In particular, laminae V and VI were densely labeled throughout the cortex. This includes the cells of lamina Vb which in rodents and higher mammals project subcortically but not, in general, transcallosally. Regional variations in the pattern of labeling in laminae V and VI reflected cytoarchitectonic differences among cortical areas. In contrast, the variable pattern of labeling in more superficial laminae reflected regional differences in the degree of interhemispheric connectivity. Regions of dense labeling in superficial layers alternated in a complex fashion with regions of little or no labeling. In densely labeled areas, HRP filled neurons above laminae V and VI resided predominantly in lamina III, with fewer in lamina IV and very few in lamina II. Small areas within motor, parietal, temporal and occipital cortices (Rose, 1912) contained few or no HRP filled cells in the superficial layers. By analogy with patterns of callosal connections in other mammals, these regions correspond to primary motor, somatosensory, auditory, and visual cortices, respectively.

In conclusion, the complex patterns of neocortical interhemispheric connections in the pallid bat indicate that, as in other placental mammals, regional variations in these connections probably reflect specializations for sensory and motor processing. It is interesting to note that the continuous pattern of labeling in laminae V and VI of Microchiroptera resembles that seen in neonatal rodents. This pattern suggests a neotonized state for the deeper cortical layers in the pallid bat. Further, the relative lack of a dense callosal projection from superficial laminae suggests that cells in these layers preferentially mediate ipsilateral cortico-cortical information processing. Supported by NIH NS21484 (to ML).

203.7 PROJECTIONS FROM THE BASAL FOREBRAIN TO THE CORTEX DEMONSTRATED BY ANTEROGRADE TRANSPORT OF PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). N. J. Woolf, M. C. Hernit*, and L. L. Butcher. Dept. of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

In order to trace the trajectories and to study the terminal endings of basal forebrain projections to the cortex, the plant lectin PHA-L was iontophoretically injected (Gerfen & Sawchenko, Brain Res., 290:219, 1984) into various regions of the basal forebrain containing cholinergic somata. PHA-L was demonstrated by an immunofluorescent protocol employing a fluorescein isothiocyanate second antibody. In order to compare the total number of neurons taking up PHA-L to cholinergic somata accumulating the anterogradely transported label, tissue sections were additionally reacted with monoclonal antibodies directed against choline acetyltransferase (ChAT), which was visualized with a rhodamine-conjugated secondary antibody.

Following injections of PHA-L into the magnocellular preoptic area many fibers could be traced to the olfactory bulb, amygdala, and the pyriform cortex. A large bundle of fibers entered the olfactory tract and continued to the bulb. In the anterior olfactory nucleus a few fibers could be traced leaving the bundle. Scattered fibers emanating from the magnocellular preoptic area coursed laterally to innervate the amygdala and pyriform cortex. Most of the magnocellular preoptic cells that took up the lectin did not contain ChAT, and only a few PHA-L cells were also immunoreactive for ChAT. It is likely, therefore that many fibers observed in the olfactory bulb, amygdala, and pyriform cortex were not cholinergic. A few fibers probably were cholinergic, however, since cholinergic as well as non-cholinergic cells of the magnocellular preoptic area are known to project to the olfactory bulb, amygdala, and pyriform cortex.

Injections of PHA-L into medial portions of the substantia innominata resulted in the labeling of numerous fibers coursing through the medial septal nucleus and diagonal band area. A few of these fibers continued dorsally to innervate the anterior cingulate cortex. Some coursed caudally in the cingulate to innervate medial and posterior cingulate, as well as the visual cortex. Many cells labeled with lectin were also co-labeled for ChAT. Thus, it is likely that these cortical fibers demonstrating PHA-L were cholinergic.

PHA-L injected into the nucleus basalis resulted in the labeling of fibers in the frontal cortex. These fibers did not appear to have any regular orientation. Several bifurcations in these fibers were noted. PHA-L was co-localized with ChAT in some basalis cells suggesting that the frontal cortex fibers were cholinergic.

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203.8 THE CONNECTIVITY OF TWO SUBREGIONS OF THE INFERIOR PARIETAL LOBULE WITH THE PULVINAR COMPLEX OF THE THALAMUS IN THE RHESUS MONKEY. L.J. Lobeck*, J.C. Lynch and S.G.P. Hardy. Dept. of Anatomy, U. Miss. Med. Center, Jackson, MS 39216.

The cortex of the inferior parietal lobule (IPL) and the pulvinar complex of the thalamus share many functional properties. Neurons in both regions fire during active reach, hand manipulation, saccadic eye movements, interested fixation of gaze, and combinations of the above. Neural activity in both regions is strongly affected by the animal's level of attention. The inferior parietal lobule sends dense projections to the pulvinar and receives reciprocal projections from it. The projection to the pulvinar has been reported to end in multiple dense patches separated by terminal-free regions, but the interpretation of this observation has been complicated by the use of multiple injections. We have studied the pattern of corticothalamic terminations in the pulvinar and relationship of the thalamocortical cell bodies to these terminal zones using large single placements of tracers. We have also compared the corticothalamic connectivity of the convexity of the IPL (area PGc) with that of the IPL cortex buried within the inferior bank of the intraparietal sulcus (area POa). These two cortical areas differ cytoarchitecturally as well as in their afferent and efferent connectivity with other regions of the cortex and with the superior colliculus.

Eight hemispheres of six monkeys were studied using anterograde transport of tritiated amino acids and anterograde and retrograde transport of horseradish peroxidase (HRP). Injections of tritiated amino acids were made in five hemispheres and placements of HRP gel were made in three hemispheres. Large single placements of either tracer in PGc resulted in multiple patches of labeled axons within the lateral, medial, and oral nuclei of the pulvinar. These terminal zones were characterized by dense horizontal bands of labeled axons and terminals separated by less heavily labeled or unlabeled zones. Large single placements in POa resulted in single, generally smaller, zones of label extending across the lateral and medial pulvinar nuclei. Only faint labeling was observed in pulvinar oralis following POa placements. Retrogradely labeled thalamocortical cell bodies were found predominantly in clusters that were almost totally restricted to the regions of labeled corticothalamic axons. These findings suggest that the two subregions of the inferior parietal lobule that we have studied have different patterns of connectivity with the pulvinar complex. There is a close relationship between the corticothalamic terminal regions and the thalamocortical cell bodies that connect pulvinar and IPL. Furthermore, the heterogeneous distribution of areas of connectivity with the IPL within each pulvinar nucleus suggests that each of these three nuclei are composed of functionally specialized subregions. [Supported by grants from NIH (EY-04159) and the Vaughan Stroke Research Trust]

203.9 DISJUNCTIVE DISTRIBUTION OF MEDIODORSAL THALAMIC AFFERENTS IN THE PREFRONTAL CORTEX OF RHESUS MONKEYS. M. Giguere and P. Goldman-Rakic. Yale University School of Medicine, Section of Neuroanatomy, 333 Cedar Street, New Haven, CT 06510

Although the reciprocal connections between the mediodorsal (MD) nucleus and prefrontal association cortex in primates are well established and their basic topographic organization worked out, the details of laminar and tangential distribution of MD afferents in primate prefrontal cortex are not known. In the present study, we employed injections of WGA-HRP in different subdivisions of MD in rhesus monkey in order to determine: i) the layer(s) in which MD afferents terminate; ii) the location of the cells of origin of the reciprocal cortico-thalamic pathway; and, iii) whether the terminal fields of this pathway - like that of the cortico-cortical afferents - is organized in bands of a specific width.

(i) With injections confined to the medial (MDmc) and lateral (MDpc) portions of MD labeled terminal were present in layer IV and deep layer III in the ventral and dorsolateral regions of the prefrontal cortex, respectively. No terminal label was found in layers I, superficial III, V or VI in any part of the prefrontal cortex following these injections.

(ii) Analysis of retrograde transport reveals labeled cortico-thalamic neurons mainly in layer VI with some labeled cells situated also in the superficial part of layer V. The distribution of retrogradely labeled cells corresponds to the location of anterogradely labeled terminal fields, and, in addition, fluctuations in the density of labeled cells parallel changes in terminal field density. In contrast, in the anterior cingulate and supplementary motor cortex, which lay outside the traditional borders of prefrontal cortex, reciprocity was totally absent. These areas also contain high concentrations of retrogradely labeled neurons but received only sparse reciprocal projections from MD.

(iii) The projection from MD to the cortex formed a disjunctive pattern of bands ranging in width from 0.3 to 1.3 mm. These bands were evident only when injections were circumscribed; when the injection involved more than a single moiety of MD or extended into a larger area of the thalamus, such as the adjacent VA-VL complex, the pattern of the terminal labeling was more diffuse and tangentially extensive throughout layers III and IV. These data suggest that the thalamic inputs from the nuclear subdivisions of the intrinsic association nuclei and/or from the motor relay nuclei of the thalamus may interdigitate in the prefrontal cortex.

In conclusion, MD has reciprocal connections with the prefrontal cortex that exhibit precise laminar specificity. In addition, the disjunctive patterning of thalamocortical terminals supports the concept of a modular prefrontal architecture.

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203.10 SOMATOSENSORY REPRESENTATION IN PRIMATE PREFRONTAL CORTEX: CONNECTIONS OF THE PRINCIPAL SULCUS WITH S-I, S-II, AND ADJACENT AREAS OF THE FRONTOPARIETAL OPERCULUM. T.M. Preuss and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Although it is commonly believed that primate prefrontal association cortex does not receive direct projections from primary sensory cortex, several studies have demonstrated connections between prefrontal cortex and the frontoparietal operculum, a region containing the intraoral representation of S-I (Robinson & Burton, J. Comp. Neurol., 192: 43, 1980). We have examined prefrontal-opercular connections in macaques using frontal lobe injections of HRP or tritiated amino acids, making particular reference to cytoarchitectonic and physiological maps of the opercular region. In four cases with injections that included the ventral rim of the principal sulcus (Walker's area 46), dense anterograde and retrograde labeling occurred over the rostrocaudal extent of the frontoparietal operculum, including the deep gustatory area, the intraoral representation of S-I, and S-II. The heaviest labeling in S-II occurred in its rostral and superficial portions, corresponding to the orofacial representation (Robinson & Burton, 1980). Within the S-I complex, labeling occurred mostly in areas 1-2. Anterograde and retrograde labeling in the operculum took the form of rostrocaudally oriented stripes or columns. Cells projecting to prefrontal cortex were found mostly in layers III and V; terminal labeling was heaviest in layer I, followed by layer III.

Labeling was also found in several areas adjacent to the operculum, including the anterior and middle insular cortex, which receives input from the principal gustatory thalamic nucleus (VPMpc); the ventral precentral cortex (Von Bonin & Bailey's area FCBm), which contains the representation of the laryngeal muscles (Hast et al., Brain Res., 73: 229, 1974); and areas 2 and 7b of the rostral inferior parietal lobule, where cells have trigeminal receptive fields (Robinson & Burton, J. Comp. Neurol., 192: 69, 1980).

This study demonstrates that primate prefrontal cortex has strong reciprocal connections with primary somatosensory cortex as well as with other areas (S-II, insula, deep gustatory cortex) which receive input from principal somatosensory or gustatory thalamic relay nuclei. Further, the finding that the ventral rim of the principal sulcus is connected with cortical subdivisions representing intra- and perioral regions, the laryngeal muscles, and taste, suggests that an area of frontal association cortex in monkeys contains an orofacial representation. The functions of this region may include the voluntary control of facial movements in feeding and/or communication.

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203.11 GEOMETRY AND QUANTITATIVE CHARACTERISTICS OF DENDRITIC ARBORS OF THALAMOCORTICAL PROJECTION NEURONS IN THE CAT VA AND VM. S. Al-Hussain and K. Kultas-Ilinsky. Dept. of Anatomy, Univ. of Iowa College of Med., Iowa City, IA 52242

Dendritic geometry and branching patterns are important in understanding the conduction properties of the nerve cells. Very little is known, however, about these parameters in regard to neurons in the motor thalamic nuclei. In the present study various quantitative and qualitative parameters of the dendritic arbors of thalamocortical projection neurons in Golgi impregnated preparations were compared in the ventral anterior (VA) and ventral medial (VM) thalamic nuclei. The parameters measured were those descrbed by Hillman (The Neurosci. 4th Study Progr. MIT Press, 1979, p. 477) and included: the number, width (W), and length (L) of consecutive order dendritic branches, correlation between the soma size and the sum of dendrite diameters, type of branching, ratio between cross sectional diameters of daughter branches (R), branch power (n) and the segment taper (a).

The results indicate that in both nuclei the diameters of consecutive order dendritic branches vary and wide overlap occurs between W of primary (1D) secondary (2D), and tertiary (3D) dendrites. Positive correlation was found between the surface area of somata and the sum of dendrite diameters of cells in the VA whereas in the VM it was less pronounced. The mean number of 1D per cell in VM was 7 ranging from 5 to 10. In the VA the number was slightly higher. Branching pattern of different order dendrites on the same cell was also quite variable. Most of the dendrites divided dichotomically whereas about 1/3 of dendrites in each TCPN studied gave rise to tufts of 3-5 daughter branches. Both in the VA and VM for about 25% of primary dendrites R = 1 whereas in the rest it ranged from 1.2 to 2.3. Almost all dendrites of the VM neurons regardless of type of branching had $n < 2$. In the VA, however, n for about 60% of dichotomically branching segments was < 2, for the rest $n \geq 2$. The values were reversed for the tufted type dendrites: 66% had $n > 2$, and the rest < 2. The length of dendritic segments was also very variable. The L of 1D in VM ranged from 5-30 μm, 2D length varied from 5-20 μm and the 3D L from 5-90 μm. In VA wider range of variations (5-100 μm) in L of all segments was observed. No tapering of dendritic segments was found in either VA or VM neurons.

These findings demonstrate that the dendritic arbors of TCPN in the motor thalamus display great diversity. The fact that diverse branching patterns are found in dendritic trees of individual cells suggests that conductance properties of different dendrites of the same cell may not be same. The comparison of the morphological parameters of TCPN in the nuclei indicates that in the VA individual neurons display more variability in different parts of their dendritic arbors as compared to the VM. Supported by NIH NSR0119280.

203.12 DESCENDING PROJECTIONS ORIGINATING WITHIN THE NUCLEUS BASALIS IN THE RAT. Elizabeth L. Weiss and Richard W. Rieck. Dept. of Anatomy, Tulane Medical School, New Orleans, Louisiana 70112.

The nucleus basalis, Ch4, (Mesulam, et al., Neurosci., 10: 1185, 1983) is known to provide an extensive cholinergic projection to all areas of the neocortex. In addition, neuronal loss within the nucleus basalis may be the basis of many of the severe deficits observed in patients with senile dementia of the Alzheimer's type (Whitehouse, et al., Ann. Neurol., 10:122, 1981). Furthermore, recent evidence suggests that another cholinergic component of the basal forebrain, i.e., the diagonal band of Broca, may also contribute a descending projection into the thalamus. Thus, the presence of a descending projection from the nucleus basalis to the dorsal thalamus was investigated in the present study. Injections of retrograde tracers, i.e., 1.7% wheat germ agglutinin-horseradish peroxidase (WGA-HRP) or 30% HRP, were placed electrophoretically within the dorsal thalamus. Retrogradely labeled neurons are located within the rostral portions of Ch4 following placement of injections within the stria medullaris and the mediodorsal nucleus. In contrast, labeled neurons are located within intermediate levels of Ch4 following placement of injections within the centromedian-parafascicular complex. These thalamic projecting neurons are either located within the ansa pedundularis or are immediately ventral to the globus pallidus. Thalamic projections, however, apparently do not originate within the Ch4 component that is interdigitated between the putamen and the globus pallidus. The retrogradely labeled neurons within Ch4 have a perikaryal morphology that is consistent with that of acetylcholinesterase and choline acetyltransferase positive neurons within the same region, i.e., large multipolar isodendritic cell types. Furthermore, the same cellular type within the nucleus basalis is known to project to the neocortex. These data suggest that the nucleus basalis, as other basal forebrain cholinergic systems, may also contribute a subcortical projection to specific targets within the thalamus. Thus, the specific subcortical projections of the nucleus basalis may contribute to the symptomatology associated with certain degenerative disease states. Supported by BRSG 532802 (R.W.R.).

203.13 TOPOGRAPHY OF DORSAL RAPHE PROJECTIONS TO NEOSTRIATUM. J.W.Boja* and H.K.Kulmala (SPON: J.Reinglass), Pharmacology Program, NorthEastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The neostriatum receives a large, mainly serotonergic projection from the dorsal raphe nucleus (DRN). Whereas most serotonergic projections to the forebrain are topographically organized, no such topography has been reported in DRN projections to neostriatum. Indeed, the same DRN cells have been shown to project to medial and lateral striatum. However, the density of serotonergic innervation of neostriatum is organized in a rostro-caudal gradient, with the highest concentration in the tail of this nucleus. We have determined whether a rostro-caudal topography is evident in this projection.

Male Sprague-Dawley rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and placed in a Kopf stereotaxic instrument. Injections of 0.2-0.5 ul of a 25-35% horseradish peroxidase (HRP) solution were made via a 1 ul Hamilton microsyringe at one of three rostrocaudal levels of the neostriatum. Following 48-72 hrs, animals were killed under deep anesthesia by transcardial perfusion of saline followed by 1.5% glutaraldehyde - 1% paraformaldehyde and ice cold 10% sucrose. Forty, 50 um thick sections were cut on a CO_2-freezing microtome and reacted with tetra-methyl benzidine - hydrogen peroxide to localize the HRP.

Labeled neurons were evident in the rostral 2/3rds of the DRN after striatal HRP injections. Very few median raphe neurons contained HRP. These few probably were labeled by the small amount of HRP evident along the needle tract in the cortex overlying the striatum. Rostral injections labeled cells more medially in the DRN. Caudal injections led to label in DRN cells in the dorsal cluster and lateral wings of the DRN. More cells were labeled after caudal injections than after rostral striatal injections.

It is concluded that a rostro-caudal topography is evident in the projection from the DRN to the neostriatum.

Supported in part by a research grant from the Tourette Syndrome Association.

204.1 AN ELECTRONIC MORPHOMETRY AND MAPPING ANALYSIS MICROSCOPY SYSTEM (EMMA) FOR THE QUANTITATIVE AND COMPARATIVE STUDY OF NEURAL STRUCTURES. Warren G. Young, John H. Morrison, and Floyd E. Bloom, Div. Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

As methods for the selective demonstration of neurons and neural circuitry have expanded, the morphologically oriented neuroscientist requires methods to measure and map structures and compare them with existing anatomic data. Existing commercially-developed systems are expensive and lack analytic flexibility. Therefore, we have developed a general analytic system to acquire accurate morphological date quickly, compare them with previously acquired data, and an enlargeable text data base of neuronal microscopic circuitry, transmitters, and other functional properties. EMMA uses the Digital Equipment Corp. LSI 11/73 super microcomputer combined with a Zeiss Inverted ICM 405 microscope through highly accurate optical position sensors on the X,Y and Z axes, and through a high resolution video interface. EMMA can accurately measure and record structural elements from 1 micron to 50 mm. The live video images from the microscope are displayed on a monitor, with overlaid menus of program commands and computer-generated tracings of desired structures. A separate color monitor displays cumulated computer graphic data. With this configuration we can reconstruct the arborization of most neuronal structures, determine their macro-regional distribution, and compare these cells for 2- or 3-dimensional morphological and positional properties with previously analyzed fields. The image may also be digitized for storage or mathematically processed to enhance or subtract images, or to assign colors to gray levels. In a typical application, EMMA can render a 2-dimensional distribution of cytochemically-defined soma versus fibers in a single field, contingent only on the resolving power of the cytochemical method. If the density or size distribution of the reactive structures is discriminable, EMMA can automatically estimate the density and frequency of the two defined structural components. EMMA users can also consult several online anatomic databases while analyzing specimens. Frontal and sagittal atlas sections of brain structures can be overlaid onto the televised microscopic specimen to assist in identifying neuronal structures, and to provide templates onto which cell body or neuropil data may be mapped, stored and compared. A text-oriented data base of known anatomic structures, indicating afferent projections, efferent projections, as well as transmitters and functional properties are tied to selected anatomic regions. (Supported by grants from MacArthur Foundation and the Sam and Rose Stein Charitable Trust).

204.2 THE IMMUNOHISTOCHEMICAL DISTRIBUTION OF SOMATOSTATIN 28 (SS28) AND SOMATOSTATIN 28(1-12)(SS28(1-12)) IN MONKEY PREFRONTAL (PFC) AND TEMPORAL (TC) CORTICAL REGIONS. M.J. Campbell, D.A. Lewis*, J.H. Morrison. Div of Preclin. Neurosci. & Endocrinol, Scripps Clinic and Res. Fdn., La Jolla, CA 92037.

We have used antisera that selectively recognize the prosomatostatin-derived peptide fragments SS28 and SS28(1-12) to characterize their immunohistochemical distribution in the PFC (including Area 24) and TC of the cynomolgus and squirrel monkey. These antisera demonstrates that SS28 is largely restricted to cell bodies, whereas SS28(1-12) is preferentially localized in neuronal processes and terminals. Although it was not invariant there was a characteristic laminar pattern of fiber staining. The densest terminal arborization was in layers I, II and superficial III. Deep III and IV were traversed by radial fibers with little arborization. V and VI contained both radial fibers and a moderately dense terminal plexus. There were fewer labelled fibers in the white matter. These fibers tended to run parallel to the pial surface until they swept into layer VI and assumed a radial orientation. This radial orientation was most striking in the TC where it was often possible to follow fibers from the white matter or infragranular layers to superficial layer III. Somatostatin-positive cells were found throughout all cortical layers and the white matter. The largest number were located in layers II and superficial III and deep V and VI. This bimodal distribution was more pronounced in TC than in PFC. There were regional differences in cell body density which were paralleled by differences in fiber density. For example, in PFC, area 24 had the greatest cell and fiber density, area 46 the lowest and area 9 had an intermediate density of both. However all regions of the PFC and TC had far greater density of cells and terminals than primary visual cortex. These regional patterns imply that intrinsic cortical systems can exhibit substantial regional variation. Many stained cells had a Golgi-like quality displaying both dendritic processes and a presumed axonal process. These neurons represented a diverse morphological group that included examples of established cell types such as Martinotti and basket cells. As in the rat (Morrison et al., Brain Research 262, 1983) and the human (Morrison et al., Nature 314, 1985) the antisera to SS28 and to SS28(1-12) allowed for a much more extensive characterization of the distribution and morphology of both cell bodies and processes than antisera to SS-14. The complexity and the density of this heterogeneous group of SS-containing neurons suggest that they are likely to play an important role in several aspects of intrinsic circuitry of the neocortex. Supported by AG0513, MacArthur Foundation, Sam and Rose Stein Charitable Trust, K1-00519, AA07456-01, and AA06420.

204.3 AN EXPERIMENTAL ANALYSIS OF THE INTRACORTICAL SOMATOSTATIN-CONTAINING NEURONS. J.H. Morrison, S. Scherr, M.J. Campbell, and D.A. Lewis. Scripps Clinic and Research Foundation, La Jolla, CA 92037

Prosomatostatin derived peptide fragments somatostatin-28(SS28) and somatostatin 28(1-12) (SS28 (1-12) have been shown to have a preferential intracellular distribution in the rat neocortex. SS28 is located primarily in cell bodies whereas SS28(1-12) is located in fibers and terminal processes. In the rat neocortex both the cell bodies and terminal plexuses have their highest density in a supragranular band and infragranular band. There are long radial fibers that traverse deep III, IV and superficial V. Also, there are labelled cells and fibers present in the sub-cortical white matter. In order to further characterize the branching patterns of specific axons and sources of the axonal plexuses, we initiated an experimental analysis of the somatostatin system. Three techniques were used in combination with somatostatin immunohistochemistry: 1) small iontophoretic injections of phaseolus vulgaris-leucoagglutinin (PHA-L), which fills cells locally and is transported anterogradely, and 2) true blue injections (transported retrogradely), and 3) carefully placed undercut lesions. The PHA-L was localized with an anti-PHA-L made in guinea pig, followed by FITC-conjugated anti-guinea pig IgG, allowing for double-labelling with peptide antisera made in rabbit (Gerfen and Sawchenko, Brain Research, 1985). The iontophoretically applied PHA-L formed an injection site 100-300 um in diameter, and labeled cells within a 1 mm radius that had a dendritic process extending into the injection site. When the PHA-L loaded cells were visualized simultaneously with anti-SS12 fine radial SS12-positive processes were seen in association with pyramidal cell apical dendrities. The true blue double-labelling studies demonstrated that there were multiple sources for both the infra- and supragranular terminal fields. Following superficial application of true blue, three major somatostatin-containing cell classes were double-labelled; 1) cells with elongated, horizontally disposed dendrites at the VI-white matter border, 2) small multi-polar cells throughout VI directly below the injection site, and 3) multi-polar cells within layers II and III 300-1200 um from the injection site. Following the layer VI undercut experiments, the supra- and infragranular terminal plexuses were not decreased in density, implying that most of the somatostatin-containing terminals are intrinsic in origin. We conclude that horizontally and radially projecting somatostatin containing cells are present in neocortex, and layer III pyramidal cell apical dendrites may be one of the primary targets of radially projecting, multi-polar somatostatin cells. Supported by AG0513, MacArthur Foundation, K1-00519, AA07456, and AA06420.

204.4 DETERMINATION OF BIOLOGICALLY RELEVANT QUANTITIES OF NEUROTENSIN AND ITS FRAGMENTS USING ISOCRATIC OR GRADIENT ELUTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION. R. Rivest, A. Drumheller*, S. St-Pierre* and F. Jolicoeur. Departments of Psychiatry and Pharmacology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.

High Performance Liquid Chromatography coupled with electrochemical detection (HPLC-ECD) has become the method of choice for the determination of many electroactive substances in biological samples. The sensitivity of this method often surpasses that achieved with either UV or fluorometric detection. Recently it has been shown that peptides containing tyrosine and/or tryptophan residues are electroactive and can be detected amperometrically (Bennett et al., Life Sci, 29:1001, 1981). In this study we investigated the potential of using HPLC-ECD for the separation and quantification of neurotensin, a 13 amino acid peptide containing two tyrosine residues at positions 3 and 11. Results indicate that under isocratic conditions less than 300 pg of neurotensin can be separated and detected within 10 min. The sensitivity of this assay greatly surpasses that of HPLC-UV detection and approaches that achieved with radioimmunoassay techniques.

In a parallel study the use of a modest gradient to separate and quantify biologically relevant fragments of neurotensin including NT1-8, NT1-10 and NT9-13 was examined. Several mobile phase compositions and gradient profiles were tested and compared in terms of resolving power and sensitivity. Results indicate that less than 50 ng of neurotensin and fragments can be separated and detected within 25 min using 0.05 M ammonium acetate (pH 4.2) /acetonitrile (90/10 v:v) as equilibrating buffer and increasing the concentration of acetonitrile stepwise until neurotensin eluted (23% acetronitrile). Although the gradient method outlined here is 100 to 200 x more sensitive than HPLC-UV detection, extraneous noise and baseline drift generated by the gradient increased the detection threshold approximately 100 fold as compared to that found using isocratic elution.

Finally, in preliminary experiments we have examined the ability of this procedure to detect neurotensin and selected fragments in rat brain. In whole brain homogenates peaks having the same retention time as synthetic neurotensin, NT 1-8 and NT 1-10 were observed. We are presently examining the regional distribution of neurotensin-like electroactivity to see if it resembles that of the immunoreactivity of the peptide.

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204.5 RE-EVALUATION OF THE DISTRIBUTION OF ANGIOTENSIN IN RAT BRAIN BY PAP IMMUNOCYTOCHEMISTRY USING NICKEL INTENSIFICATION: COMPARISON TO THE DISTRIBUTION OF RENIN SUBSTRATE. M.S.Brownfield* and B. Poff*. (SPON: S. Lorens). Department of Physiology, University of California, San Francisco, CA 94143 and Department of Structural and Functional Sciences, University of Wisconsin, Madison, WI, 53706.

Biochemical studies have shown that a renin substrate (RS) from which angiotensin (ANG) can be liberated in vitro is present in the brain and cerebrospinal fluid of rats. It has been suggested that this material could be the precursor of brain ANG. A map of the distribution of brain ANG has been evolving as a result of several immunocytochemical (ICC) studies. However, ICC studies of brain RS are lacking and so a knowledge of its cellular origins and how they may relate to those of ANG is unknown. In this study, we have (1) evaluated the distribution of brain ANG using the peroxidase-antiperoxidase method in combination with nickel intensification (PAP-Ni) of the final reaction product, and (2) compared the distribution of ANG to that of RS. Cryostat sections were processed using two specific ANG (DE, courtesy D. Ganten and UW-MB- #R29) and one RS (gift of J. Bouhnik) antibodies at dilutions of 1:750 to 1:1500 and 1:8,000 to 1:20,000, respectively.

The PAP-Ni method stained immunoreactive ANG much more intensely and with a much greater distribution than has been reported previously using peroxidase methods. Colchicine treated rats (100 ug ivt) revealed the presence of a large number of neuronal perikarya in hypothalamic parvocellular and magnocellular nuclei and lateral hypothalamus. The latter appeared to give rise to an intense fiber system (as shown in untreated rats) projecting through the lateral hypothalamus to numerous areas including (but not limited to) periventricular structures, the amygdalaoid complex, preoptic area, nucleus of the solitary tract, spinal cord and cerebellum. Lesser numbers of immunopositive cells are also found in several of these apparent terminal fields.

The distribution of RS in areas also occupied by ANG was overwhelmingly glial, in contrast to the neuronal localization for ANG. Moreover, most brain areas contained reactive glial cells, but were devoid of ANG. Substantial background staining was encountered which under the electron microscope was shown to be intercellular.

These results demonstrate that the source of RS is glial and that it is morely widely distributed than ANG. Glial to neuronal contacts might supply RS to many neurons directly, or they could take it up from the interstitium. Whether they produce ANG would depend on whether they contain renin. Alternatively, RS might have a more general role in the brain that might not be related to ANG production. (Supported by the UWGS and NIH HL 29714).

204.6 QUANTITATIVE AUTORADIOGRAPHIC DETERMINATION OF ANGIOTENSIN-CONVERTING ENZYME DISTRIBUTION IN RAT BRAIN WITH ^{125}I-MK351A, A SPECIFIC INHIBITOR. F.M.A. Correa, L.M. Plunkett* and J.M. Saavedra. Section on Clinical Psychopharmacology, NIMH, Washington, DC 20205-1000

Quantitative determination of angiotensin converting enzyme (ACE, kininase II) can be accomplished by dissecting brain nuclei with the "punch" technique and incubating the homogenates with the ACE substrate hippuryl-His-Leu [glycine-1-^{14}C] (Brain Res.309: 389-392, 1984). Recently ACE has been localized in the brain by incubating brain sections with the ACE inhibitor [^{3}H]-Captopril (PNAS, 81:1599-1603, 1984). Although [^{3}H]-labelled compounds have the advantage of improved neuroanatomical localization, enzyme quantitation cannot be determined in individual brain structures.

We report a quantitative autoradiographic method for the kinetic analysis of ACE in discrete brain areas using a ^{125}I-ligand. ACE was localized, levels were quantitated and enzyme kinetics determined by incubation of tissue sections with ^{125}I-MK351A (Merck, Sharp, Dohme). Compound 351A is a p-hydroxybenzamidine derivative of MK521, which is the Lys-Pro analog of MK422, the active di-acid form of the specific ACE inhibitor enalapril (MK421). Compound MK521 was used as a displacer.

^{125}I-MK351A binding sites were observed throughout the CNS with a very localized distribution. The density of ACE sites was found to be higher in the choroid plexus and the subfornical organ (320±22 and 166±38 fmol/mg protein). A good correlation was observed between ACE-site density and areas rich in AII receptors. High densities of ^{125}I-MK351A sites were observed in the supraoptic and paraventricular nuclei, the median eminence, the area postrema and the nucleus tractus solitarius (48±8, 35± 12, 43±8, 58±4, 39±2 fmol/mg protein), areas which are involved in important physiological processes such as cardiovascular regulation. In addition, a large part of ACE binding is present in areas not involving the angiotensin system, such as the nigrastriatal system. ACE distribution was found to be relatively homogenous throughout the caudate-putamen (average value of 104± 21 fmol/mg protein), the globus pallidus (50±6 fmol/mg protein) and the substantia nigra pars reticularis (177±33 fmol/mg protein). Scatchard analysis indicates the existence of two slightly different groups of binding sites with significantly different dissociation constants. Structures such as choroid plexus and subfornical organ display a Kd of around 0.8nM whereas areas such as caudate-putamen and globus pallidus have a Kd of around 2.0mM.

The use of ^{125}I-MK351A autoradiography allows the quantitation of ACE-sites in tissue, since it interacts on a one to one molecular basis with the enzyme. Our results point to a possible heterogeneity among dipeptidyl carboxipeptidases in the brain.

204.7 **THYROTROPIN-RELEASING HORMONE (TRH) PROJECTION TO THE HIPPOCAMPAL FORMATION VIA THE FORNIX.** W.C. Low, T.G. Hill*, and M.J. Kubek. Depts. of Physiology and Biophysics, Anatomy, and Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

Thyrotropin-releasing hormone (TRH) and TRH receptors have previously been localized throughout the limbic system and other areas of brain. Recent studies in our laboratory have demonstrated sustained elevations of TRH within the hippocampal formation (HF), amygdala (AY), and cortex (CTX) as a result of electroconvulsive shock (Life Sci., 36:315-320, 1985; and this meeting). TRH within these areas of brain may be intrinsic and/or originate from TRH-containing nerve cells within the hypothalamus and/or brainstem. The fornix is a possible pathway by which TRH or its synthesizing enzyme(s) can reach the HF. To test this hypothesis unilateral lesions of the right fornix and overlying neocortex were made by aspiration in male Sprague-Dawley rats (200-350 gm). Control animals received aspirated unilateral lesions of the neocortex overlying the right fornix sparing its fibers. Two weeks after the lesion, animals were decapitated and brains were removed, dissected, weighed and frozen on dry ice. Tissues were extracted with acetic acid and TRH was quantified by a specific radioimmunoassay. TRH content was expressed as pg/mg wet weight (mean +/- SEM). Lesions of the fornix resulted in a highly siginificant decrease in HF TRH in comparison to sham operated controls (6.2 +/- 0.39 vs. 2.06 +/- 0.21; $p < 0.001$). In contrast, a significant increase in cortical TRH was observed following the fornix lesion (1.27 +/- 0.20 vs. 2.75 +/- 0.18, $p < 0.005$). No significant changes were found in the anterior, medial or posterior hypothalamus, amygdala or septum. These results support the hypothesis of an extrinsic source of hippocampal TRH via the fornix. The amount of TRH remaining in the HF after fornix lesions may reflect levels intrinsic to the hippocampus or a second TRH fiber projection. The increase in cortical TRH was unexpected and may be due to a sprouting of the severed TRH fibers. This phenomenon remains to be determined along with the source of HF TRH innervation. Supported by the Veterans Administration Research Service (M.J.K.), and PHS grants AM-28260 (M.J.K), RR-5371F (W.C.L.), and AG-5575 (W.C.L.).

204.8 THE AMYGDALA IS A SOURCE OF CHOLECYSTEKININ OCTAPEPTIDE FOUND IN THE PONTINE PARABRACHIAL NUCLEUS OF THE CAT. T.H. Burke, B.G. Kirol*, B.E. Maley, and W.M. Panneton Depts. of Anatomy, Univ. of Kentucky, Lexington, Ky. 40536 and St. Louis University, St.Louis, Mo. 63104.

Cholecystekinin Octapeptide (CCK-8), a peptide found in both gut and the central nervous system, has been implicated in the regulation of vasomotor tone. Both the amygdala and the parabrachial nucleus have been shown to contain CCK-8, and also are components of central cardiovascular control. Since large numbers of CCK-8 containing neurons are found in the amygdala, while none are found in the parabrachial nucleus it was therefore hypothesized that the CCK-8 immunoreactivity found in the parabrachial nucleus originates in the amygdala.

In this study, CCK-8 immunoreactivity was identified by means of the peroxidase, antiperoxidase (PAP) method. Adult cats were injected with 0.01 μl of horseradish peroxidase (HRP) in the parabrachial nucleus. After four days, 240 μl of colchicine were injected intracisternally, and the animals were allowed to survive for two additional days. They were then perfused transcardially with 4% paraformaldehyde in Sorenson's phosphate buffer pH 7.2. 50 μm frozen sections were collected, pretreated with cobalt chloride to enhance the HRP staining, and then reacted with 3,3' diaminobenzidine containing 0.3% hydrogen peroxide prior to employing the free floating PAP procedure of Sternberger.

Following injections into the parabrachial nucleus, the amygdala was examined for labelled neurons. The amygdala contained neurons which exhibited either black HRP granules or the brown reaction product of CCK-8 immunoreactivity. In addition, several cells also contained both the black HRP reaction product and the brown immunostaining of CCK-8 immunoreactivity.

These results indicate that at least some of the CCK-8 immunoreactivity in the parabrachial nucleus arises from neurons in the amygdala. The central regulation of cardiovascular control contains several loops of which the amygdala-parabrachial loop is a part. The presence of CCK-8 in this loop suggests its use as the neuropeptide involved in cardiovascular regulation of the amygdala and parabrachial nucleus.

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204.9 PEPTIDERGIC EFFERENTS FROM THE INTERCALATED NUCLEI OF THE AMYGDALA TO THE PARABRACHIAL NUCLEUS IN RAT. T.S. Gray and M.M. Moga*. Dept. Anat., Loyola Stritch Sch. Med., Maywood, IL 60153

The peptide content of the intercalated nuclei (ICN) of the amygdala and their projection to the parabrachial nucleus was studied using the combined retrograde transport-immunofluorescence method. Stereotaxically-guided injections of 50nl of fast blue retrograde tracer were made into the parabrachial nuclei of 150-300gm male rats. Bilateral injections of colchicine were administered to all animals 36-48hr prior to sacrifice. Post-fast blue injections survival periods were 9-12 days. Animals were overdosed with sodium pentobarbital, and then fixed via transcardial perfusion with 4.0% phosphate buffered paraformaldehyde. Brains were cut into 20um coronal sections. Alternate sections were processed using antibodies to neurotensin (NT), somatostatin (SS), corticotropin releasing factor (CRF) or enkephalin (ENK). Immunoreactivity was visualized using rhodamine conjugated antirabbit immunogammaglobulin. One set of sections was counterstained with cresyl violet.

In animals with fast blue injections centered within the lateral parabrachial nucleus, retrograde labeling was observed within a distinct cluster of cells in the rostral amygdala. This cluster corresponded in shape and location to an anterior group of intercalated cells first described in the rat by Guardjian ('28). Most rostrally, the retrograde labeling appeared as a plate of cells just ventral to and aligned with the posterior limb of the anterior commisssure. In more caudal sections, the retrograde labeling appeared as a tightly-packed sphere and was located adjacent to the external capsule. Distinct clusters of NT-immunoreactive cells were seen in corresponding positions to the retrogradely labeled cells. Many NT-immunoreactive cells were double-labeled with fast blue. SS-, CRF- and ENK-immunoreactive cells were diffusely distributed within the ICN. Only a few CRF- and SS- cells were retrogradely labeled. Retrogradely labeled ENK-immunoreactive neurons were never observed. Both SS and ENK terminal immunoreactivity densely filled the ICN. NT- and CRF-immunoreactive fibers were sparsely distributed within the ICN.

Results of this study suggest that NT, and to a lesser degree, CRF and SS, are contained with ICN neurons which innervate the parabrachial nucleus. ENK and SS terminals surround parabrachial efferent cells within the ICN. To our knowledge, the present study is the first to identify an efferent pathway originating from the intercalated nuclei of the amygdala. Our results indicate a functional link between the ICN and the lateral subdivision of the central nucleus of the amygdala based on their similar peptidergic projections to the parabrachial nucleus. (Supported by NIH grant NS 20041)

204.10 HYPOTHALAMIC PEPTIDERGIC EFFERENTS TO THE PARABRACHIAL NUCLEUS IN THE RAT. M.M. Moga* and T.S. Gray. Dept. Anat., Loyola Stritch Sch. Med., Maywood, IL 60153

Previously, we found that the parabrachial nucleus receives substantial corticotropin releasing factor, neurotensin, and somatostatin projections from both the central nucleus of the amygdala (CNA) and the bed nucleus of the stria terminalis (BNST). In this study, we examine the contribution and content of hypothalamic peptidergic efferents to the parabrachial nucleus using the combined retrograde transport-immunofluorescence technique.

Fifty nanoliters of the retrograde tracer fast blue was injected into the parabrachial nucleus of 150-300g male rats. Animals were treated with bilateral injections of colchicine 36-48 hours prior to sacrifice. Post-fast blue survival periods were 9-12 days. Animals were overdosed with sodium pentobarbital, and then perfused transcardially with 4.0% phosphate buffered paraformaldehyde. The brains were cut into 20um coronal sections. Four series of sections, each with a different primary antibody, were processed for each animal. A number of primary antibodies were tested including: neurotensin (NT), corticotropin releasing factor (CRF), somatostatin (SS), met-enkephalin (ENK), vasoactive intestinal peptide, substance P, cholecystokinin, oxytocin (OXY), and vasopressin. Immunoreactivity was visualized with rhodamine conjugated antirabbit immunogammaglobulin.

Retrogradely labeled neurons were found in the lateral hypothalamus, medial and lateral preoptic areas, arcuate nucleus, paraventricular nucleus, dorsal hypothalamus, and periventricular nucleus. Neurons labeled with both fast blue and rhodamine immunofluorescence were located predominantly in the lateral hypothalamus. Preliminary cell counts indicate that approximately 12% of the NT-, 9% of the CRF-, and 8% of the SS-immunoreactive neurons found in the lateral hypothalamus project to the PBN. Less than 1% of the NT-, CRF-, SS-, OXY- and ENK-immunoreactive neurons in the PVN were retrogradely labeled with fast blue. A few cells in the medial preoptic area were double-labeled with fast blue and either ENK or NT. Still fewer were double-labeled for CRF or NT in the lateral preoptic area.

In summary, the lateral hypothalamus is the major hypothalamic source of CRF, NT, and SS terminals to the PBN. The PVN peptidergic contribution to the PBN is minor. In the CNA and BNST, a high percentage of CRF-(57% and 28%, respectively), NT-(50% and 23%), and SS-(42% and 22%) immunoreactive neurons project to the PBN. In conclusion, the results from all three of our studies (i.e., CNA, BNST and hypothalamus) indicate that the CNA and BNST are the major forebrain sources of CRF, NT and SS terminals to the PBN. (Supported by NIH grant NS 20041)

204.11 THE MEDIAL PREOPTIC AREA RECEIVES A MAJOR SUBSTANCE P PROJECTION FROM THE REGION OF THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN THE RAT. Charles W. Malsbury, Dept. Psychology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada, A1B 3X9

Autoradiographic tracing studies have revealed long-axon projections from the ventromedial nucleus (VMN) to a number of forebrain and midbrain sites. The VMN contains numerous substance P-immunoreactive cell bodies (Ljungdahl et al., Neuroscience, 1978, 3, 861) and some of the VMN projection sites have a substantial substance P (SP) innervation. The origins of the SP innervation of most of these areas are not known.

Immunocytochemistry (ICC) was used here to explore the possibility that VMN projection neurons contribute to the SP innervation of these areas. Adult female rats received large unilateral radiofrequency lesions of the VMN region and were killed 6 days later. The brains were processed for ICC using the PAP method. Known VMN projection sites including septal area, bed nuc. of the stria terminalis, medial preoptic area (MPOA), medial amygdala, peripeduncular nucleus, and midbrain and pontine central gray were examined for a reduction of SP staining on the side of the brain ipsilateral to the lesion. The only site where an obvious reduction of staining was observed was the MPOA, including the periventricular POA. Takatsuki et al. (Exp. Brain Res., 1983, 53, 183) reported that lesions rostral to the VMN, in the ventral part of the medial anterior hypothalamus, reduced SP staining in the MPOA. Given the present results, it is possible that their lesions interrupted ascending axons from VMN to MPOA. Further studies combining retrograde tracing of MPOA afferents with SP ICC will be necessary to precisely identify the cells of origin of this innervation.

The functional significance of this SP projection is not known, but it is possible that it is involved in the control of mating-induced prolactin release, as lesions of either VMN or MPOA, or knife cuts between these areas, disrupt this response (Gunnet & Freeman, End. Rev., 1983, 4, 44).

204.12 THE DEVELOPMENT OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE HYPOTHALAMUS OF THE GOLDEN HAMSTER. JOAN M. MURNANE, FRANK D. SABATINO*, ROGER A. HOFFMAN*†, JOHN K. MCDONALD. Departments of Anatomy, Emory Univ. Sch. of Med., Atlanta, GA 30322, and †Biology, Colgate Univ., Hamilton, NY 13346.

High concentrations of Neuropeptide Y (NPY) have been found in the hypothalamus of several species. Previous studies in the rat have demonstrated that neurons in the intergeniculate leaflet (IGL) project NPY labeled axons to the suprachiasmatic nucleus (SCN) (Moore et al., Cell Tiss. Res., 1984). Injections of NPY into the SCN of the Golden hamster (Mesocricetus auratus) phase shift circadian activity rhythms (Albers & Ferris, Nsci. Lett., 1984). In addition, injections of NPY into the third cerebral ventricle of ovariectomized rats decreased plasma levels of luteinizing hormone (LH) and growth hormone (GH), while directly stimulating LH and GH release from pituitary cells in vitro (McDonald et al., PNAS, 1985).

In view of these reports, we have investigated the distribution of NPY-like immunoreactivity in the hypothalamus of adult and developing Golden hamsters. Adult and neonatal animals of both sexes at 2, 3, 6, 8 and 11 days after birth were anesthetized and perfused through the aorta with fixative containing 4% paraformaldehyde, 0.1 M DL-lysine and 0.01 M sodium periodate in 0.05 M phosphate buffer. The brains were sectioned at 40 µm with a freezing microtome and alternate sections were stained with cresyl violet to identify nuclear landmarks. Conventional immunohistochemical methods were used with our own rabbit anti-porcine NPY serum at a dilution of 1:1500. Incubation of the antiserum with 10^{-6} M synthetic porcine NPY prevented staining.

In the adult, labeled perikarya were observed in the arcuate nucleus, and in the ventral lateral geniculate nucleus and IGL of the thalamus. High concentrations of NPY immunoreactive fibers were seen in the SCN, and also in the preoptic (medial and median), paraventricular, periventricular, dorsomedial and lateral hypothalamic nuclei. With the notable exception of the SCN, a network of labeled fibers was present in these nuclei on postnatal day 2 which gradually attained the adult pattern of staining by day 11. The SCN displayed 1 or 2 fibers on the second postnatal day and a striking increase in the number of labeled fibers between days 3 and 11, at which time the adult pattern was observed. These results provide a description of NPY-like immunoreactivity in the hypothalamus of the adult and developing Golden hamster. The functions of NPY in these hypothalamic nuclei remain to be determined. However, the gradual increase in NPY labeled fibers in the SCN during the first eleven days of postnatal life may have important implications for the development of circadian rhythms. Supported by NIH HD 19731, Emory Univ. Research Fund and †Faculty Development Council, Colgate Univ.

204.13 NPY LOCALIZATION IN MONKEY HYPOTHALAMUS. C.L. Shen and R.Y. Moore. Dept. of Anatomy, Col. of Medicine, Natl. Cheng Kung University, Taiwan, Rep. of China and Departments of Neurology and Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, New York, 11794.

Neuropeptide Y (NPY) is a 36 amino acid peptide originally isolated from porcine brain by Tatemoto ('82). It has a wide distribution in rat brain with highest concentrations occuring in hypothalamus (Emson and Quilt, '83). Although no detailed analysis of NPY distribution in hypothalamus has been published, it is likely that a prior study demonstrating avian pancreatic polypeptide (APP) like immunoreactivity in the rat hypothalamus (Card et al., '83) actually showed NPY since the APP antibody actually demonstrates NPY (Moore et al., '84). In the present study, the distribution of immunoreactive structures was identical with the two antibodies and cross blocking studies have shown that they both demonstrate NPY.

Hypothalami were provided by Dr. Anita Hendrickson, University of Washington Regional Primate Center. The animals were perfused with periodate-lysine-paraformaldehyde and 30 um coronal sections through the hypothalamus were processed using the peroxidase-anti-peroxidase procedure of Sternberger ('79).

Immunoreactive axons and terminals were present throughout the hypothalamus, however, with the exception of the ventral division of the bed nucleus of the stria terminalis and the arcuate nucleus , no immunoreactive cells were evident. This contrasts with previous findings in the rat (Card et al., '83) and probably reflects differences in methodology since immunoreactive cells were most effectively demonstrated in rats pretreated with colchicine. Axons containing NPY are densest in the periventricular zone extending from the preoptic area to the caudal tuberal area. A dense plexus of immunoreactive axons is present in the medial preoptic nucleus, ventral tuberal area, arcuate nucleus and in the lateral hypothalamus, particularly in the zone surrounding the fornix. Other areas of hypothalamus are more lightly innervated. In general, the pattern of innervation is quite similar to that of noradrenergic afferents arising from lateral tegmental cell groups. Since many of the lateral tegmental noradrenergic neurons also contain NPY (Everitt et al., '84), these data suggest that a significant portion of the NPY system that we have identified in the hypothalamus may arise from brainstem cell groups which colocalize noradrenaline and NPY. Hypothalamic axons which do not arise from brainstem noradrenergic neurons but exhibit NPY immunoreactivity are also apparent in our material. This is illustrated by thin varicose immunoreactive axons in the suprachiasmatic nucleus that presumably arise from the lateral geniculate nucleus (Card and Moore, '82). Supported by NIH grant NS-16304.

204.14 ULTRASTRUCTURAL FEATURES OF THE HYPOTHALAMIC CORTICOTROPIN RELEASING FACTOR (CRF)-IMMUNOREACTIVE NEURONAL SYSTEM OF LONG TERM ADRENALECTOMIZED RATS. W.K. PAULL and Zs. LIPOSITS (SPON: J.D. Dexter). Department of Anatomy, University of Missouri-Columbia, School of Medicine, Columbia, MO 65212.

Recent light microscopic immunocytochemical studies (Merchenthaler et. al., Reg. Peptides 5:295-305,1983; Paull and Gibbs, Histochemistry 78:303-313,1983) indicated that following long term adrenalectomy (ADX) the paraventriculo-infundibular CRF-system is characterized by an increased hormone content. The present study deals with the ultrastructural aspects of adrenalectomy induced alterations of the hypothalamic CRF-system. The peroxidase-antiperoxidase-complex (PAP)-immunocytochemical method was used in a preembedding manner for the detection of CRF immunoreactivity in both long term (2-3 weeks) adrenalectomized and adrenalectomized-dexamethasone treated (100 µg/100 g.b.w., 24 hours before sacrifice) administered male rats.

CRF-positive neurons located in the medial and dorsal parvocellular subnuclei of paraventricular nucleus (PVN) were completely filled with hypertrophied rough endoplasmic reticulum (rER). The rER demonstrated considerable dilatation of the cisternae. Neurosecretory granules (NSG), in the vicinity of active Golgi complexes, measured 80-120 nm diameter. The CRF-neurons also contained an increased number of multivesicular bodies. CRF dendrites possessed labeled smooth endoplasmic reticulum (sER) and microtubules. Within the PVN, CRF-positive neurons received both axo-somatic and axo-dendritic synapses. In addition, ephaptic contacts were observed to occur between CRF-immunopositive and other CRF-immunopositive, as well as CRF-immunonegative parvocellular cells. The area of apposition was increased due to the retraction of glial processes and the cell membranes were coupled by puncta adherentia.

Axons within the median eminence contained CRF positive NSG's, small vesicles and hypertrophied sER. The preterminal portions of the axons were characterized by a dominance of NSG's, while the greatly enlarged terminals possessed few NSG's. Within the palisade layer the axon terminals made frequent contact directly on the abluminal basal lamina in that ependymal processes were retracted. Occasionally, CRF positive terminals were observed within the pericapillary space of portal vessels.

The short-term glucocorticoid administration further increased the hormone content of paraventricular CRF neurons in adrenalectomized animals.

These data suggest, that: 1) CRF-production is adrenal steroid dependent. 2) Removal of adrenal glands results in hypertrophy of organelles associated with protein synthesis and transport in CRF neurons. 3) Glial and ependymal elements possibly take part in the regulation of the paraventriculo-infundibular CRF-system. 4) In adrenalectomized rats the effect of short term glucocorticoid treatment is opposite to that of the long term administration of the hormone. Supported by NIH grant NS 19266 to WKP.

204.15 INFLUENCE OF THE CENTRAL NUCLEUS OF THE AMYGDALA ON THE CONTENT OF CORTICOTROPIN-RELEASING FACTOR IN THE MEDIAN EMINENCE. L. Dêsy*, S. Beaulieu, M.-C. Tonon*, H. Vaudry*, G. Pelletier and N. Barden (Spon: M. Beaulieu). MRC Group in Molecular Endocrinology, CHUL, Quebec, G1V 4G2, CANADA and Groupe de Recherche en Endocrinologie moléculaire, Faculté des Sciences de Rouen, Mont-Saint-Aignan, FRANCE.

High concentrations of corticotropin-releasing factor (CRF) in the amygdaloid central nucleus (ACE) of different species have been reported by various groups. We have also accumulated evidence that the ACE exerts a stimulatory action on ACTH secretion in response to stress (S. Beaulieu and al., this meeting). The present study was designed in order to investigate whether or not this stimulatory action of the ACE was mediated via modification of the CRF concentration in the median eminence.

Ovariectomized Sprague-Dawley rats (250-300g) were either left intact or submitted to bilateral electrocoagulation lesions of the ACE one or two weeks before perfusion with Bouin's fixative under ketamine anesthesia. Some of the intact and lesioned animals received an intraventricular injection of colchicine (30 µg in 15 µl of isotonic saline) 48 hrs before perfusion. Brains were removed, post-fixed for 18 hrs in the same fixative and embedded in paraffin. Slices (7 um thickness) were mounted on slides and incubated with a specific anti-rat CRF antibody or an anti-rat neurophysin antibody according to the standard peroxidase-anti-peroxidase procedure.

The CRF-like immunoreactivity was markedly decreased in the median eminence of rats bearing bilateral lesions of the ACE performed either one or two weeks before perfusion. Lesion of the ACE did not alter the neurophysin-like immunoreactivity in the median eminence, indicating the integrity of the efferent neurophysin-containing fibers of the supraoptic and paraventricular hypothalamic nuclei. Moreover, in comparison with intact colchicine-treated animals, the number of cells containing CRF-like immunoreactive material in the hypothalamic paraventricular nucleus was higher in colchicine-treated rats bearing two weeks old lesions of the ACE.

These results substantiate the hypothesis that the ACE can influence the content of CRF-like material in the median eminence. Finally, they also indicate that long-term (2 weeks) bilateral lesions of the ACE may trigger an enhancement of the synthesis of CRF in the paraventricular nucleus of the hypothalamus in order to compensate for the decreased CRF content in the median eminence. (Supported by the MRC)

204.16 DISTRIBUTION OF CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRF-LI) IN MICRODISSECTED AREAS OF THE RAT BRAIN. P. B. Chappell*, M. A. Smith, C. M. Anderson*, G. Bissette, C. D. Kilts, and C. B. Nemeroff. (Spon: G. R. Marsh) Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, N. C. 27710

CRF-LI was measured in thirty-six rat brain regions by modification of a radioimmunoassay described in detail elsewhere (Meth. Enzymol. 103:565, 1983) using an antiserum raised in rabbits against synthetic ovine CRF which is directed toward the C-terminal region of the peptide (amino acids 31-41) and a tracer of ^{125}I-Tyr-CRF, purified by HPLC, with the lowest detectable level of CRF-LI being 2.5 pg/tube. Subjects were ten adult male Sprague-Dawley rats in which care had been taken to minimize stress by two weeks of daily handling prior to decapitation. Brains were rapidly removed and frozen on dry ice. Thirty-six brain nuclei were then micropunched from 300 µm coronal brain slices using the microdissection technique of Palkovits. Brain samples were sonicated in 1N HCl and centrifuged; duplicate aliquots of supernatant were lyophilized and assayed for CRF-LI. Protein was determined by the method of Lowry.

Concentrations of CRF-LI, expressed as picograms CRF/mg protein, were detected in all brain nuclei sampled. The highest concentrations were found within the hypothalamus in the median eminence/arcuate nucleus (5054 ± 674), the paraventricular nucleus (498 ± 44), and the periventricular nucleus (316 ± 15). The next highest levels of CRF-LI were found in the dorsal (238 ± 28) and the median (241 ± 17) raphe nuclei and the dorsal motor nucleus of the vagus (193 ± 15). The locus ceruleus which is rich in norepinephrine cell bodies contained 148 ± 9 pg/mg. CRF-LI was well represented in the central (192 ± 17), cortical (148 ± 21), and medial (110 ± 10) amygdaloid nuclei and in the bed nucleus of the stria terminalis (136 ± 14). In the dorsal and ventral hippocampus and in all cerebrocortical regions examined, relatively low levels (12 to 28 pg/mg protein) of CRF-LI were detected. In the midbrain/brainstem, the ventral tegmental area (134 ± 11), the periaqueductal grey (144 ± 18), and the substantia nigra, zona compacta (190 ± 13) were found to contain appreciable concentrations of CRF-LI.

These RIA findings are generally concordant with previous RIA studies of CRF-LI distribution in rat brain as well as with the results of immunocytochemical studies. CRF-LI was found to be widely distributed in the rat CNS as well as in the hypothalamus, and appreciable concentrations were detected in major serotonergic and adrenergic nuclei suggesting that CRF may be involved in the central integration of endocrine, behavioral, and autonomic responses to stress. (Supported by NIMH MH-39415)

204.17 RELATIONSHIP OF CRF-IMMUNOSTAINED CELLS AND MAGNOCELLULAR NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS.
D.T. Piekut and S.A. Joseph. The Neuroendocrine Unit, University of Rochester School of Medicine, Rochester, NY 14642.

Following the isolation and synthesis of corticotropin releasing hormone (CRF), immunocytochemical studies have elucidated the anatomical identification and localization of this peptide in rat brain. In the paraventricular nucleus (PVN) of hypothalamus, a dense accumulation of immunostained neurons containing CRF has been demonstrated in the parvocellular component of this nucleus. A potential modulating role of neurohypophyseal hormones (vasopressin, oxytocin) in the pituitary-adrenal axis has been speculated. In our studies of the PVN, a new immunocytochemical technique in which the glucose oxidase-antiglucoseoxidase complex (GAG) is combined with the PAP or ABC complex is employed to visualize two antigens with contrasting colors in the same tissue section. This dual antigen immunocytochemical staining protocol is utilized to study a potential relationship of perikarya containing oxytocin, vasopressin and CRF. Brains from (a) normal untreated, (b) colchicine treated and (c) adrenalectomized animals are utilized. Results indicate that within the confines of the nucleus separate and distinct populations of cells containing the immunoreactive (ir) elements are seen within the magnocellular and parvocellular divisions in PVN of rat hypothalamus. Examination of dual stained vibratome sections is extended to the one micron level and in both thin and thick sections, results are consistent in the normal untreated, colchicine treated and adrenalectomized rat. CRF-ir cells are for the most part concentrated in the medial parvocellular component of PVN. Immunostained CRF neurons are present in the ventral medial portion of the posterior magnocellular (pm) division in which oxytocinergic neurons predominate; juxtaposed CRF-ir and oxytocin-ir perikarya are identified in the colchicine treated and adrenalectomized animals. Few CRF immunostained cells are seen in the dorsal lateral portion of the pm division in which vasopressin-containing cells are localized. However, an intimate anatomical proximity between CRF-ir and VP-ir perikarya is evident in brains of adrenalectomized animals. The latter is localized in the medial parvocellular division of PVN, an area normally VP-ir poor except in the adrenalectomized rats. This extension of VP-ir cells into this CRF-rich region and the very close approximation between the two cells bodies suggests potential cell to cell communication following pertubation of the brain-pituitary-adrenal axis.

204.18 CRF-IMMUNOREACTIVE NEURONS WITHIN THE INFERIOR OLIVE PROJECT TO THE FLOCCULUS AND DORSAL AND VENTRAL PARAFLOCCULI. S. Cummings, R. Wade and B. Sharp*. Depts. of Anatomy and Medicine, University of Minnesota, Minneapolis, Minnesota 55455

Corticotropin releasing factor (CRF) has been demonstrated by RIA and immunohistochemistry to be widely distributed throughout the central nervous system, in extrahypothalamic regions as well as within the hypothalamic-hypophyseal axis. One rather unexpected observation was the presence of CRF-immunoreactive (CRF-IR) fibers within the cerebellum, an area in which few peptides have been localized. In the present study have examined CRF-IR fibers within the flocculus and dorsal and ventral paraflocculi of cat and monkey cerebellum. In these regions the CRF-IR fibers appear to be intimately associated with the cell bodies and dendrites of Purkinje cells.

There are several nuclei within the brainstem which provide input to the cerebellum and also contain CRF-IR cell bodies. Therefore, we initiated a tract tracing study combining the retrograde transport of the fluorescent dye Fast Blue with the peroxidase-anti-peroxidase method of immunohistochemical detection of CRF in order to explore the possible projections of CRF-containing fibers from these regions. Fast Blue (1%) was injected into the right flocculus and paraflocculi of randomly bred cats (1.5-2.0 kg). After 5 days, colchicine (500 ug) was injected intracisternally. The animals were perfused transcardially with buffered paraformaldehyde 48 hr later. Sections were observed under UV illumination and Fast Blue-labeled cells were photographed. Subsequently, the sections were processed for immunoperoxidase staining using a primary antiserum directed against rat CRF, and then re-photographed. We were able to demonstrate populations of both Fast Blue-labeled cell bodies and CRF-IR neurons within the medial vestibular nucleus, n. prepositus hypoglossi, n. raphe dorsalis, locus ceruleus, and the inferior olivary nucleus. However, comparisons of negatives and photographs revealed co-localization of CRF immunoreactivity and Fast Blue only within neurons of the inferior olive.

These results demonstrate that CRF-IR fibers arising from cell bodies within the inferior olive project to the flocculus and dorsal and ventral paraflocular lobules of the cerebellum. Because the inferior olive is known to be the region from which climbing fibers project to these ventral cerebellar lobules, we suggest that the CRF-IR fibers may represent a subpopulation of such climbing fibers. A study to determine the ultrastructural identity of these CRF-IR fibers is in progress. Demonstration that ovine CRF binding sites are likewise present in the cerebellum, (Wynn et al. Peptides 5:1077, De Souza et al. J. Neurosci. in press) suggests a possible role here for CRF as a neurotransmitter. Supported by DA02148.

204.19 IMMUNOLOCALIZATION OF PEPTIDES CONTAINED WITHIN PROGLUCAGON IN RAT CENTRAL NERVOUS SYSTEM. C. Jin*, A.C. Towle*, V.K.M. Han*, M. Hynes, J.G. Simmons*, J.M. Lauder and P.K. Lund*, School of Medicine, University of North Carolina, Chapel Hill, N.C. 27514.

A Rat pancreatic cDNA was previously shown to encode a large molecular weight proglucagon containing a glicentin related polypeptide (GRPP), glucagon, intervening peptide I (IP-I), glucagon-like-peptide I (GLP-I), intervening peptide II (IP-II) and glucagon-like peptide II (GLP-II) (Heinrich et al. Endocrinol. 115, 2176,1984). GRPP, glucagon and GLP-I are co-localized in the rat pancreatic A cells which is consistent with the structure of proglucagon predicted from the cDNA. In rat brain the predominant glucagon-like immunoreactant (GLI) was shown to correspond to glicentin (Tager et al, PNAS 77, 6229, 1980) which contains GRPP, glucagon and IP-I. In the present study we investigated the distribution of peptides present in proglucagon in rat central nervous system. Rats were perfused with Bouins fixative and 10μ paraffin sections prepared. Coronal brain sections were stained with antisera to GRPP (R64), glucagon (R32) and GLP-I (R1). Immunostaining was detected by the Avidin-biotinylated-peroxidase complex (ABC) method. GRPP and glucagon antisera stained many fibers in medial hypothalamus and mediodorsal thalamus but stained few or no fibers in cortex, striatum and brain stem. In contrast, GLP-I antisera stained only a few fibers in medial hypothalamus. The similarity of staining pattern of thalamic and hypothalamic fibers with GRPP and glucagon antisera is indicative of cosynthesis of these peptides in a brain proglucagon, and axonal transport of both peptides. The low abundance of hypothalamic fibers containing GLP-I immunoreactivity, compared with GRPP and glucagon, raises several possibilities about proglucagon synthesis and processing. Brain and pancreas may synthesize the same glucagon precursor but post-translational processing of proglucagon in brain may result in (a) segregation of GLP-I from GRPP and glucagon so that few neurons transport GLP-I to axonal projections, or (b) transport of GRPP, glucagon and GLP-I to axonal projections but different rates of release and/or metabolism of GLP-I or (c) transport of GRPP, glucagon and GLP-I to axonal projections but lack of recognition of the axonal molecular form of GLP-I in most fibers, by the GLP-I antiserum. Alternatively,hypothalamic neurons or neuronal subpopulations could synthesize a different glucagon mRNA and precursor than pancreas resulting in fewer fibers containing GLP-I immunoreactants compared with GRPP and glucagon. Consistent with this hypothesis, we have detected a 1300 base glucagon-related mRNA in rat brain compared with the characterized 1100 base pancreatic glucagon mRNA. Further investigation of brain glucagon mRNAs, and of the distribution of GRPP, glucagon and GLP-I immunoreactants in neuron cell bodies of colchicine treated rats, should provide further information about proglucagon synthesis and processing in central nervous system.

205.1 CHANGES IN SPONTANEOUS SINGLE UNIT ACTIVITY IN THE PUTAMEN FOLLOWING AREA 4 and 6 ABLATION IN PRIMATES. J.W. Aldridge, S. Gilman & G.W. Dauth, Dept. of Neurology, Univ. of Mich., Neuroscience Lab Bldg., 1103 E. Huron, Ann Arbor, MI., 48104.

The purpose of this study is to examine the changes in spontaneous single unit activity in the Putamen (PUT) after ablation of the motor cortex. The PUT receives a large component of its afferents from ipsilateral motor cortical areas 4 & 6 of Brodmann. The projection is believed to use glutamate as its transmitter. Intracellular studies show that this projection produces EPSP-IPSP sequences that dominate other inputs.

Two M. fascicularis primates were used. Under general anesthesia one control animal and one experimental animal were prepared for chronic single unit recording. In the experimental animal area 4 and 6 were ablated by sub-pial aspiration. Recording chambers were fixed stereotaxically to the skulls of the animals to allow daily recording sessions using tungsten electrodes. Daily recording with the animals sitting quietly in the awake state began 1 week after implantation. After accessible areas of PUT were explored, small lesions were made to mark electrode tracks, the animals were sacrificed and the brains were fixed and sectioned for histological reconstruction of tracks. Only units recorded within PUT were included.

Data were collected for 127 units in the control animal. The data from the experimental animal consist of 82 units collected in the period 8 to 31 days after the cerebral cortical ablation. During this period there was no recovery in the ability of the animal to use the affected limbs. The PUT units in the control animal fired with a mean interspike interval of 571 ms and a modal interspike interval of 16 ms, which indicates a highly skewed distribution. These values reflect the low firing rate and bursting nature of unit activity normally found in the PUT. Units in the animal with the cortical ablation had a mean interspike interval of 302 ms and a modal interval of 111 ms. Thus, there is a decrease in interspike interval (increased firing rate) of 41% and an increase in the modal interval of 594%. These changes demonstrate a new pattern of activity after ablation of areas 4 and 6 in which the units fire in more regular spike trains with fewer and less intense bursts. Loss of an important cortical influence has left the neurons to fire in a pattern that may now be determined by other afferents and the intrinsic properties of the neurons. The greater firing rate indicates that the net change may be decreased suppression due to loss of the potent collateral inhibition induced in putamenal neurons by cortical excitation.
Supported in part by grants from NIH (NS 19613, NS 15655) and the United Cerebral Palsy Research & Educational Foundation (R231).

205.2 ELECTROPHYSIOLOGICAL DEMONSTRATION OF AN INHIBITORY PALLIDOSUBTHALAMIC PATHWAY IN PRIMATES. R.D. HUFFMAN AND L.P. FELPEL. Div. of Neuropharmacology, Dept. Pharmacology, Univ. Texas Health Science Center, San Antonio, TX 78284.

Anatomical studies have demonstrated that the pallidal input to the subthalamic nucleus originates exclusively from the lateral pallidal segment of globus pallidus. Stimulation of globus pallidus in rat and cat has been shown to suppress neuronal activity in subthalamic nucleus (Brain Res. 35:67, 1971; 264:255, 1983); however, excitation of subthalamic and zona incerta neurons following globus pallidus stimulation has also been reported (Brain Res. 35: 67, 1971; Br. J. Pharmac. 65:511, 1979). Stimulation of the medial pallidal segment in monkey has also been reported to depress spontaneous discharge of subthalamic neurons (Brain Res. 111:241, 1976). In the present study, we have employed extracellular microelectrode recording techniques to assess the effects of pallidal stimulation on the activity of subthalamic and zona incerta neurons in primates. Adult Macaca mulatta (2) or Macaca fascicularis (2) monkeys anesthetized with either pentobarbital or chloralose-urethane were employed. Bipolar concentric stainless steel electrodes (6) were employed to stimulate the two pallidal segments. Extracellular recordings were obtained from the 4M NaCl-filled barrel of 2 or 5 barrel glass micropipettes. Glutamate or acetylcholine were ejected microiontophoretically onto subthalamic and zona incerta neurons to provide a steady level of neuronal activity. The effect of pallidal stimulation was assessed on the activity of 12 subthalamic and 21 zona incerta neurons. Two types of inhibition of subthalamic neuronal discharge were characteristically observed following stimulation of the lateral pallidal segment. The most common response consisted of a prolonged depression of neuronal discharge (437 ± 51 msec, n=5). The second type of response consisted of an inhibition-excitation-inhibition sequence. The initial inhibitory period (69.5 ± 16.2 msec, n=3) terminated abruptly in a rebound excitation which was then followed by a second period of inhibition of variable latency and duration. The average latency to inhibition as indicated by suppression of neuronal discharge following pallidal stimulation was 4.8 ± 0.4 msec; the discharge of several subthalamic neurons was suppressed without any evidence of post-stimulus neuronal discharge. Stimulation of the caudal half of the lateral pallidal segment resulted in suppression of activity in the lateral part of the caudal half of the subthalamic nucleus. Stimulation of the medial pallidal segment also evoked inhibition of activity in subthalamic nucleus, but putaminal stimulation occasionally resulted in excitation followed by inhibition. No zona incerta neurons were found that responded either with excitation or inhibition in response to stimulation of either pallidal segment. (Supported in part by NIH Grant NS 14091).

205.3 BEHAVIORALLY CONTINGENT ACTIVITY OF PUTAMEN NEURONS DURING LEARNED MOVEMENT IN THE MONKEY (Macaca fuscata). M. KIMURA
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There are two categories of neurons in the primate putamen; type I with tonic discharges (3-7 Hz) and type II with low spontaneous discharges (< 2 Hz). The type I cells respond to sensory cue for a movement only when the animal initiates the movement by the sensory cue and type II cells exhibit phasic time-locked discharges to the body movement (Kimura et. al., Proc. Nat. Acad. Sci., 81:4998,1984). The present study aimed to examine the activities of these two types of neurons during repetitive arm and orofacial movements triggered by sensory stimulus, especially whether the type II cells have behaviorally contingent responses or not.

Neuronal activity was recorded in the left putamen and somatotopic organization was identified by microstimulation (300 Hz, 10 pulses, < 200 μA) which evoked discrete movement of body musculatures. Two movement tasks were used; (1) 3 repetitive flexion-extension of right elbow across the target for juice reward triggered by the visual command (AM), and (2) repetitive licking movements to consume juice reward triggered by a solenoid click (FR). Type I cells (n=77) showed either excitation followed by inhibition or inhibition in response to the solenoid click (latency 70 msec, 56 cells) which accompanied juice reward in AM and/or FR and to the visual command (90 msec, 38 cells) triggering arm movements in AM. These responses to the sensory cue were evoked only when the monkey initiated learned orofacial or arm movements by the cue. Type II cells (n=67) showed phasic discharges locked to the somatotopically related body movement. However, most type II cells in orofacial area exhibited phasic discharges at only initial period of repetitive licking movements triggered by the solenoid click and 60 % of type II cells in arm area discharged at only first extension or flexion phase of 3 repetitive flexion-extension movements of elbow triggered by the visual cue. All these type II cells showed almost no response at spontaneous movement without the sensory cue. The rest (40 %) of type II cells in arm area exhibited phasic time-locked activities to every 3 repetitive arm movements in AM. The onset of activities of 55 % of all the type II cells in arm area preceded (mean, 46 msec) the EMG activities in m. biceps and triceps brachii. These behaviorally contingent nature of both type I and type II putamen cell activities suggested a role of the putamen in linking sensory stimuli to behavioral responses.

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205.4 CAUDATE UNIT ACTIVITY DURING JAW MOVEMENTS. C. Manetto*, J.S. Schneider, T.I. Lidsky, College of Vet. Med., VPI, Blacksburg, VA 24061.

Neuronal activity was recorded extracellularly in the caudate nucleus (CN) of partially restrained, awake cats. Jaw position and electromyographic (EMG) activity from masticatory muscles were monitored. Rhythmic jaw and tongue movements were elicited by periodically delivering a small quantity of milk to the cat via a tube resting against its upper lip. 60% of CN units changed firing rate during some phase of ingestion. The majority of ingestion-related CN units showed activity changes that were associated with the situational and stimulus events controlling ingestion rather than jaw movements per se. The firing of 49% of these cells was temporally associated with the milk contacting the perioral tissue rather than the subsequent tongue and jaw movements. All of these cells were sensitive to tactile stimulation and had perioral receptive fields. It seems plausible that the ingestion-related activity of this population reflects stimulation of the somatosensory receptive fields by the milk. An additional 16% of cells with ingestion responses had activity that was temporally-related to milk delivery but had no perioral tactile sensitivity. Conceivably these rate changes were conditioned responses.

The activity of 14% of ingestion-related units was temporally associated with jaw muscle activation. Firing rates changed at or after the onset of EMG activity increases. During ingestion, jaw muscle EMG shows rhythmic phasic bursts that are time-locked to the cyclic opening and closing of the jaw. CN units showed no relation to these individual EMG bursts: rather, unit activity changes were tonic. In addition, there was no association between the patterns of CN unit responses and the latency or magnitude of oropharyngeal movements. Moreover, the activity of these neurons typically returned to baseline rates well before the cessation of ingestional movements. Only 22% of these cells had perioral receptive fields.

The remaining 21% of responsive units had activity related to both sensory and motor aspects of ingestion. All had perioral sensitivity but showed responses that were time-locked to EMG activation. However, unit responses were only associated with milk-triggered jaw movements rather than all jaw movements. Thus, these cells showed sensory-related movement responses.

These data underscore the importance of the CN to sensory aspects of motor control. In this connection it should be noted that perioral receptive fields were seen in 70% of the cells with ingestion-related activity. The manner in which the various cell types described here interact as well as the way in which CN processing ultimately influences movement remains to be determined.
(Supported by NS 21418)

205.5 GLOBUS PALLIDUS NEURONS RELATED TO PARAMETERS AND MODES OF MOVEMENT. J.W. Mink and W.T. Thach. Depts. of Anatomy & Neurobiology, Neurology & Neurosurgery, and The McDonnell Center for Higher Brain Function, Washington U. Sch. Med., St. Louis, MO, 63110.

The discharge of single neurons in the globus pallidus was recorded in a rhesus monkey trained to perform 5 wrist movement tasks designed to dissociate several movement modes and parameters. All tasks were performed by flexing and extending the wrist with opposing or assisting torque loads (0.2 Nm). The 5 tasks included (1) a visually cued step tracking task, (2) a visually guided hold-ramp-hold tracking task, (3) a self-paced hold-ramp-hold task with delayed alternation, trained velocity, and no visual feedback of wrist position, (4) a visually guided rapid sinusoidal tracking task, and (5) a self-paced rapid sinusoidal movement without visual feedback of wrist position. Wrist position and velocity were monitored during all recordings and wrist, arm, shoulder and back EMG was monitored periodically. Neurons were recorded from both segments of the globus pallidus, but were similar in the present analysis and are considered together.

The 46 task-related globus pallidus neurons recorded to date showed activity patterns that were highly task dependent. The discharge of 41 neurons (89%) changed during visually guided step tracking movements, 21 neurons (46%) changed during visually guided ramp tracking movements, 20 neurons (44%) changed during the visually guided sinusoidal movement, 4 neurons (9%) changed during the self-paced ramp movement, and 7 neurons (15%) changed during the self-paced rapid alternating movement. 19 neurons (41%) were related to 1 task only and 5 cells (11%) were related to all tasks. For the remaining 22 neurons (48%), the relation of the discharge of a given neuron to one task did not predict its relation to other tasks. The task-dependent differences in the discharge of pallidal neurons was not due to differences of wrist velocity or position or to task-dependent differences of muscle activity.

These results address controversies concerning basal ganglia function. First, we saw no systematic relation to position or velocity, per se; second, more neurons were related to visually guided than to self-paced movements; and third, many pallidal neurons (46%) were related to step tracking ("open-loop") movements and not to ramp tracking ("closed-loop") movements. (Supported by NIH grants RO1 NS12777, T32 GM07200, NS15070, and The McDonnell Center.)

205.6 DIRECTIONAL AND BIDIRECTIONAL SIGNALS FOR MOVEMENT CONTROL IN PALLIDAL AND CEREBELLAR NEURONS. W.T. Thach, J.W. Mink, and S.A. Kane. Depts. Anatomy & Neurobiology, Neurology & Neurosurgery, and The McDonnell Center for Higher Brain Function, Washington University Medical School, St. Louis, MO, 63110.

Do signals relating to movement direction exist in the basal ganglia and cerebellum? Two rhesus monkeys were trained to perform hold-ramp-hold and hold-step-hold visually guided movements by flexing and extending the wrist with and against uniform torque loads (0.2 Nm). In one monkey recordings were made in the globus pallidus internal segment (GPi), external segment (GPe), and deep cerebellar nuclei during ramp tracking and step tracking. In a second monkey neurons were recorded in cerebellar cortex and deep nuclei during ramp tracking only. Wrist position, velocity, force and EMG were recorded simultaneously.

Task related neurons were classified as directional if the discharge rate was reciprocal for opposite movements or increased or decreased during movement in one direction only, bidirectional if the discharge rate increased or decreased during movement in both directions, and mixed if it was directional under one load and bidirectional under the other.

During step tracking, 10 GPi, 31 GPe, and 15 cerebellar nuclear neurons were related to the task. Of the GPi cells, 8 were directional, 1 bidirectional, and 1 mixed. Of the GPe neurons, 10 were directional, 18 bidirectional, and 3 mixed. Of the cerebellar nuclear cells, 1 was directional, 10 bidirectional, and 4 mixed.

During ramp tracking, 4 GPi, 17 GPe, 28 cerebellar nuclear, and 17 cerebellar Purkinje cells were related to the task. In GPi 3 neurons were directional, 1 bidirectional, and 0 mixed. In GPe 5 neurons were classified as directional, 8 bidirectional, and 4 mixed. In cerebellar nuclei, 6 neurons were directional, 14 bidirectional, and 8 mixed. In cerebellar cortex, 1 Purkinje cell was directional, 12 bidirectional, and 4 mixed.

These data suggest that a majority of GPi cells carry information related to direction of movement, independent of which muscles are used to make that movement. These data also confirm previous observations from this laboratory that in steps and ramp tracking most cerebellar neurons have a bidirectional signal. The GPe appears to carry both a bidirectional and a directional signal. We have suggested previously that the bidirectionality of cerebellar neurons is related the activity of spindles afferents, (Schieber and Thach, 1980) but whether the bidirectional GPe cells are also related to spindle afferents is not known. None of the neurons we recorded in globus pallidus and cerebellum had discharge patterns that were obviously related to the EMG of wrist, arm, or back muscles. (Supported by NIH grants RO1 NS12777, T32 GM07200, NS15070, and The McDonnell Center).

205.7 DISTINCTIVE AND TOPOGRAPHICALLY ORGANIZED MOTOR FUNCTIONS OF THE RAT'S LATERAL STRIATUM. M. Pisa. Depts. of Neurosciences and Psychiatry, McMaster Univ., Hamilton, Ontario, Canada, L8N 3Z5.

The aim of this study was to examine the hypothesis of regional heterogeneity of motor functions in the rat's striatum. After being trained in tasks of food-reinforced tongue protrusion and forelimb reaching, male Wistar rats were anesthetized with Equithesin (3.5 cc/kg, i.p.) and assigned to groups (N=6) for bilateral stereotaxic injections of either the axon-sparing somatodendrotoxin ibotenic acid (7.5 ug in .5 ul of PBS vehicle solution) or an equal volume of vehicle solution into either of these regions of the rostral striatum: dorsomedial (DMS), dorsolateral (DLS), ventrolateral (VLS), dorsomedial and ventromedial (DMS + VMS), dorsolateral and ventrolateral (DLS + VLS). Tongue extension and forelimb extension performance in the food-reaching tasks, and times spent on 'normal' (extended) and 'abnormal' (stooped) postures of the neck and trunk during manipulation and eating of 5 g pellets were examined on postoperative Days 2, 15 and 30. The lesion sites were verified histologically after behavioral testing. The behavioral results were as follows: 1) compared with the control rats, the rats with VLS lesions showed a chronic impairment of both tongue and forelimb extension, with no reliable alteration of head and trunk posture during food manipulation; 2) the rats with DLS lesions showed chronic alterations of both forelimb extension and neck-trunk posture, with no reliable impairment of tongue extension; 3) the rats with DLS + VLS lesions showed chronic impairments of tongue extension, forelimb extension, and neck-trunk posture, and these impairments were more severe than those shown by the rats with DLS lesions or VLS lesions; 4) the rats with either DMS lesions or DMS + VMS lesions showed no reliable chronic alterations in any of the measured motor parameters.

These findings are interpreted as follows: 1) in the rat, the lateral, but not the medial, rostral striatum facilitates extension movements of the tongue and the forelimbs, and extension posture of the neck and trunk; 2) the lateral striatum displays a topographically organized gradient of segmental motor control, with the tongue being represented ventrally, the neck and trunk dorsally, and the forelimb in both ventral and dorsal regions; 3) within the lateral striatum, the motor impairments caused by large lesions are more severe than those caused by more localized lesions (regional, mass action effect). The present findings are consistent with anatomical data (Wise and Jones, JCN, 175: 129, 1977) indicating that, in the rat, the striopetal projections of the sensorimotor cortex mainly terminate in lateral regions of the striatum (supported by the MRC of Canada. M.P. is an OMHF Scholar).

205.8 EFFECTS OF ELECTROLYTIC LESIONS IN THE SUPERIOR COLLICULUS, VENTROMEDIAL THALAMUS, OR MIDBRAIN RETICULAR FORMATION ON BEHAVIORAL RESPONSES TO INTRANIGRAL MUSCIMOL. A.A. Baumeister and G.D. Frye, Department of Medical Pharmacology and Toxicology, Texas A&M University, College Station, TX 77843.

Bilateral injection of muscimol into the substantia nigra produces stereotyped behavior, self-injurious behavior (SIB), and analgesia in rats (Pharmacol Biochem Behav, 21(1984), 89; Soc Neurosci Abstr, 10(1984), 414). Among the nigral efferent pathways that may mediate these effects are projections to the superior colliculus (SC), the mesencephalic reticular formation (MRF), and the ventromedial thalamus (VMT) (Brain Res, 175(1979), 191). In the present studies the role of these structures in the behavioral effects of intranigral muscimol was evaluated. Male Sprague Dawley Rats (180-220g) were anesthetized with sodium pentobarbital, and a stainless steel electrode (0.2mm diameter) insulated except at the tip (1mm) was stereotaxically placed in the SC (A 1.75, L^{+} 1.5, V 5), MRF (A 1.0, L^{+} 1.5, V 6.5) or VMT (A 5.5, L^{+} 1.25, V 7.25). A 2mA anodal current was then passed through the electrode for 5 seconds. After two weeks, the animals received bilateral intranigral injections of muscimol and behavior was recorded as previously described (Pharmacol Biochem Behav, 21(1984), 89). In unoperated controls intranigral microinjection of 30ng or 60ng of muscimol produced SIB in 5/10 and 12/17 animals, respectively. Lesions of the SC or MRF completely blocked muscimol-induced SIB. Muscimol produced SIB in 9/11 animals with VMT lesions, and in 6/9, 14/21, and 7/10 animals with sham electrode placement in the SC, MRF, or VMT, respectively. Lesions of MRF increased muscimol-induced stereotyped rearing, sniffing, head nodding, and locomotion. Lesions of the SC increased muscimol-induced rearing and locomotion but blocked stereotyped gnawing. On the hot water tail-flick test, the analgetic effect of muscimol was suppressed by lesions of the MRF. In animals with lesions of the SC the muscimol-induced analgesia was increased at 90 minutes but decreased at 180 minutes from the intranigral injection, suggesting little change in the overall analgetic effect. VMT lesions had no effect on stereotyped behavior, but potentiated muscimol-induced analgesia. These results suggest that analgesia and stereotyped gnawing play an important role in SIB induced by intranigral muscimol. The MRF appears to be important in the expression of muscimol-induced analgesia, while the SC appears to be important in the expression of stereotyped biting and gnawing. The VMT does not appear to play a role in the stereotyped behavior or SIB produced by intranigral muscimol, but may be involved in muscimol-induced analgesia. (Supported by HD-18668 & AA-06223.)

205.9 RAPHE NUCLEI LESIONS BLOCK LATERAL HABENULA INDUCED TURNING BEHAVIOR

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Anatomical evidence indicates that the lateral habenula (LH) projects to the dorsal (DR) and medial (MR) raphe nuclei (Herkenham, M. and Nauta, W.J., J. Comp. Neurol.,187:19, 1979; Stern, W.C. et al,Neuropharmacol.,20:979, 1981). It has been reported that an electrolytic lesion of LH produces turning behavior induced by apomorphine systemic administration (Ahlenius, S., et al, Exp.Brain Res.,47:270, 1982). The aim of the present experiment was to investigate:a) if turning to apomorphine persists after a more specific lesion using kainic acid into LH and b) to find out if the raphe nuclei are involved in the expression of the motor behavior induced by LH stimulation.

Male Wistar rats (150-175 g) were anesthetized with Halothane (0.8-2.0% in 95% O_2- 5% CO_2) and fixed to a stereotaxic apparatus. Kainic acid was used to lesion the LH (50 ng/0.1 µl) or the DR or MR (0.1 µg/0.1 µl). Apomorphine (2.0 mg/kg i.p.) was administered a week after the lesion. Haloperidol (1.0 mg/kg) 30 min prior apomorphine was also administered to a group of lesioned rats. Kainate (1.0 µg/ 0.3 µl) was acutely administered into LH as an stimulating agent. In this case, as soon as the animal recovered from anesthesia the behavior was quantified. The total time for surgical procedure,from exposure to anesthesia to waking up did not exceed 15 min.

As previously reported, apomorphine induced turning behavior in animals with a lesion in LH (60 ± 5,C), DR (61 ± 11, I) or MR (113 ± 29, C), (X ± SD, C= contra, I= ipsilateral, turns/ 30 min). This behavior was blocked by haloperidol. Acute injections of kainate into LH,induced turning (378 ± 34, I) which was abolished after either a lesion in DR (4.4.± 3, I) or MR (0.2 ± 0.4, C).

It can be concluded that the raphe nuclei are necessary for the motor expression induced by LH stimulation. Since it has been suggested that LH may exert some control on raphe neurons innervating the basal ganglia (Soubrie, P. et al, Brain Res., 47: 270, 1982), it remains to be explored if the basal ganglia provides another link for the motor output of LH-induced turning behavior.

This experiment was partially supported by a grant from CONACYT, PNCCBNA 001888.

205.10 DEMONSTRATION OF BEHAVIORAL DA-SUPERSENSITIVITY AFTER CHRONIC HALOPERIDOL TREATMENT USING THE ROTATIONAL MODEL

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To date, behavioral supersensitivity, attributable to striatal DA activity, after chronic antipsychotics has been studied exclusively by quantifying agonist-induced stereotypies with rating scales. The developement of a striatal efferent lesion that blocks the expression of striatally mediated behaviors by Marshall and Ungerstedt (Eur. J. Pharm. 41 361-367, 1977), enables the use of the rotational paradigm to measure neuroleptic-induced DA supersensitivity. This is the first demonstration of increased apomorphine (APO) induced rotational behavior after chronic haloperidol (HAL) in the striatal output lesion model.

Thirty-two C57BL/6J mice were administered an electrolytic lesion in the internal capsule at 6 weeks of age. One week after surgery animals were screened for rotational behavior with 2 mg/kg amphetamine (IP). The mice were separated into 2 groups with approximately equal levels of rotational behavior. One group received 2.5 mg/kg HAL for 30 days, while the other group received vehicle, both in drinking water. The mice were then tested in a 4X4 Latin Square (LS) design, each treatment 1 week apart with doses 0.117, 0.46875, 1.875, and 7.5 mg/kg APO (IP). Mice were maintained on HAL throughout the testing phase of the experiment and 10 days after withdrawal of HAL animals were tested in the same LS design to study the decline of the hypersensitivity. Surgical procedures, behavioral testing, and curve fitting procedures have been described elsewhere (Br. Res. 330 358-363, 1985).

The estimated ED_{50}'s obtained from best fitting logistic curves for control and treatment group in response to APO were 7.3 and 0.99 mg/kg respectively, indicating approximately 7 fold increase in sensitivity. Ten days after HAL withdrawal, animals' response had already diminished and by 17 days post-withdrawal appeared to have reached control levels of response.

Potentially the most interesting finding is the rapid loss of the supersensitivity after withdrawal. This does not agree well with previous reports of longer lasting supersensitivity after chronic HAL and also indicates that increased rotational behavior is evident despite continued HAL administration.

205.11 THE EFFECTS OF SELECTIVE DOPAMINE RECEPTOR SUBTYPE MANIPULATION ON STEREOTYPIC BEHAVIOR.

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Behavioral responses associated with selective dopamine receptor subtype manipulation were studied by treating mice with systemic or intraventricular administrations of selective dopamine agonists and antagonists. The behavioral response to a systemic injection of either 1.0, 5.0, or 10.0 mg/kg of LY 171555, a selective D-2 agonist, was characterized and compared to stereotypic behavior produced by pretreating mice with 1.0 mg/kg of SCH 23390, a selective D-1 antagonist, and a subsequent injection of either 1.0, 5.0, or 10.0 mg/kg of apomorphine (APO), an agonist at D-1 and D-2. SCH 23390 blocked the expression of the oral behavior associated with the higher doses of APO. However, other components of stereotypic behavior, e.g. stereotypic sniffing and an unusual splayed posturing, were still present. Mice injected with any one of the three doses of LY 171555 exhibited similar stereotypic sniffing and posturing. The consistency between the stereotypic behavioral ratings for these two treatments indicate that selective D-2 stimulation produces a reliable behavioral response.

Behavioral responses associated with D-1 receptor stimulation were also studied in mice pretreated with an intraventricular administration of sulpiride (SULP) or spiperone (SPIP), both selective D-2 antagonists, and a subsequent injection of APO. It was recently shown that intraventricular injections of low doses of SULP or SPIP enhance APO-induced stereotypic behavior, characterized by intense licking and biting, and simultaneously produce catalepsy. Though scopolamine reduces and pilocarpine increases catalepsy in these mice, neither treatment blocks the enhanced APO-induced stereotypic behavior or the unusual oral component. These data are consistent with Christensen et al. (Life Sci., 34 1529-1540, 1984) who report that D-1 receptor activity is relatively insensitive to cholinergic manipulation. In a related study we pretreated mice with systemic injections of SPIP in a dose range between 0.0001 - 1.0 mg/kg to examine the effects on APO-induced stereotypic behavior. Doses between 0.005 - 0.01 mg/kg of SPIP enhance APO-induced stereotypic behavior and doses between 0.005 - 0.075 mg/kg produce intense, vacuous licking and biting while also exhibiting an increase in locomotor activity. Doses at or greater than 0.1 mg/kg blocked expression of stereotypic behavior while locomotor activity was substantially attenuated at doses greater than 1.0 mg/kg.

These data suggest that both D-1 and D-2 receptor stimulation mediate the expression of stereotypic behavior and that dopaminergic antagonism at one DA receptor subtype influences the effects produced by stimulation of other DA receptor subtypes.

205.12 STRIATO-NIGRAL ROTATIONAL MODEL IN THE RAT: INTERACTION OF NUCLEUS ACCUMBENS AND STRIATUM.

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Apomorphine-induced Rotational behavior in rodents bearing lesions of striatal efferents (predominantly striato-nigral pathway) provides a control measurement for rotation in response to stimulation of "normosensitive" dopamine (DA) receptors. Subsequent lesion of the contralateral nigro-striatal pathway leads to a 10-40 fold increase in sensitivity to apomorphine. Most such experiments utilize 6-OHDA placements which destroy DA afferents to the nucleus accumbens as well as the striatum. In order to determine whether depletion of accumbens DA contributes to the high estimates of supersensitivity we examined apomorphine-induced rotational behavior after 6OHDA placements which have differing effects on accumbens DA.

Sprague-Dawley rats received electrolytic lesions (2mA,20 sec) directed at the striato-nigral pathway at the tip of the internal capsule. All animals were subsequently screened for rotational behavior with both amphetamine and apomorphine to confirm that the lesion effectively interrupted the striatal output. A dose-respone curve for rotational behavior to apomorphine was then obtained (9-doses at equal log-intervals from .0156 to 8 mg/kg). Subjects then received micro-infusions of 6-OHDA (8ug/2ul) into the MFB, the tail of the caudate nucleus, or bilaterally into the nucleus accumbens. The MFB placement destroys both the accumbens and striatal DA input, while the placement at the tail of the caudate spares the nuclues accumbens DA. Two weeks later, a second dose-response curve was obtained using the same dose-range as above with the exception of the highest dose.

Rats with depletion of both striatal and accumbens DA showed a marked increase in rotation relative to that after the striato-nigral lesion alone (12-20 fold shift in the ED50 depending upon method of calculation). Striatal depletion alone also increased rotational behavior, but only by a factor of 2-4 fold. Bilateral accumbens DA depletion surprisingly led to a reduction, rather than enhancement of rotation, suggesting that either the interaction of accumbens DA activity with that in striatum occurs via striatal efferents other than those lesioned in this study or that nucleus accumbens contributes a directional and activational component to rotational behavior. Regardless, these data show that quantitative estimates of supersensitization are highly sensitive to accumbens depletion. The 2-4 fold shift following selective striatal denervation is much more consistent with the typical 20-40% increase in DA receptor number.

205.13 LACK OF BROMOCRIPTINE-INDUCED ROTATIONAL BEHAVIOR AFTER UNILATERAL STRIATAL EFFERENT LESION R.K. Easley, R.J. Mandel, and P.K. Randall (Spon. D.F. Lindsley). Depts. Psychology and Physiology and Biopyhsics, U.S.C. Andrus Gerontology Ctr., Los Angeles, CA 90089-0191.

Bromocriptine (BROMO) was first regarded as a direct acting dopamine (DA) agonist because its injection resulted in contralateral rotation in a unilaterally 6-OHDA lesioned rat. However, BROMO induced rotation is inhibited by pretreatment with the tyrosine-hydroxylase inhibitor, a-methyl-p-tyrosine (AMPT). This experiment was designed to examine whether BROMO-induced rotation exhibits the same characteristics when the rotation is mediated by normosensitive DA receptors (DAr).

Eight, 6-week-old, male, C57BL/6J mice were lesioned in the striatum with 6-OHDA. This group was included to demonstrate that mice respond to BROMO in a similar manner as do rats. Electrolytic lesions of striatal efferent pathways were administered to 16 mice and the behavioral effectiveness was confirmed with both amphetamine (AMPHET) and apomorphine (APO) screening tests. All lesion and behavioral testing procedures have been described previously (Mandel and Randall, Br. Res., 330 358-363, 1985). The 6-OHDA lesioned group received 5.0 and 10.0 mg/kg of BROMO (SC) while the "output" lesioned group received vehicle, 5.0, 10.0, and 30.0 mg/kg BROMO (SC). Both groups were tested in a crossover design for each dose with 100.0 mg/kg (IP) AMPT 2 Hr. and 1/2 Hr. before BROMO.

As expected, animals with unilateral 6-OHDA lesions responded to BROMO by rotating contralaterally after a 90-120 minute onset delay. This effect was inhibited by AMPT pretreatment. Mice with unilateral striatal output lesions, however, did not show an asymmetry in response to BROMO at any dose as compared to vehicle injection but did show behavioral hyperactivity at similar onset latency as the 6-OHDA group. The hyperactivity was also completely attenuated by AMPT pretreatment.

These data indicate that BROMO is behaviorally active as a DA agonist but its action is clearly different from AMPHET or APO in preparations with either normo- or supersensitive DAr. The peculiar characteristics of BROMO-induced behavior may be related to its apparent selectivity for the D-2 DAr (Gershanik et al., Br. Res., 261 358-360, 1983). Such selectivity may predispose BROMO to affect particular striatal efferents (Herrera-Marschitz and Ungerstedt, Br. Res., 323 269-278, 1984) in different preparations or, equally plausible, BROMO may preferentially stimulate DAr in the n. accumbens in the output model.

205.14 ROTATION IN RATS AFTER UNILATERAL INFUSION OF HEMICHOLINIUM-3 INTO THE CAUDATE NUCLEUS. L.L. Wing, K.E. Asin and D. Wirtshafter. Dept. of Psychology, Univ. of Illinois at Chicago, P.O. Box 4348, Chicago, IL 60680.

Considerable evidence supports the existence of an interaction between central cholinergic and dopaminergic systems. For example, anticholinergics both attenuate the catalepsy induced by dopamine (DA) receptor blockers in experimental animals and reduce some of the symptoms of Parkinson's disease in man. Although data from a number of other behavioral situations are also compatible with the notion of a cholinergic/dopaminergic blance, little is known as to the anatomical locus of this interaction. It is possible that cholinergic mechanisms within the striatum itself may modulate the effects of dopaminergic activity or it is possible that the interaction might take place elsewhere.

In an effort to further investigate the role of cholinergic striatal functioning in behavior, we made unilateral infusions of the reversible choline uptake blocker hemicholinium-3 (HC-3) into the caudate nucleus of rats and observed spontaneous and amphetamine-induced rotation in these animals. Unilateral infusions of one microliter of distilled water containing various doses of HC-3 (10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, 0.075, 0.03, 0.015, 0.003, 0.0015 and 0.00075 ug) or the vehicle alone were made into the caudate nucleus of rats which were operated on under ether anesthesia. Biochemical studies have demonstrated that intracranial injections of HC-3 produce a depletion of brain acetylcholine (ACh) which is maximal between 3 and 4 hours following injection.

Spontaneous rotation was observed for a ten minute period 3.5 hours post-operatively. Animals were then given an intraperitoneal injection of 4 mg/kg amphetamine, and tested 15 minutes later for 10 minutes. Net rotation scores were calculated by subtracting the number of ipsilateral turns from the number of contralateral turns. No net spontaneous rotation was observed in vehicle-treated controls or in HC-treated animals. When tested after an injection of amphetamine, however, animals treated with doses of HC-3 greater than or equal to 0.003 ug displayed significant net contralateral rotation (e.g., 0.3 ug HC-3: mean net rotation=20.8), while animals treated with lower doses of HC-3 or with vehicle showed no such effect.

Another group of rats treated with 0.3 ug HC-3 or the vehicle alone and tested 24 hours post-operatively did not demonstrate either spontaneous or amphetamine-induced rotation, thus demonstrating the transient nature of the effect of HC-3.

These results support the notion of DA/ACh interactions in the striatum.

Data from apomorphine-induced rotation studies will also be presented.

Supported by UIC Campus Research Board.

205.15 SHORT-TERM EFFECTS OF DOPAMINE-DEPLETING BRAIN LESIONS ON SPONTANEOUS ACTIVITY OF TYPE II STRIATAL NEURONS: RELATION TO LOCAL STRIATAL DOPAMINE LEVELS AND BEHAVIOR. W.B. Orr, T.W. Gardiner, E.M. Stricker, M.J. Zigmond, and T.W. Berger. Departments of Psychology and Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Striatal dopamine (DA) depletion in animals results in profound deficits in sensorimotor and consumatory behaviors, but only if the loss of DA is greater than 90% (Zigmond and Stricker, Life Sci. 35: 5-18, 1984). Electrophysiological studies show that the spontaneous activity of striatal neurons increases after treatments that deplete striatal DA. If striatal neuronal activity is related to the behaviors disrupted by DA-depleting lesions, changes in spontaneous activity of striatal neurons should occur only when DA depletions exceed 90%.

Spontaneous activity of single striatal neurons (n=687) was recorded 5 to 9 days after rats were given intraventricular (ivt) injections of 100 ug (n=5), 180 ug (n=9) or 250 ug (n=12) 6-hydroxydopamine (6-HDA), or intranigral injection of 16 ug (n=12) of 6-HDA, to induce different levels of DA depletion. Vehicle-injected (ivt, n=12) and nontreated (n=15) control groups also were prepared and used for recording. All except nontreated animals also received pretreatments of pargyline (40 mg/kg, ip) and desipramine (25 mg/kg, ip). Behavioral testing included a latency measure of front paw akinesia and daily consumption of water and food. Because this laboratory has shown that 6-HDA administered ivt results in a medio-lateral gradient of DA depletion (Onn et al., Soc. Neurosci. Abstr. 8: 743, 1982), cells within both medial and lateral regions of striatum were recorded from all groups. Type II striatal neurons were located by their orthodromic activation in response to neocortical stimulation. Striatal tissue punches were removed from medial and lateral regions for subsequent determination of DA concentration.

Type II neuron activity was significantly increased in medial and lateral regions of striatum only when local DA depletions exceeded 90%. Additionally, severe disruption of sensorimotor and consumatory behaviors always was associated with medial striatal depletions of greater than 90%. For example, animals given 180 ug 6-HDA showed behavioral dysfunctions even though no change was observed in the spontaneous activity of Type II neurons in lateral striatum and DA depletions in that region were 89.9% of vehicle-injected controls. Thus, spontaneous activity in medial, but not lateral, Type II striatal neurons appears to be related to the behaviors disrupted by DA-depleting brain lesions. Supported by NIH (NS19608).

205.16 A STRATEGY FOR SEPARATING BEHAVIORALLY-RELATED VS. DRUG-RELATED CHANGES IN UNIT ACTIVITY IN FREELY MOVING RATS. M.O. West, A.J. Michael, J.K. Chapin and D.J. Woodward. Univ. of TX Health Science Center, Dallas, TX.

We have previously shown that the activity of units in the rat neostriatum during locomotion increases following injection (i.p.) of d-Amphetamine (d-A) (Michael et al., Neurosci. Abstr. 1984, 10:350). This effect was presumably mediated by direct actions of d-A in the nigrostriatal system, i.e., to augment dopaminergic activity. However, even though locomotor behavior following d-A injection appeared similar to control locomotion (following saline injection), drug-induced changes in movements during locomotion could alternatively have been responsible for a portion of the increased striatal unit activity. Our current aim is to develop a strategy for separating behaviorally-related vs drug-related changes in observed unit activity.

An essential feature of the strategy is to minimize behaviorally-related differences by generating behaviors before drug injection which were similar to those exhibited by the animal after drug injection. This was accomplished by utilizing a simple treadmill locomotion paradigm in conjunction with doses of d-A known to increase locomotor behavior in rats (0.5-1.0 mg/kg). However, since these doses produce behaviors in addition to locomotion (e.g., rearing, or increased sniffing and exploring), a further tactic is to examine correlations between these discrete behaviors and unit activity. The magnitude and direction of such correlations indicate the extent to which changes in unit activity are a direct result of behavior. Alternatively, the absence of such correlations indicates that changes in unit activity may be due to a more direct action of the drug on the neural circuit under investigation.

In initial applications of this procedure, we have focused on rearing behavior (the animal's shifting to a vertical posture supported on its hindlimbs), which consistently increases in frequency following d-A administration. Peri-event histograms of caudate unit activity were constructed around the onset of rearing. Neither the magnitude nor the direction of changes in unit activity related to rearing could have accounted for the increased firing rates following d-A administration. This evidence, in addition to the fact that locomotor movements were similar before and after drug injection, suggests that the increased caudate unit activity was not related to observable changes in behavior. Rather, the increased firing rates may have been due to drug-induced changes in the animal's internal behavioral state or to more direct changes in central catecholaminergic activity. We anticipate that this approach will prove useful in a continuing analysis of synaptic activity in the basal ganglia. Supported by DA02338, NIAAA 3901, and the Biological Humanics Foundation.

205.17 ANATOMICAL, PHARMACOLOGICAL AND PHENOMENOLOGICAL CHARACTERISTICS OF MUSCIMOL-INDUCED CIRCLING ELICITED FROM THE VENTRAL MESENCEPHALON: COMPARISONS WITH MORPHINE-INDUCED CIRCLING. Larry J. Holmes* and Roy A. Wise. (SPON: Michael Bozarth) Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec.

In a previous study we reported that unilateral ventral mesencephalic morphine injections induces naloxone-sensitive, dopamine-dependent contralateral circling. The best site was localized in the ventral tegmental area. The behaviour involved forward locomotion and resembled exploration with a bias in the direction of turning. The present study investigated the anatomical, pharmacological and phenemonological characteristics of muscimol-induced circling elicited from the same brain regions. Muscimol (25,50 or 100 ng) was applied unilaterally to the ventral mesencephalon in rats; it caused contralateral or ipsilateral picrotoxin-sensitive circling, depending on the site of injection. Muscimol induced strong contralateral circling when applied to the rostral or caudal substantia nigra pars reticulata (SNR) and weak ipsilateral circling when applied to the zona compacta of the substantia nigra (SNC); only the ipsilateral circling was blocked by pimozide pretreatment. There was very little circling noted when injection sites were located outside of the medial substantia nigra; when circling was recorded from extra-nigral sites, it was only with the highest dose of muscimol. The phenomenological characteristics of muscimol-induced contralateral circling differed from morphine-induced contralateral circling in several ways: 1) in contrast to morphine-induced circling, muscimol-induced circling was accompanied by a strong postural asymmetry (even when the dose was adjusted to produce the same rate of circling) 2) muscimol-induced circlers reared less often than morphine-induced circlers but engaged in more stereotypic mouth movements 3) whereas the direction of morphine-induced circling could be reversed by environmental contingencies, muscimol-induced circling could not. These studies suggest that three types of circling can be elicited from the ventral mesencephalon. Morphine-induced contralateral circling is throught to activate dopaminergic cells, muscimol-induced ipsilateral circling is thought to inhibit dopaminergic cells and muscimol-induced contralateral circling is thought to result from activation of striato-nigral GABAergic systems which seem to serve as output pathways mediating dopamine-induced circling.

CEREBELLUM: ANATOMY, PHARMACOLOGY AND CELLULAR PHYSIOLOGY

206.1 FUNCTIONAL TOPOGRAPHY OF THE HUMAN CEREBELLUM, DEMONSTRATED WITH POSITRON EMISSION TOMOGRAPHY. Peter T. Fox* and Marcus E. Raichle, (Departments of Neurology and Radiology), (Washington University School of Medicine, St. Louis, MO 63110)

Physiological activation within the human cerebellum during motor tasks and sensory stimulation was observed in 15 normal volunteers using positron emission tomographic (PET) measurements of regional brain blood flow (rBF). 15-O-labeled water administered by intravenous bolus was used as a blood flow tracer (J Nucl Med 24:782). Each subject underwent a series of 8 sequential, 40-sec, BF emission scans with an inter-scan delay of 8-10 min (for isotope decay). No stimulus was given during the initial or final scans in a series. Activation paradigms were performed during the intervening 6 scans, with task onset preceeding scan initiation by 40-60 sec. A finger movement task (FM; n = 15) consisted of bilateral, 2 Hz, alternating flexion-extension of the fingers (opening & closing the hands). An eye movement task (EM; n = 7) consisted of alternating (left-right), 2 Hz, large amplitude (90 degrees) saccadic eye movements. A finger stimulation task (FS; n = 8) consisted of passively-received vibration (2 mm amplitude; 130 Hz) of the pads of all fingers on one or both hands. All responses were computed as the % change in rBF from the resting-state value (J Neurophysiol 51:1109). Anatomical localization within PET images was determined stereotactically (J Comput Assist Tomogr 9:141).

Focal increases in cerebellar BF occurred during each of the three tasks. Digital-task (FM, FS) responses lay in anterior, superior cerebellum, probably in anterior-lobe cortex. In both instances, the responsive loci were parasagittal (mean = 13 mm from midline), with no significant difference in location of FM and FS responses. Response magnitudes did differ, with FM responses (mean = 20%) exceeding FS responses (mean = 8%), although cerebral responses to the two tasks were equal (mean = 29%). Unilateral task performances (FS) produced solely-ipsilateral cerebellar rBF responses, and solely-contralateral responses in cerebral sensorimotor cortex. The oculomotor task (EM) induced a single, midsagittal response in the posterior vermis (mean = 13%). Thus, a consistent topography was observed, with digital activation lying lateral and anterior to oculomotor activation.

To our knowledge, this is the first demonstration of physiological activation of the human cerebellum by any technique. A technique allowing rapid, non-invasive mapping of human cerebellar responses to voluntary movement and sensory stimulation now exists.

206.2 AN ANALYSIS OF THE RECURRENT COLLATERALS OF PURKINJE CELLS IN ZONE B OF THE CAT'S VERMIS. G.A. Bishop, D.L. O'Donoghue and J.S. King. Department of Anatomy and Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio 43210.

Purkinje cells in Zone B of the cat's vermis were physiologically characterized on the basis of their receptive fields and intracellularly injected with horseradish peroxidase. The distribution pattern of the collaterals of these cells was reconstructed from serial sections and illustrated in camera lucida drawings. Typically, one or two primary collaterals arise from the Purkinje cell axon as it courses through the granule cell layer. Primary collaterals recur toward the Purkinje cell-granule cell border and arborize into four to six secondary branches which form a varicose plexus along the bases and apices of adjacent Purkinje cells. In addition, some beaded branches distribute to: 1) the superficial and deep granule cell layer; and 2) the lower molecular layer. Two distinct patterns of distribution are observed with respect to the sagittal and transverse distribution of the collaterals. In one pattern, the collaterals are confined to the immediate area of the cell of origin, extending approximately 400 um in the sagittal plane and 200 um in the transverse plane. The second pattern is more complex. The major portion of the collateral arborizes within the immediate vicinity of the parent cell in both planes of section. However, one or two branches course up to 480 um medial or lateral to the cell of origin. Often, these latter branches terminate in the same transverse plane as the major portion of the collateral. However, in other cases, beaded branches arborize in spatially remote areas in both the sagittal and transverse planes relative to the cell of origin. In general, the sagittal distribution pattern for both classes of collaterals does not form a continuous band as there are regions which are devoid of varicosities interspersed between varicosity rich areas. Data derived from physiological studies (Oscarsson, TINS, '79) have suggested that Zone B of the cat's vermis can be subdivided into 5 microzones. The present anatomical account suggests that the collaterals of Purkinje cells could subserve different functions in processing information in Zone B. One population may process information within a single microzone. Purkinje cells with the second, more extensive pattern of distribution may not only participate in intrazonal circuitry, but also have the potential of participating in the local circuitry of adjacent microzones and possibly of adjacent zones. The presence of varicosities in all three cortical lamina and their discontinuous pattern of distribution suggest that the collaterals have multiple discrete foci of influence on diverse populations of cortical neurons. (This work was supported by NS 18028 and 08798).

206.3 INDUCED RESPONSES OF TRANSMEMBRANE POTENTIAL TO EXTERNALLY APPLIED ELECTRIC FIELDS IN TURTLE PURKINJE CELLS. C. Y. Chan, J. Hounsgaard* and G. Nicholson. Panum Inst. Neurophysiol., Copenhagen Univ. (Denmark); Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Externally applied low-frequency electric fields modulate the firing rate of turtle cerebellar neurons (Chan & Nicholson, 1985, J. Physiol. in press). Since Purkinje cells have nonhomogeneously distributed membrane properties, the modulation mechanism was investigated. We studied the effect of 0.1 Hz sinusoidal electric fields on the transmembrane potential (TMP) and electrophysiological responses in Purkinje cells.

Constant fields (5 mV/mm–250 mV/mm) were induced in the pia-ventricle axis of an isolated turtle cerebellum bathed in Ringer solution. TMP was determined at various depths by subtracting extra- from intracellular potential recorded at matched depths. TMP recorded from deeper levels (>210 μm deep) showed depolarization when the field was ventricle-directed (V-phase) and hyperpolarization when the field was pia-directed (P-phase). The opposite occurred for upper levels. The degree of polarization was greater at distal or proximal than at mid levels of the Purkinje cell. TMP was generally field strength-dependent, but distal TMP showed a flattening or reversal of the depolarization at P-phase when presumed Ca^{2+} spikes appeared; deep TMP showed similar flattening of depolarization at V-phase when presumed Na^{+} spikes occurred at high rate, or during inactivation. The minimum field eliciting Na^{+} spikes was 2-4 times lower than that for Ca^{2+} spikes. Outward currents injected through the intracellular electrode also induced flattening of otherwise sinusoidal TMP. Injected currents had synergistic or compensatory interaction with the field, consistent with simple polarization of membrane by the field. At all levels, responses from climbing fiber (CF) stimulation were diminished in the V and increased in the P phase, suggesting the response to be an EPSP generated proximally, but near the peak of the P-phase, the response usually failed, suggesting CF conduction block. Responses to surface parallel fiber stimulation were small but larger at the V than P phase and showed no failures. Mossy fiber responses were much smaller than CF responses and were largest at the P phase, sometimes showing an IPSP after the EPSP.

These results show that applied fields can directly modulate the behavior of neurons through interaction with nonhomogeneous membrane properties. Additionally, the use of such extrinsic fields provides a potent tool for the study of localized membrane excitability. [Supported by grant NS-18287 from NINCDS].

206.4 BURST FIRING PROPERTIES OF DEEP CEREBELLAR NUCLEI CELLS STUDIED IN THE ISOLATED GUINEA-PIG BRAINSTEM CEREBELLUM, IN VITRO. M. Mühlethaler* and R. Llinas (SPON: L. Kaufman). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Electrophysiological properties of the deep cerebellar nuclei (CN) were investigated by intracellular recordings from the in vitro guinea pig brainstem cerebellar preparation (Llinás et al., Fed. Proc. 40:2240, 1981). These neurons are known to receive a GABA-mediated monosynaptic IPSP from Purkinje cells (Ito et al., Exp. Brain Res. 10:64, 1970). To evoke such IPSPs, an array of concentric bipolar stimulating electrodes was introduced in white matter overlaying the nuclei. Stimulation at these sites generated IPSPs in CN cells which were occasionally preceded by an antidromic invasion and/or a small EPSP. The EPSPs probably resulted from the activation of climbing or mossy fiber collaterals to the nuclei. The IPSP displayed a powerful summation at frequencies in the 300-500 Hz range and could be reversed by injection of hyperpolarizing current. In the presence of harmaline, which induces oscillations in the olive, the occurrence of spontaneous oscillatory IPSPs in CN neurons indicated the preservation of the entire olivo-cerebellar circuitry (Mühlethaler & Llinás: Soc. Neurosci. Abst., 1984). These spontaneous IPSPs could also be reversed by hyperpolarizing current. More interesting, however, was the fact that these IPSPs were followed by a rebound excitation in the form of a burst of action potentials. This rebound which is voltage- and time-dependent could be mimicked by injection of hyperpolarizing pulses through the recording microelectrode. Depolarizing current pulses delivered at different levels of membrane hyperpolarization revealed a low threshold calcium spike (LTS) similar to that originally described in the olive by Llinás & Yarom (J. Physiol. 315:549, 1981). However, the amount of hyperpolarization needed to reach threshold for de-inactivation was 10-20 mV negative to rest potential, resembling the rebound described in Substantia nigra (SN) where it appears to be of dendritic origin (Llinás et al.: Brain Res. 294:127, 1984). LTS in CN are probably calcium-mediated; they are TTX-resistant but completely abolished by cobalt, as are the LTS in the olive, thalamus and SN. From the above, we propose that the CN, as the thalamus (Jahnsen & Llinás, J. Physiol. 349:205, 1984), have two modes of signalling, (1) as a relay nucleus for mossy and climbing fiber collateral inputs modulated by Purkinje cell inhibition, and (2) as an oscillator when olivary neurons fire in synchrony. Indeed the resultant spike bursts produce powerful IPSPs in the nuclear cells due to Purkinje cell activity. This serves to activate the LTS, resulting in a rebound excitation which may serve as a resonant throughput, most probably involved in phasic motor organization. [Supported by USPHS grant NS13742 from NINCDS]

206.5 SOME PROPERTIES OF AN ORGANOTYPIC CULTURE MODEL OF THE CEREBELLUM R. Kapoor*, C. B. Jaeger and R. Llinás (SPON: S. M. Simon). Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

We have maintained rat cerebellar slices in organotypic cultures using the roller tube technique (Gähwiler: J. Neurosci. Methods 4:329, 1981). Modifications were used in an effort to duplicate some of the morphological and physiological observations made in the mature in vivo cerebellum and in acute cerebellar slices. Thus, 450 μm parasagittal slices were taken from rats aged P9-12. Our studies were performed following 5-10 days culture in vitro, when the slices were less than 100 μm thick, but the overall foliar architecture was preserved.

The intradendritic and intrasomatic recordings obtained from neurons in the cultures were in every way similar to those described for somatic and dendritic impalements of Purkinje cells in acute cerebellar slices (Llinás & Sugimori: J. Physiol. 305: 171 & 197, 1980). The mean resting potential was 62.1 mV for an n of 11 ± 7.19. The input impedances were somewhat higher than previously reported, with a mean of 57 MΩ (n = 8 ± 11.2). Selective channel blockers showed that both Na- and Ca-dependent action potentials were present. Indeed, dendritic spikes were characterized by two types of electroresponsive properties. All-or-nothing fast spikes of low amplitude which were blocked by tetrodotoxin (TTX) and were previously demonstrated to be generated at somatic level and electrotonically conducted to the dendrite. The second type of spike was larger in amplitude and in duration, was not TTX-sensitive but was blocked by 0.5 mM Co or Cd added to the bath. This dendritic Ca electroresponsiveness is also quite similar to that originally described in the mature cerebellar slice. At somatic levels the large action potentials were Na-dependent and Ca spikes were electrotonically conducted from dendrites. The neurons also showed anomalous rectification and a high level of spontaneous excitatory and inhibitory unitary postsynaptic potentials. These potentials could be reversed by outward and inward transmembrane current injection, respectively. Moreover, IPSPs evoked by local stimuli showed a similar time course as those in vivo.

Intracellularly labelled Purkinje cells had oriented dendritic trees and characteristic dendritic spine structures. Their morphology resembled that of age P10 Purkinje cells in vivo. Other neuronal and non-neuronal cell types were revealed in the slice cultures by silver stains and specific localization of astroglia.

Short-term slice culture, as performed in this study, may be employed advantageously in the preservation of physiological response patterns and of morphological structure in this cortex. [Research supported by USPHS grant NS14732 from NINCDS]

206.6 DIRECT DEMONSTRATION OF DENDRITIC INHIBITION IN CEREBELLAR PURKINJE CELLS IN VITRO. M. Sugimori and R. Llinás. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York NY 10016.

Intrasomatic and intradendritic recordings were obtained in guinea pig cerebellar slices (Llinás & Sugimori: J. Physiol. 305, 1980). In addition to voltage recording, glutamic acid and GABA-filled microelectrodes were simultaneously utilized for iontophoresis in order to test the interactions between a putative excitatory and inhibitory transmitter on neuronal integration. As reported previously (Sugimori & Llinás: Soc. Neurosci. Abst., 1983), iontophoresis at dendritic level can generate tetrodotoxin-resistant calcium-dependent spikes. These responses are characterized by either a gradual plateau-like potential and/or by the generation of clear all-or-none spikes which can be shown to be generated at different sites in the dendrite. Here we report that iontophoresis of GABA from a second electrode in the immediate vicinity of the first was shown to block very specifically the calcium-electroresponsiveness (both the plateau and the dendritic spike components). This powerful inhibition could not be totally accounted for on the basis of increased conductance to chloride. This suggests that GABA may have, in addition to an inhibitory effect via chloride conductance, a direct effect on voltage-dependent calcium channels. In order to test this possibility, experiments were done such that the conductance change produced by GABA inhibition could be measured either at somatic or dendritic level. Following the application of picrotoxin, which blocks the GABA-dependent chloride channels (Takeuchi & Takeuchi: J. Physiol. 205, 1969) a marked reduction of the chloride conductance change was observed; however, a sizeable inhibition of calcium spikes could still be seen. Finally, in situations where the g_{Na} was not blocked, glutamic acid injection at the dendritic level was accompanied, at somatic level, by both sodium- and calcium-dependent spikes. Upon iontophoresis of GABA and the dendrites, there was a marked decline in calcium electroresponsiveness without obvious modification of the sodium spike properties observed at the soma. It is suggested, therefore, that dendritic inhibition of Purkinje cells by GABA can regulate chloride conductance as well as have a certain degree of direct action on the voltage-dependent calcium channels. [Supported by USPHS grant NS13742 from NINCDS]

206.7 DIVERSITY AMONG PURKINJE CELLS IN THE MONKEY AND RAT CEREBELLUM. J.M. Gossels*, C.L. Chatot*, B. Blanchard Owens*, M.P. Ogren* and V.M. Ingram. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA, and Dept. of Ophthalmology, Children's Hospital, Boston, MA 02115.

A monoclonal antibody (B1) produced as an antibody against rat embryonic forebrain membranes shows specific and striking immunohistochemical staining of Purkinje cells in the monkey cerebellum in a pattern of broad parasagittal alternating bands of B1 positive and B1 negative cells. In addition, some neurons of the deep cerebellar nuclei, the motor cortex and the spinal cord stain positively.

The adult rat cerebellum also shows B1 staining of neurons of the deep cerebellar nuclei and selective staining of Purkinje cells. The rat Purkinje cell dendrites, unlike those of the monkey, do not stain. In neonatal rats the cerebellar neurons are not yet reactive.

The parasagittal banding pattern of B1 positive Purkinje cells in the monkey is quite different from the patterns for other antigens - neurotransmitters, neuropeptides, etc. - previously reported by others in the rat. For example, in the vermis of the adult monkey B1 positive Purkinje cells cluster at the edges of the lobes, whereas the center is occupied almost exclusively by B1 negative cells. Our findings in the adult rat resemble our findings in the adult monkey and are also different from those reported in the literature for other antigens.

206.8 **CEREBELLAR TRANSMITTER RECEPTORS: AUTORADIOGRAPHIC LOCALIZATION IN NEUROLOGICALLY MUTANT MICE.** A. Rotter and A. Frostholm. Department of Pharmacology, California College of Medicine, University of California, Irvine, CA 92717.

An important problem in chemical neuroanatomy concerns the identification of transmitter receptor phenotypes of specific neuronal populations, and the cellular factors influencing their expression. To address these questions we have chosen the cerebellum as a model system. The cerebellum is particularly suitable since it contains several types of transmitter receptors, and it has a limited number of neuronal cell types whose connections are known. Neurological mouse mutants with specific defects in cerebellar structure are available, which make it feasible to correlate the absence of a specific cell group with the loss of a particular population of receptors. In addition, the consequences of disrupted cerebellar circuitry on the expression of receptor phenotypes can be studied.

We have studied the distribution of transmitter receptors in the cerebellae of "weaver", "staggerer", "Purkinje cell degeneration (pcd)" and "reeler" mutants. Autoradiography of receptor bound ligands was used to visualize receptors for muscarinic acetylcholine (^{3}H-quinuclidinyl benzilate), adenosine (^{3}H-cyclohexyladenosine), benzodiazepine (^{3}H-flunitrazepam), gamma-aminobutyric acid (^{3}H-muscimol), histamine (^{3}H-mepyramine) and noradrenaline (^{3}H-dihydroalprenolol) following procedures described by Young and Kuhar (Brain Res. 175,255, 1979). In all cases, cerebellar sections from mutant mice were mounted side by side with sections from normal background strain mice to assure identical processing for the two groups.

In the "weaver" cerebellum, in which granule cells have degenerated, a reduction in the density of muscarinic acetylcholine, adenosine and GABA receptors, no change in beta-adrenergic receptors and a slight increase in the density of benzodiazepine and histamine receptors were observed. In the "pcd" cerebellum, benzodiazepine, beta-adrenergic and histamine receptors were reduced in density, while muscarinic acetylcholine, adenosine and GABA receptor levels were unaffected. In the "staggerer" cerebellum, granule cells fail to form synapses with Purkinje cells, this is probably due to a defect in the latter. Receptor densities are normal in this mutant with the exception of benzodiazepine and GABA receptor levels which were decreased. In the "reeler" cerebellum, there is extensive neuronal malpositioning; however, the majority of synaptic connections maintain their specificity. Receptors in "reeler" display normal densities, despite a disruption in their laminar distribution. Our studies describe the cellular localization of transmitter receptors in the rodent cerebellum, and demonstrate that receptor densities are influenced by intercellular connections. Supported by USPHS grant NS 18089.

206.9 AN ANALYSIS OF MONOCLONAL GLUTAMATE IMMUNOREACTIVE NEURONS IN THE DEEP CEREBELLAR NUCLEI AND THEIR PROJECTIONS TO THE RED NUCLEUS AND THE THALAMUS. P.L. Monaghan*, A.J. Beitz, J.E. Madl*, A.A. Larson (SPON: R.E. Phillips). University of Minnesota, St. Paul, MN 55108.

The deep cerebellar nuclei (DCN) provide the major output of the cerebellum. The excitatory neurotransmitters responsible for this output have yet to be identified. Using a monoclonal antibody to carbodiimide-fixed glutamate in conjunction with polyclonal antisera to glutaminase (GLNase) and aspartate aminotransferase (ATTase), we have examined whether glutamatergic neurons are present in the DCN. Three Sprague-Dawley male rats were perfused intracardially with 4% paraformaldehyde and glutaraldehyde (0.1%-0.2%) and sections were subsequently reacted with either the GLNase or AATase antibody. Three additional rats were perfused with a solution of 5% carbodiimide - 0.5% glutaraldehyde in phosphate buffer followed by 5% glutaraldehyde. The sections were post-fixed in 5% glutaraldehyde, washed and then reacted with the monoclonal glumate antibody. Sections incubated with either the polyclonal or monoclonal antibody were subsequently incubated with the avidin biotinylated peroxidase procedure and reacted with diaminobenzidine to visualize the immunoreactive cells. Glutamate-like, GLNase-like and AATase-like immunoreactive profiles were found in the medial, interpositus and lateral nuclei. A combined retrograde transport immunohistochemical technique was used to determine if glutamatergic neurons of the DCN project to the red nucleus or the thalamus. Horseradish peroxidase (HRP) was injected into the ventroposterior nucleus of the thalamus or the red nucleus and a retrograde transport-immunohistochemical procedure was performed as previously described (Beitz, A.J., Neurosci. 7:2753, 1982). Following injections of tracer into these two areas, HRP labeled neurons were found in all three deep cerebellar nuclei. Retrogradely labeled neurons and neurons containing both retrogradely transported HRP and glutamate or glutaminase immunoreactivity were most prominent in the interpositus and lateral nuclei. These results suggest that glutamatergic neurons project to the thalamus and the red nucleus. Further studies attempting to localize putative neurotransmitters in the DCN are currently in progress using a monoclonal antibody against carbodiimide-fixed aspartate. We thank Dr. R. Altschuler for providing the antisera against glutaminase and aspartate aminotransferase. This work was supported by NIH grants NS19208 to A.J.B. and NS17407 to A.A.L.

206.10 ELECTROPHYSIOLOGICAL EFFECTS OF 3-(3-HYDROXYPHENYL)-N-(1-PROPYL) PIPERIDINE (3-PPP) ON SPONTANEOUSLY ACTIVE CEREBELLAR PURKINJE NEURONS. [1]M. Lee, [2]A. L. Gundlach, [1]J. C. Strahlendorf.

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Although 3-PPP has been reported as a selective dopamine autoreceptor agonist in the central nervous system, recent autoradiographic studies revealed that localization of specific 3-PPP binding sites did not parallel that of dopamine neurons (Gundlach, A. L., et al., Neurosci. Abstr., 1984; Largent, B. L., et al., Proc. Natl. Acad. Sci. 81:4983-4987, 1984). Moreover, localizations of (+)-[^{3}H]3-PPP binding sites were identical to those of σ receptors, which mediate psychotomimetic effects of some opiates. Specific (+)-[^{3}H]3-PPP binding sites were labelled in many brain areas, including limbic, midbrain, brainstem, and cerebellar regions. In the cerebellar cortex, high receptor labeling was found in the Purkinje cell layer.

On the basis of these anatomical studies, this study was designed to evaluate the effects of iontophoretically applied (+)-3-PPP and (-)-3-PPP on extracellularly recorded Purkinje cells in urethane-anesthetized rats.

In the majority of Purkinje cells tested (31 of 39 cells), (+)-3-PPP (0.05M) elicited inhibition. It had no effect on 3 of the remaining cells, but elicited a biphasic effect (slight inhibition followed by excitation) or excitation in the other 5. Iontophoretic applications of (-)-3-PPP elicited inhibitions in 13 of 19 Purkinje cells. It had no effect on 6 cells. In the majority of cases, (-)-3-PPP induced less inhibition of Purkinje cells than did (+)-3-PPP with the same currents. Continuous applications of haloperidol blocked or attenuated the inhibitory effects of (+)-3-PPP consistently in 8 of 8 cells tested. Spiperone attenuated the inhibitory effects of (+)-3-PPP to a lesser degree than did haloperidol.

Currently, we are examining whether the observed effects of (+)-3-PPP and (-)-3-PPP on Purkinje cells are direct or occur indirectly through other neurotransmitter systems in the cerebellum. Preliminary results from studies using the cationic inhibitors of synaptic transmission manganese and cobalt suggest that presynaptic sites may not be the main sites of the observed effects of 3-PPP in the present study. (Supported by NIH grant RO1-NS 19296 to J.C.S.)

206.11 ENHANCEMENT OF CEREBELLAR PURKINJE CELL (PC) COMPLEX DISCHARGE (CD) ACTIVITY BY MICROIONTOPHORETIC SEROTONIN. J. C. Strahlendorf, H. K. Strahlendorf, and M. Lee. Dept. of Physiol. and Med. and Surg. Neurol., Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

We have reported previously that serotonin (5-HT) applied iontophoretically to cerebellar PCs elicited one of three effects: inhibition (62%), biphasic (27%), and excitation (11%), effects that appeared to be correlated with the initial firing rates of the cells. Specifically, PCs that responded to 5-HT with increases in firing rate had significantly slower predrug firing frequencies than those that were suppressed by 5-HT. These previous studies have addressed the actions of serotonin on simple spike (SS) activity. The present study was designed to monitor the effects of iontophoretic 5-HT on the absolute number of CDs and mean post-complex-discharge interval (MPCI) and to correlate these effects with predrug spontaneous activity in urethane-anesthetized rats. The major finding of the present study is that iontophoretically applied 5-HT increased the number of CDs markedly (average 94.0%, n=132) in the majority (75%) of PCs tested. The remaining 25% of the cells showed an average 23% decrease in the number of complex discharges. A correlation was apparent between the actions of serotonin on CD activity ($r=0.799$, $p<0.05$) and the initial SS firing rate of the PC. Specifically, a greater percentage of the faster firing cells, which are as a class characterized by fewer initial CDs, responded to 5-HT with an increase in complex activity, and increases were progressively greater in magnitude. The other component of the CD pattern analyzed in this study was the MPCI phase. It became evident that in the control state, slower firing cells exhibit higher MPCI values, whereas faster firing cells have characteristically lower MPCI values ($r=0.987$). PCs evidencing lower MPCI values were those in which 5-HT increased the MPCI value preferentially, whereas those cells in which 5-HT depressed MPCI values exhibited the higher predrug MPCI values. Furthermore, it was apparent that with increasing MPCI values, the proportion of cells displaying a shortening in the MPCI increased ($r=0.939$), and the reverse situation was evident, also. The 5-HT antagonists methysergide and metergoline antagonized 5-HT-induced enhancements of the numbers of CDs, whereas ketanserin failed to alter the response, suggesting a degree of receptor specificity. It would appear that the effect of 5-HT arises from an enhanced efficacy of junctional events at the climbing fiber-PC synapse such that in the presence of 5-HT, normally subliminal climbing-fiber volleys become adequate to produce a complex spike in the Purkinje cell. (Supported by NIH grant RO1-NS19296 to J.C.S.)

206.12 INCREASED RESPONSIVENESS OF CEREBELLAR PURKINJE CELLS TO THE FACILITATORY EFFECT OF IONTOPHORETICALLY APPLIED SEROTONIN ON COMPLEX SPIKE ACTIVITY IN THIAMINE DEFICIENT RATS. H.K. STRAHLENDORF, J.C. STRAHLENDORF AND M. LEE. Medical and Surgical Neurology and Physiology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430

Thiamine deficiency (TD) is believed to be an important etiologic factor in Wernicke's encephalopathy. TD decreases the high affinity uptake for serotonin (5-HT) in rat cerebellar synaptosomes and alters cerebellar 5-HT metabolism (Plaitakis et al, Ann. N. Y. Acad. Sci., 378, 1982). We have previously demonstrated that Purkinje cells (PC) from TD rats show qualitative and quantitative changes to iontophoretically applied 5-HT. These actions were manifest as a shift from mixed simple spike responses of excitation and inhibition in normal rats to solely inhibitory actions that were elicited by significantly lower amounts of 5-HT in TD rats (Lee et al, Brain Res. 327, 1985). As part of a larger, continuing study on the effects of 5-HT on cerebellar PC in normal rats, we have recently found that iontophoretically applied 5-HT increases the number of spontaneously occurring complex spikes (CS) (Strahlendorf et al, this Volume). The present experiments extend these studies to TD rats. Male rats were fed a thiamine-free diet and received daily subcutaneous injections of pyrithiamine 0.5 mg/kg. Controls were either pair-fed the identical diet with thiamine replenishment or were fed standard rat chow. After 15 days the TD group displayed severe neurological impairment. All animals were anesthetized with urethane and prepared for extracellular single neuron recording and microiontophoretic application of 5-HT. Numbers of CS spontaneously occurring in 120 second epochs were compiled by interspike interval histogram software before, during and after 5-HT ejection. Currents were adjusted to produce minimal effects on simple spike firing (5-30nA). TD markedly reduced the number of CS which could be recorded from PC: Control = 56 $\pm$ 4 per 120 sec or 0.47 Hz, n=176; TD = 9 $\pm$ 2 per 120 sec or 0.08 Hz, n=52. The post CS pause did not differ in the two groups: Control = 47.9 $\pm$ 2.5 msec; TD = 42.0 $\pm$ 6.0 msec. 5-HT enhanced the number of CS in both groups, however the increase in the TD rats was markedly greater: Control = 94 $\pm$ 9% increase; TD = 325 $\pm$ 70% increase. These data provide additional support for our previous findings of enhanced sensitivity of PC to 5-HT in TD. (Supported in part by NIH RO1 NS 19296)

206.13 RETROGRADE TRACING STUDIES REVEAL THE ORIGIN OF ASCENDING PROJECTIONS TO THE BASILAR PONTINE GRAY IN THE RAT. R.J. Kosinski, S.A. Azizi, B. Border and G.A. Mihailoff, Dept. of Cell Biology, Univ. Texas Health Science Ctr., Dallas, Texas 75235.

Previous orthograde tracing studies have described the topographic distribution of spinal (Sp), dorsal column nuclear (DCN) and spinal trigeminal nuclear (Vsp) projections to the basilar pontine gray (BPG) in rats. The present study examined the origin of these ascending brainstem projections to the BPG as well as the origin of other novel pontine afferent projections. Under halothane anesthesia and through a ventral surgical approach, the BPG was unilaterally injected with a 2% solution of WGA/HRP (0.02 ul-0.08 ul). Following a survival period which ranged from twelve to thirty hours, animals were sacrificed, perfused and the tissue processed according to routine HRP histochemical methodologies.

Retrogradely labeled Sp, DCN and Vsp neurons were usually distributed contralateral to the injection sites except for a few cells observed within the ipsilateral Vsp. Although Sp labeling was relatively sparse, large round and fusiform shaped cells were localized to the T_{12}-L_3 segments of Clarke's column. While labeled neurons were seen throughout all levels of the DCN, particularly for the cuneate nucleus, the location of most labeled cells corresponded to levels at and caudal to the obex. Labeled neurons within the DCN included round to oval dorsally located neurons as well as more ventrally positioned small oval, fusiform and triangular shaped cells. Labeled Vsp neurons were confined to the interpolaris subdivision and included a variety of round to multipolar shaped cells that were primarily localized to the dorsal half of this nucleus. Additional labeled neurons were observed near the central canal at caudal medullary levels and within caudal and medial portions of the contralateral external cuneate nucleus. Other labeled somata were seen within various regions of the reticular formation including some scattered cells within the medullary and pontine raphe nuclei. Reticular formation labeling included contralaterally located cells just medial to the labeled cuneate and Vsp neurons, ipsilaterally located cells dorsolateral to the inferior olivary complex (IOC) and bilaterally distributed cells dorsal and lateral to the pyramids at levels rostral to the IOC.

Results thus confirm the topography of Sp, DCN and Vsp projections to the BPG in the rat as well as provide evidence for additional novel pontine afferent sources. Furthermore, the existence of contralaterally labeled neurons within the DCN and the Vsp as well as the column of Clarke and the external cuneate nucleus might provide the substrate for certain peripheral somatosensory inputs described on the basis of related electrophysiological studies. Supported by NS-12644 and BNS 80-0453.

206.14 THE ORIGIN OF DESCENDING PROJECTIONS TO THE BASILAR PONTINE NUCLEI AS REVEALED BY RETROGRADE TRACING STUDIES IN THE RAT. G. A. Mihailoff, S. A. Azizi, R. J. Kosinski, B. Border and D. J. Woodward, Dept of Cell Biology, Univ. TX Hlth Sci Ctr, Dallas TX 75235.

This study was undertaken to determine the origin of descending afferent projections to the basilar pontine gray of the rat from the cerebral cortex, diencephalon, mesencephalon and the deep cerebellar nuclei. The pontine gray was exposed under halothane anesthesia by a ventral surgical approach. Various areas of the basilar pons of 10 rats received small or large hydraulic injections of WGA-HRP (2% solution in H20). Brain sections were processed using the TMB procedure. Computer assisted serial reconstruction was employed to accurately visualize the totality of projections. Only cases in which HRP did not spread to the overlying crus cerebri, medial lemniscus or reticular formation were used in this analysis.

The basic trend of projections noted was as follows. Within the cerebral cortex groups of neurons were localized from caudal to rostral in the secondary visual (areas 18 and 19) and auditory cortices, the somatosensory cortices and bilaterally within the motor cortex. In addition, clusters of labeled cells were observed in the cingulate and entorhinal cortices. The labeled cortical neurons were almost exclusively of the pyramidal type and were restricted to layer V of the cortex. Within the diencephalon a number of cells were observed in the zona incerta as well as the mammillary, lateral and anterior hypothalamic nuclei. The mesencephalic projections to the pontine nuclei include HRP-labeled neurons in the substantia nigra, pretectal complex, and ventral lateral geniculate. Several regions within the periaqueductal gray contained labeled somata as well as the dorsal tegmental and dorsal raphe nuclei. Also, intensely labeled neurons were found in the perirubral area and retrorubral fields. The midbrain reticular formation including nucleus cuneiformis as well as the region medial to the mdial geniculate nucleus also exhibited labeled somata as did the superior and inferior colliculi. Lastly, labeled neurons were observed in the deep cerebellar nuclei, particularly the dentate and interposed nuclei. Although many of these connections have been established in earlier studies, several new basilar pontine afferent projections are demonstrated in the present study and these include the substantia nigra, periaqueductal gray, perirubral areas, and the reticular formation. These observations emphasize the diversity of afferent systems projecting to the pontine nuclei and point out the complex integrative function that must be served by pontine neurons in the transfer of information to the cerebellum. (Supported in part by grants DA-2338, NS-12644 and the Biological HumanicsFoundation)

206.15 RESPONSES OF CAUDAL PONTINE GRAY TO CORTICAL AND PERIPHERAL SOMATOSENSORY STIMULI: CONVERGENT INPUTS TO THE BASILAR PONS S. Ausim Azizi, R. J. Kosinski, D. J. Woodward and G. A. Mihailoff. The University of Texas Hlth Sci. Ctr, Dallas, TX 75235

In this series of experiments we have carried out an electrophysiological investigation of somatosensory afferents to neurons of the basilar pontine gray (BPG). Bipolar concentric stimulating electrodes were implanted ipsilaterally into the primary motor face (MF), motor forelimb (MFL), sensory face (SF) and sensory forelimb(SFL) cortical areas as determined later by histological verification of the electrode tracts. Other electrodes were implanted bilaterally into each vibrissal pad (Vb), both forelimbs (FL) and contralateral hindlimb (HL). The basilar pons was exposed by a ventral surgical approach under halothane anesthesia. Conventional electrophysiological recording and stimulation apparatus including a computer for building on-line histograms was used. In 175 BPG neurons (17 rats), evoked responses to electrical stimuli (doule pulses .2 ms duration, 1.5 ms interpulse interval, 40-250 μA) of different regions of the cerebral cortex and/or the periphery were recorded. At threshold currents, responses were elicited (single or multiple spikes) following cortical stimulation with latencies of 6 - 8 ms. BPG neuronal responses to peripheral electrical stimulation often consisted of two components occuring at latencies of 5 and 12 ms respectively. Of the 175 BPG neurons, many responded to stimulation of either a single cortical area: MFL (28), SFL (15), SF (13) or to a single peripheral locus: Vb (7), FL (10) and HL (7), whereas a substantial number of cells (81/175) responded to electrical stimulation of two or more locations. Such locations involved either different cortical zones or a combination of cortical and peripheral areas. Within the later category several cells (23) responded to stimulation of corresponding cortical and peripheral areas. In the three most recent cases, natural stimuli were applied to body zones responsive to electrical stimlulation. A total of nineteen cells responded to natural stimuli and exhibited either discrete cutaneous (light brushing) or deep receptive fields (squeezing and limb manipulation). BPG neurons reponsive to cutaneous stimuli tended to respond to electrical stimulation of the corresponding sensory cortical area, whereas neurons responsive to deep stimuli tended to be responsive to motor cortical stimulation.

These data indicate that neurons in the caudal BPG receive discrete as well as diffuse assortments of descending and ascending sensory and motor inputs. Although, these inputs correspond to previous anatomical tracing studies, the convergence of these affents suggests that BPG neurons may be involved in more complicated sensorimotor integrative functions than previously thought. (Supported in part by DA-2338, NS-12644 and the Biological Humanics foundation)

206.16 CERTAIN BASILAR PONTINE AFFERENT SYSTEMS ARE GABA-ERGIC, B. Border*, R. Kosinski, S.A. Azizi, and G. Mihailoff, (SPON: T. Cope), Cell Biology, Univ. Texas Hlth Sci Ctr., Dallas, 75235

Previous light and electron microscopic immunocytochemiocal studies performed in this lab have demonstrated the presence of glutamic acid decarboxylase (GAD), a key enzyme in the synthesis of gamma-aminobutyric acid (GABA), in neuronal somata, axons, and axon terminals within the basilar pontine nuclei (BPN) of the rat. Although these findings suggest that some labeled terminals might arise intrinsically from the population of GABA-ergic pontine neurons, the distribution of the labeled terminals suggests that some might take origin from a source extrinsic to the BPN. In order to identify the extrinsic source of GAD-positive terminals, a double-label strategy was employed that involved both retrograde transport of HRP and immunocytochemical visualization of GAD. In this procedure, WGA/HRP was injected into the BPN from a ventral approach to demonstrate retrograde axonal transport without damaging afferent axons coursing through the pontine tegmentum. After a 12-30 hour survival period, animals were perfused with fixative, and the brain removed and sectioned (30μm) on a Vibratome. Tissue was first reacted with TMB in order to visualize the HRP reaction product which was subsequently stabilized with cobalt chloride and nickel ammonium sulfate. Overnight incubation of tissue sections in sheep GAD antiserum was then carried out and the unlabeled antibody enzyme (PAP) method of Sternberger utilized to visualize neuronal cell bodies containing GAD. Sections were examined for cells exhibiting both the black reaction product of WGA/HRP labeling and the reddish-brown product of the immunocytochemical procedure.

Our principal findings in these studies are the following. 1) Neurons in the zona incerta were seen to contain both WGA/HRP reaction product and GAD-positive labeling. These small to medium-size somata were located predominantly ipsilateral to the pontine injection site and were observed in medial and lateral portions of the zona incerta. 2) Double-labeled neurons were also observed bilaterally in the brainstem reticular formation (ipsilateral predominance) at levels rostral and caudal to the pontine nuclei. Such neurons were diffusely distributed with some located dorsomedially and others positioned ventrolaterally. 3) The anterior pretectal nucleus at the level of the posterior commissure also contained double-labeled neurons located predominantly ipsilateral to the pontine injection site.

The present observations suggest that the origin of at least some of the GAD-positive axon terminals in the BPN of the rat arise from extrinsic sources. The function of this GABA-ergic input to the BPN is not fully understood; however, further studies will attempt to determine which pontocerebellar circuits are being affected by GABA-ergic neurotransmission. (Supported by NS-12644).

206.17 THREE-DIMENSIONAL RECONSTRUCTIONS OF THE OPOSSUM CEREBELLAR NUCLEI. P.S. Klinkhachorn* and P. Klinkhachorn.* (SPON: W. Flory). Department of Veterinary Anatomy, School of Veterinary Medicine and Department of Electrical Engineering, College of Engineering, Louisiana State University, Baton Rouge, LA 70803.

The purpose of this project is to use computer graphics to reconstruct a three-dimensional model of the opossum cerebellar nuclei. Serial sections through the cerebellum of normal adult opossums were used in this study. They were cut in the coronal plane, 40 micrometers thick, and stained with cresyl violet acetate. The outlines of four cerebellar nuclei in each section were traced from caudal to rostral directions with a microprojector. Each section was digitized as the contour line. The digitized file created only X and Y coordinates. Since the thickness of each section was known, a simple program was written to add the Z coordinate for each point. The cerebellar nuclei of the opossum, although incompletely separated from each other, can be divided into four individual nuclear masses located on each side of the midline. The most medial nucleus is large and is located adjacent to the midline. The posterior interposed nucleus is located at a more ventromedial position of the interposed complex and is closer to the medial nucleus than the anterior one, which is situated in a more dorsolateral position. Both nuclei have a somewhat rostral to caudal relationship to each other in contrast to the medial to lateral relationship as seen in primates. The most laterally located nucleus is a rounded mass of cells without any appearance of hilus or denticulations, which are present in higher mammals. It is established that each region of every nucleus appears to receive and project the nerve fibers from and to different areas of brain. The computer graphic technique will help to reconstruct these nuclei and represent them in a three-dimensional model. It is believed to be more accurate and less time-consuming than the regular histological method, in which only one plane of section can be investigated at a time.

206.18 ORGANIZATION OF CORTICONUCLEAR AND OLIVOCEREBELLAR CONNECTIONS IN THE PIGEON. J.J.A. ARENDS*.(SPON: H.P.Zeigler). West Laboratory, American Museum of Natural History, NY 10024, and Biopsychology Program, Hunter College, CUNY, NY 10021.

In order to clarify the organization of the avian cerebellar nuclei and the inferior olive, anterogradely labeled corticonuclear fibers and retrogradely labeled olivary cells were traced following small iontophoretic or pressure injections of WGA-HRP throughout the cerebellar cortex, both longitudinally and mediolaterally.

Vermal injections in lobules I through X produced a topographically organized ipsilateral projection covering 3 of the 4 purportedly separate cerebellar nuclei, i.e. the medial, intermediate and intercalate nuclei: apparently these 3 nuclei together constitute the medial cerebellar nuclear complex (CbM). Posterior lobe vermal cortex injections, particularly those in the vestibulocerebellum, also labeled peripheral parts of the superior vestibular nucleus, e.g. the lateral cerebellovestibular process (PCV). Lateral injections produced topographically organized labeling in the (ipsi-)lateral cerebellar nucleus (CbL). Termination was also present throughout the vestibular complex, excluding the tangential nucleus and the dorsolateral vestibular nucleus of Sanders (VDL). Injections in the auricular area (flocculus and paraflocculus) and lateral unfoliated cortex produced labeling in CbL and in the vestibular complex, including the tangential nucleus and VDL.

Using a subtractive approach, complementary injections in the cerebellar and subjacent vestibular nuclei showed that the cortex, excluding the vestibulocerebellum, contains at least 4 mediolateral efferent zones. Purkinje cells in the medial 2/3 of the ipsilateral cortex (zone A) were labeled after injections in CbM. The lateral 1/3 (comprising zones B, D1 and D2) was labeled after injections in CbL. Labeling was limited to zones B and D2 after injections in the dorsal part of the lateral vestibular nucleus, whereas the lateralmost D2 zone was the only cortical area labeled after injections in the medial and/or descending vestibular nuclei.

Retrograde labeling in the contralateral inferior olive (OI) after the injections in the cerebellar cortex also showed a topographical organization. After medial cortex injections narrow, elongated clusters of labeled cells were observed in the lateral part of the large dorsal lamella of OI, shifting from lateral to medial with injections ranging from lobule I through IXa. Lateral cortex injections produced a similar picture in the smaller ventral lamella of OI. These injections were never seen to label the area of OI medial to the exiting rostral cervical nerve roots, except when the lateral unfoliated cortex and the vestibulocerebellum (cortical lobules IXb and X), including the auricle, were involved as well.

These data suggest the absence of an avian homologue of the mammalian nucleus interpositus, its associated cortical C zones, and the C-zone afferent parts of the inferior olive.
Supported by NSF Grant BNS-8216604.

206.19 THE CONNECTIVITY OF A CEREBELLAR STRUCTURE, (EMINENTIA GRANULARIS PARS POSTERIOR) IN THE TELEOST APTERONOTUS LEPTORHYNCHUS. E. Sas* and L. Maler(SPON:W. Hendelman). Dept. of Anatomy, Univ. of Ottawa, Ottawa K1H 8M5 Canada

The cerebellum in teleost fish consists of the corpus cerebelli, valvula cerebelli and eminentia granularis pars anterior and pars posterior (lobus caudalis of older nomenclature). The lateral granule cell mass of the eminentia granularis pars posterior (EGp) is the most caudal region of the cerebellum; it comprises an outer granule cell layer, a Purkinje cell layer, an inner molecular layer, and a particular cell type, the eurydendroid cells, which give origin to cerebellar efferents, and probably represent the deep cerebellar nuclei of other species.

I) Injections of HRP into different locations (rostral, caudal, dorsal, ventral) and depths of this cerebellar region, resulted in labeled fibers in the following locations: a) around eurydendroid cells and molecular layer of ipsilateral eminentia granularis pars posterior; b) in the dorsal molecular layer of ipsilateral and contralateral electrosensory lateral line lobe, in a stratified lamellar fashion; c) n. praeeminentialis dorsalis, pars principalis and pars lateralis; d) contralateral torus semicircularis layer 8b.

II) Cells projecting to the lateral granule cell mass of EGp were labeled in: a) lateral line ganglion; b) lateral portion of contralateral inferior olive; c) lateral reticular nuclei, bilaterally; d) n. raphe posterioris, bilaterally; e) n. prepositus, bilaterally; f) n. raphe dorsalis, bilaterally; g) n. praeeminentialis pars principalis and pars lateralis; h) a cell group within the ventral efferent bundle of contralateral n. praeeminentialis; i) acousticolateral area, bilaterally.

The connections of the lateral cell mass of the eminentia granularis pars posterior are highly topographical and due to the laminar segregation of its afferents, specific structures appear labeled according to the depth of the HRP injection. The medial cell mass of the eminentia granularis pars posterior, receives somewhat similar inputs to those of the lateral granular cell mass; in addition these granule cells receive a unique input from certain subnuclei of the pretectal region. Particularities in the connectivity of the lateral and medial granular cell masses of EGp will be discussed with special reference to the possibility of corollary discharge in this system.

VESTIBULAR SYSTEM II

207.1 EFFECTS OF CEREBELLAR STIMULATION ON VELOCITY STORAGE IN THE MONKEY D. Solomon*, T. Raphan and B. Cohen (SPON: J. Goldfarb). Depts. of Neurology and Physiology, Mt. Sinai Sch. of Med., NY 10029 and Computer & Information Science, Brooklyn College, CUNY, Brooklyn., NY 11210

Tilting the head during post-rotatory nystagmus or optokinetic after-nystagmus (OKAN) causes a reduction in slow phase velocity (SPV) that does not recover when animals are brought back to the upright position. This indicates that this maneuver had caused a rapid discharge or "dump" of central vestibular system activity that produces slow phases of nystagmus. The same occurs during visual suppression. The origin of activity responsible for the dumping process has been localized to the nodulus and uvula (Waespe et al. Science 1985). This suggests that it may be possible to suppress nystagmus and reduce stored central activity by nodular and uvular stimulation. To study this the region of the posterior vermis was electrically stimulated in alert rhesus monkeys with constant currents of 60-100 microamps at frequencies of 150-400 Hz for periods up to ten seconds. Trains of pulses caused a rapid loss in SPV during per- or post-rotatory nystagmus and OKAN, shortening the duration of the response. SPV did not recover at the end of stimulation, suggesting that central activity responsible for the nystagmus had been lost. The loss of SPV was directionally specific, occurring when slow phases were to the stimulated side. The latency of the decline in velocity was about one second, similar to the response to tilt. The time constant of decline in SPV varied as a function of stimulus duration. This was consistent with the decline after visual suppression, and was significantly faster than the decline in SPV in darkness without suppression. Contralateral SPVs were generally enhanced by stimulation, leading to a longer response than in the absence of stimulation. With the animal in darkness and the eyes stationary, stimulation caused eye movements to either side, depending on electrode location. This was followed by contralateral SPVs that had the characteristic time course of post-rotatory nystagmus or OKAN. Vertical eye velocities were also induced. The discharge of stored activity could not be accounted for by a simple summation of the ongoing SPV with that induced by stimulation. Stimulation during the initial step in eye and head velocity had little effect on the response.

These data show that stimulation of the posterior vermal area that includes the nodulus and uvula can evoke nystagmus "dumping" similar to that elcited by visual suppression or tilt suppression. Presumably the ipsilateral output projections of the nodular and/or uvular Purkinje cells inhibit cells in the vestibular nuclei that are responsible for producing velocity storage without affecting portions of the VOR that are responsible for generating rapid changes in velocity during compensatory eye movements.

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207.2 POSTURAL COACTIVATION AND THE HABITUATION OF SWAY STABILIZING RESPONSES. E.A. Keshner, J.H.J. Allum and C.R. Pfaltz. Dept. of ORL, Univ. Hosp., 4031 Basel, Switz.

Coactivation of pitch sway stabilizing responses and their habituation to a series of 10, 36 deg/s dorsiflexion support surface rotations was investigated in the ankle and neck muscle responses of normals and patients with bilateral peripheral vestibular deficit (BILATS). Areas under the EMG curves were calculated for the avg. of the first 3 and next 7 trials of each muscle at latencies determined from individual responses. Short latency (SL) responses appeared in soleus (SOL) at 60 ms and tibialis anterior (TA) at 84 ms. Medium latency (ML) responses of SOL (125 ms) and TA (122 ms) were measured for a 75 ms window and long latency (LL) responses for the following 75 ms. The same procedure was used for ML trapezius beginning at 116 ms, and longus capitus at 128 ms. Average head angular acceleration amplitudes, recorded from an angular accelerometer mounted on a helmet, and incremental torque exerted on the platform were measured. Habituation was assessed through t-tests for paired comparisons. Correlations were calculated between the muscles, and bivariate regressions performed to assess the actions of TA and SOL on foot torque, and the neck muscles on head acceleration.

Significant decreases occurred across trials in the muscles of normals and BILATS in all but the SL responses. ML and LL responses of the BILATS were significantly smaller than those of normals. Muscle response decrements were reflected in decrements of torque and head acceleration. Strong correlations existed between the ankle muscles at both ML (r = 0.81) and LL (r = 0.88) with eyes open and closed. TA and SOL actions were highly correlated with foot torque for normals and BILATS at SL (r = 0.85), ML (r = 0.88), and LL (r = 0.61). ML neck muscle correlations were strong in normals (r = 0.83). Correlations between neck muscles and head acceleration in normals (r = 0.61) increased when initially tested with eyes closed (r = 0.83).

Our results demonstrate that habituation is not dependent on an intact vestibular system. It is essential to account for coactivation of antagonistic muscles acting across a joint to adequately describe sway stabilization. Separate pathways appear to control the stabilizing responses at the ankle and neck.

207.3 DOES VISION AFFECT OCULAR COUNTERROLLING? S.G. Diamond and C.H. Markham. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Ocular counterrolling (OCR) is the reflex rotation of the eyes opposite to head rotation, governed by the vestibular otoliths. Most OCR studies are conducted in ambient light, and any contribution of vision to this response is assumed to be negligible. This study was an attempt to systematically evaluate what effect light and vision might have on this otolith reflex response.

Six normal subjects, two men and four women, aged 24 to 65, were each studied in three protocols in counterbalanced order.

1) Standard condition in which subjects were rotated in ambient room light at constant velocity of 3°/s about their naso-occipital axis, twice to 90° right and left. Central vision was occupied by the camera which rotated with the chair. Peripheral vision of the room was unobscured.

2) White mode. Peripheral vision was obscured by draping the entire tilting chair in a white tent. Light entered freely but subjects had no visual reference to verticality. Rotation was performed as in the standard condition.

3) Dark mode. Both central and peripheral vision were eliminated by conducting the trials in complete darkness.

Results of the latter two conditions were compared to those obtained in the standard procedure, using the same parameters found useful in earlier studies.

Consistency, the extent to which the second trial resembles the first. One subject showed greater consistency in the white and dark modes than in the standard; two showed less in the dark mode.

Smoothness, the absence of abrupt changes of direction of OCR. One subject had less smooth OCR in white mode. Five subjects had less smooth OCR in the dark.

Symmetry, equal amplitude of response to right and left tilt. Three patients showed less symmetry in white mode. One of these three had less symmetry in the dark also, and one had more.

Conjugateness, the tendency of the two eyes to move together. In the white mode, one subject showed less and one showed more disconjugate movement. In the dark, four subjects showed more disconjugate movement, three of these dramatically so.

Amplitude, the magnitude of the response. Four subjects showed slightly diminished response in white and three of these persons had somewhat increased amplitude in the dark.

In summary, the changes observed in OCR when peripheral or all vision was interrupted were either none or mild in no systematic way, with two exceptions. In the dark condition, the occurrence of disconjugate eye movement and less smoothness may be due to a lack of cortical fusion. These findings are not sufficient to propose that OCR studies by conducted in other than the usual ambient light conditions.

207.4 BACLOFEN AND THE VESTIBULO-OCULAR REFLEX (VOR). D. Helwig*, B. Cohen and T. Raphan. Departments of Neurology and Physiology, Mount Sinai School of Medicine, New York, NY 10029, and Computer & Information Science, Brooklyn College of CUNY, Brooklyn, NY 11210.

The dominant time constant of OKAN and of vestibular nystagmus is a measure of the dynamic properties of a mechanism in the vestibular system that stores activity related to slow phase eye velocity. This time constant was reduced in monkeys after administration of baclofen. Effects began within 15 minutes of injection and lasted for more than 12 hours, paralleling increases in blood levels of baclofen that have been reported after a similar injection (Brogden et al. Drugs 8: 1, 1974). The decrease in VOR time constant was dose-dependent between 2-8 mg/kg, the range that was tested. Generalized skeletal muscle weakness occurred at higher dosages. Analysis of the response to steps of velocity as well as to sinusoids showed that the time constant of the VOR that was originally 15-20 seconds was reduced to 6-8 seconds at higher dose levels. There was a concomitant reduction in the falling time constant of OKAN. Peak velocities during continuous nystagmus elicited by off vertical axis rotation or by pitch while rotating, which also depend on the velocity storage mechanism, were also attenuated by baclofen. In contrast, the gain of the VOR in response to steps of constant angular velocity was unchanged at all dose levels. The rapid rise in OKN was equally unaffected.

These results suggest that baclofen specifically alters the dynamics of the velocity storage mechanism without affecting the pathways that mediate the direct visual or vestibular responses. This type of decrease in the VOR time constant is brought about by repeated testing and/or by repeated exposure to conflict stimuli. The ability to habituate nystagmus disappears after the nodulus and uvula are ablated (Waespe et al. Science 228: 199, 1985). Since baclofen is a GABA-agonist believed to act primarily on GABAb synapses, GABAb synapses may be utilized by the vestibulo-cerebellum in differentially controlling cells in the vestibular nuclei specifically involved in realizing velocity storage.

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207.5 **POSITION-DEPENDENT ASYMMETRIES IN HORIZONTAL NYSTAGMUS IN THE SQUIRREL MONKEY.** **L.B. MINOR* AND J.M. GOLDBERG.** Dept. Pharm. Physiol. Sci., University of Chicago, Chicago, IL 60637.

Caloric nystagmus has a position-dependent asymmetry which cannot be explained on the basis of thermal convection (Coats, A.C., and Smith S.Y., Acta Otolaryng., 63:515, 1967). The maximum slow phase eye velocity (SPEV) of the caloric response is greater in nose up (NU) than in nose down (ND) positions. There are at least two explanations for the asymmetry: a direct thermal effect on hair cells and/or afferent nerve fibers; or a position-dependent, possibly otolith-related modulation of horizontal nystagmus. If the latter effect is involved then similar position dependent asymmetries should be seen with rotational stimuli.

Eye movements were recorded by a search coil technique from four squirrel monkeys. The SPEV in response to warm or cold air calorics was about 5x greater in the supine than in the prone position. Long-duration accelerations about the earth-vertical axis (3°/sec^2 acceleration, 60 sec) were presented in darkness after the animal had been pitched 45° NU or 45° ND. NU responses were 52% greater than ND responses. The results indicate that a position-dependent modulation of horizontal nystagmus can account for a portion, but perhaps not all, of the caloric asymmetry.

In contrast to long-duration head accelerations, sinusoidal rotations about the earth-vertical axis (0.1 Hz, 40°/sec maximum velocity) with the animal pitched to 30, 60, and 90° showed no consistent asymmetries between NU and ND positions. The presence of a positional asymmetry confined to low frequency (<0.1 Hz) rotational responses suggests that velocity-storage mechanisms may be affected by static head position. To study this possibility, velocity steps to 60°/sec in darkness were given with the animal in 45° NU or 45° ND positions. Following the decay of the vestibular nystagmus, the room was illuminated and the animal continued to rotate at 60°/sec for 30 sec, at which time the lights were again extinguished and the decay of optokinetic after-nystagmus (OKAN) was evaluated. Time constants for decay of vestibular nystagmus and OKAN were greater for 45° NU than 45° ND for all animals with the average values (in sec) being $T(VOR)_{NU}$ = 21.7, $T(VOR)_{ND}$ = 13.0, $T(OKAN)_{NU}$ = 23.6, and $T(OKAN)_{ND}$ = 15.9. When the NU and ND time constants for each animal were compared, the average ratio of 45° NU to 45° ND was 1.8 for T(VOR) and 1.5 for T(OKAN). These findings support the hypothesis that static pitches can influence velocity storage for the horizontal VOR. The differences in time constants are appropriate in magnitude to explain the asymmetry in responses to long-duration head acceleration.

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207.6 Vestibular neurons in cerebral cortex: Two populations with different locations and different activation by optokinetic stimulation. U. Schreiter*, Dept. of Neurology, Univ. of Düsseldorf, 4 Düsseldorf, W. Germany, U. Büttner, Dept. of Neurology, Univ. of Munich, 8 München, W. Germany.

Single unit recordings were performed in the cortex of awake Java monkeys during natural vestibular and optokinetic stimulation. Eye position was recorded with chronically implanted ECG-electrodes. Two populations of vestibular neurons at different locations could be identified: group A-neurons in the upper bank of the lateral sulcus (parietal operculum), group B-neurons in the intraparietal sulcus (area 7b). Both groups were activated similarly by natural vestibular stimulation (sinusoidal rotation about a vertical axis in the dark at 0.05 - 2.0 Hz and 20 - 60 deg/s max. velocity). In both groups type I (activation with rotation to ipsilateral side) and II (to contralateral side) neurons were found. Neurons showed no modulation with individual eye movements. All neurons were activated by optokinetic stimulation. Stimulation was applied in the horizontal plane either sinusoidally (0.01 - 5.0 Hz, 10 - 60 des/s max. velocity) or at constant velocity (10 - 100 deg/s). In order to elicit an activation the optokinetic and vestibular stimulus had to move in opposite directions.

The two groups showed considerable differences in their response to optokinetic stimulation. Group A-neurons responded only at low frequencies of optokinetic stimulation (below 0.2 Hz) and displayed only gradual activity changes after onset of constant velocity stimulation. Activation continued throughout optokinetic after-nystagmus (OKAN). In contrast group B-neurons responded even at high frequencies (up to 3 - 5 Hz) and showed immediate activity changes with constant velocity stimulation. There was no modulation during OKAN.

It is concluded that group A-neurons display an activity pattern similar to that found in the vestibular nuclei. For group B-neurons the visual-vestibular response properties have to derive from different, yet unknown, sources. In particular, the lack of response during OKAN indicates that group-B cortical vestibular neurons are not involved in 'velocity storage' mechanisms, which are found in vestibular nuclei neurons.

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207.7 ANATOMOPHYSIOLOGICAL CORRELATES IN THE AFFERENT ANTERIOR SEMICIRCULAR CANAL FIBERS OF THE BULLFROG. V. Honrubia, A. Kuruvilla* and S. Sitko*. Div. of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Approximately 1200 neurons ranging in diameter from 1 to 17 μm innervate the anterior semicircular canal of the bullfrog. The number of fibers (Nd) for each diameter (d) is given by the equation Nd = 446 exp (-d/2.26). The larger neurons (d > 7 μm) innervate the central region of the crista where they represent 37% of the fibers in the two central bundles. The smaller neurons predominate in the bundles at the periphery (93%). Thirty-seven neurons were intracellularly impaled with microelectrodes to measure their spontaneous activity and to inject them with horseradish peroxidase. The axon diameters, soma volumes and internodal distances of the fibers are linearly related to the coefficient of variation (CV) of the spontaneous activity. It is estimated that 92% of the neurons with fiber diameters of < 7 μm have a CV of < 0.5. All neurons innervate the same areas of the various vestibular nuclei except for the more irregular ones (CV > 0.5) which do not innervate the medial nucleus. The distribution of terminals and the length of dendritic branches along the nuclei are different for regular and irregular fibers. The response to rotational stimuli (0.0125-0.5 Hz) and the spontaneous activity of another group of 73 neurons were also obtained. The frequency response characteristics (Bode plots), middle frequency dominant time constant and sensitivity coefficient were determined. More regular fibers show a negative correlation between their CV and time constant (range 10.4 to 0.9 sec) but a positive correlation between their CV and sensitivity (range 1.2 to 5.3 [spikes/sec][deg/sec^2]). More irregular fibers have a time constant and sensitivity coefficient which are uncorrelated with the CV; there is, however, a positive correlation between the time constant (range 0.05 to 5.5 sec) and sensitivity coefficient (range 0.5 to 12 [spikes/sec][deg/sec^2]).

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207.8 GAIN REGULATION OF THE VESTIBULOSPINAL REFLEX BY THE NORADRENERGIC LOCUS COERULEUS SYSTEM. O.Pompeiano, P.d'Ascanio*, E.Horn* and G. Stampacchia*. Ist.di Fisiologia Um.,Univ.di Pisa,56100 Pisa,Italy.

The increased activity of the extensor triceps brachii during side-down tilt of the animal depends on both an increased discharge of excitatory vestibulospinal (VS) neurons and a reduced discharge of medullary inhibitory reticulospinal neurons (mRS).Since these mRS neurons are tonically excited by the dorsal pontine reticular formation (pRF), whose activity is in turn inhibited by noradrenergic locus coeruleus (LC) neurons,experiments were performed to find out whether pontine microinjections of adrenergic agonists or antagonists may modify the characteristics of the VS reflex.

In precollicular decerebrate cats,iniection of an α 2-agonist(0.25 μl of a solution of 0.012-0.15 μg/μl of clonidine) into the LC of one side at P3,L2.5 or 2.8,H-2 decreased the extensor rigidity in the ipsilateral limbs,but greatly increased the gain (imp./sec/deg) of the first harmonic component of the multiunit EMG response of the triceps brachii to roll tilt of the animal (at 0.15 Hz,±10°);however,the phase angle of the response was not modified.Similar results were also obtained after unilateral injection of an α 1-antagonist (0.25 μl of a solution of 0.1-1.0 μg/μl of prazosin) into the dorsal pRF at P2 or P3,L2.8,H-3.5 or -4.5.In both instances,the increased gain of the VS reflex did not depend on the decreased postural activity following injections,since it was still observed when an increased static stretch of the extensor muscle compensated for the reduced EMG activity.

In conclusion,the tonic inhibitory influence exerted by the noradrenergic LC neurons on the dorsal pRF was greatly depressed,either i) by microinjections into the LC of the α 2-agonist clonidine,which acts on the somatodendritic α 2-adrenoceptors by enhancing collateral inhibition of the corresponding neurons,or ii) by injection into the dorsal pRF of the α 1-antagonist prazosin,which blocks the inhibitory influence that the LC neurons exert on this pontine region,probably by activating inhibitory interneurons via α 1-adrenoceptors.In both instances, the increased discharge of the pRF neurons and the related mRS neurons decreased the postural activity in the ipsilateral limbs;moreover, the higher the resting discharge of these mRS neurons,the greater the disinhibition which affected the limb extensor motoneurons during side-down animal tilt.These motoneurons would then respond more efficiently to the same excitatory VS volleys elicited during side-down tilt, thus leading to an increased gain of the EMG response of limb extensors to labyrinth stimulation.The LC may thus exert a permissive role in the gain regulation of the VS reflex.

207.9 EFFECTS OF BEHAVIORAL ACTIVATION OF THE EFFERENT VESTIBULAR SYSTEM ON THE RESPONSE DYNAMICS OF THE HORIZONTAL SEMICIRCULAR CANAL AFFERENTS IN THE TOADFISH, OPSANUS TAU. R. Boyle* and S. M. Highstein (SPON:A.Steinacker). Marine Biological Laboratory, Woods Hole MA. 02543 and Dept. of Otolaryngology, Washington University, St. Louis, MO. 63110.

A paradigm was previously described (J. Neurophysiol. in press) for behavioral activation of the efferent vestibular system in spinalized fish. A light touch of the snout activates vestibular efferents which excite vestibular primary afferents presumably via axo-axonic synapses within the labyrinth (Sans and Highstein, Brain Res. 308: 191,1984). We have now characterized the response dynamics of horizontal semicircular canal primary afferents (HSCA) and the change in these dynamics with efferent activation. Adult toadfish were clamped in an experimental tank, perfused through the mouth with running sea water and oscillated horizontally. Dorsal craniotomy exposed the labyrinth and HSCA were penetrated with glass microelectrodes; eye retraction served as a behavioral marker of efferent activation. Resting discharge of 400 fibers ranged from 0 impulses/sec (ips) to low rates (<10ips) with irregular interspike intervals, to higher rates (>80ips) with more regular interspike intervals as reported for other vertebrate labyrinths. Sinusoidal rotation, (0.008-8.0 Hz) at various amplitudes (±0.5° to ±45°; 2-30°/s; 0.2-600°/s^2) was used to determine the sensitivity and phase of response. We compared control-response cycles to ones with efferent activation and constructed cumulative histograms of HSCA discharge. Phase and sensitivity were calculated using a sinusoidal fit by a least-squares process. Background discharge (BD) was calculated as the average ips during each cycle of rotation. Major efferent effects were on irregular HSCA; regular afferents usually did not respond to behavioral activation. To date, 12 HSCA have been analyzed in detail. In 3 HSCA with BD 2-8ips, efferent activation raised the BD 200-700% relative to control, increased the sensitivity from 1.5-2.5x and either advanced the phase re:velocity (n=1, from +47° to +78°) or retarded it (e.g. from +30° to +5°). In 4 HSCA with BD 11-43ips efferent activation raised the BD 115-145%, decreased the sensitivity 0.6-0.9x and shifted the phase re:velocity (e.g. from +40° to +25°). In 1 HSCA BD decreased from 74 to 61ips, phase shifted from +65° to +53°, sensitivity decreased 0.6x, but inhibitory cutoff was prevented. In the other HSCA with higher BD the only noticeable effect of efferent activation was brief excitatory bursts of APs. Thus behavioral activation of efferents is able to selectively modify the response dynamics of vestibular primary afferents during physiological stimulation.
(Supported by NIH NS 21055)

207.10 OCULOMOTOR AND SUBJECTIVE RESPONSES DURING CORIOLIS STIMULATION DEPEND ON GRAVITOINERTIAL FORCE BACKGROUND. J. R. Lackner, P. DiZio* and J. N. Evanoff. Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254

Lackner and Graybiel (AGARD Monograph CP-372, 1984) reported that susceptibility to motion sickness during Coriolis/cross-coupling stimulation (CCS) decreases in the free fall phase of parabolic flight and increases in the high force phase relative to ground based tests. We expected parallel oculomotor and perceptual responses to be evoked. Thus, we examined the intensity of reflexive eye movements and perceived self-motion during constant levels of CCS produced by voluntary head movements (HMs) during whole-body rotation in the alternating 20 sec periods of low (0 G) and high (1.8 G) gravitoinertial force generated in parabolic flight maneuvers.

The experiment was conducted aboard NASA's KC-135 aircraft. The seated, blindfolded subject was continuously rotated about his z-body axis at 80°/s in a chair equipped with a device that restrained HMs to the pitch plane. For at least 60 s before every HM, the subject's head was positioned 20° forward in pitch. At the next available period of constant force, the subject moved his head backwards 40° within 1 s after being instructed. Horizontal eye movements were recorded using conventional DC electro-oculography for the next 10 seconds. The subjects made pitch-up HMs alternately in 0 G and 1.8 G, rating the relative magnitude of apparent body tilt and rotation after each pair. Seven subjects participated each making between 3 and 11 pairs of HMs.

Recordings indicated no differences in chair velocity or the HM kinematics (duration, amplitude and peak velocity) between 0 G and 1.8 G, thus constant levels of CCS were acheived. All 7 subjects judged the magnitude of illusory body tilt and rotation to be less in 0 G than in 1.8 G. Both average slow phase velocity and beat frequency for the 10 s periods of nystagmus were significantly lower in 0 G than in 1.8 G. ($p < .05$).

Average Characteristics of Nystagmus

	0 G	1.8 G
Slow phase velocity (deg/s)	4.6 (s.e.=1.23)	6.8 (s.e.=.63)
Beat frequency (beats/s)	1.47 (s.e.=.22)	1.69 (s.e.=.21)

We conclude that oculomotor and subjective responses to constant levels of CCS are greater in 1.8 G than in 0 G. These differences appear to be related to both the relative provocativeness of CCS and the ability to adapt to it. The difference in the subjective responses between 0 G and 1.8 G was large while the difference in oculomotor responses was relatively small, suggesting that sensory and motor factors in addition to reinterpretation of canalicular activity are involved in responses to CCS.
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207.11 DYNAMIC RESPONSES OF CENTRAL VESTIBULAR NEURONS TO OFF-VERTICAL AXIS ROTATIONS IN CATS: EFFECTS OF VELOCITY AND AMPLITUDE OF HEAD TILT. Y.S. Chan, Y.M. Cheung* and J.C. Hwang. Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong.

The dynamic responses of static-tilt sensitive central vestibular neurons during off-vertical axis rotations were analyzed in decerebrate cats. It has earlier been shown that each unit showed modulation of firing rate, with a position dependent maximum and minimum during 360° rotation (Chan Y.S. et al., Brain Res., 1985, in press).

The effect of velocity (ranging from 1.7 to 15°/s) on the neuronal responses during constant speed 10° off-vertical axis rotations was studied. Positional responses were still observed within this range of velocity of rotation. Both the amplitude of the peak-to-peak discharge modulation and the locations of the discharge maxima were apparently insensitive to velocity in the range studied.

The relation between unitary discharge and the degree of off-vertical axis (5° - 15°) rotation was also studied over any one velocity within the specified range. There was a linear relationship between the gain of the unitary discharge modulation and the degree of off-vertical tilt, but no change in the locations of the discharge maxima was observed.

The response patterns of such central vestibular neurons during off-vertical axis rotations may well convey the information from the otolith for continuous nystagmus (Cohen B. et al., Brain Res., 276: 159, 1983) in otolith-ocular reflexes.

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207.12 ANATOMICAL CHARACTERISTICS OF THE VESTIBULAR NERVE IN THE SQUIRREL MONKEY. A. Kuruvilla* and V. Honrubia (SPON: D. S. Maxwell). Div. of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

A study was made of the dimensions and number of nerve fibers innervating each of the three semicircular canals, the utricle, the superior vestibular nerve and the whole vestibular nerve root in the squirrel monkey in order to determine whether these fibers segregate according to their diameter as they do in amphibians (Honrubia, V. et al., Laryngoscope, 94, 464, 1984). Fiber counts and dimensions were analyzed by photographing 1-µm-thick cross sections of the nerve and digitizing the outlines of the nerve fibers with the aid of a laboratory computer at more than 1000 X magnification.

The superior vestibular nerve has an average of 8850 fibers: 2850 fibers to the anterior semicircular canal, 3100 fibers to the horizontal semicircular canal, and 2900 fibers to the utricle. The posterior ampullary nerve (branch from the inferior vestibular nerve) has 3250 fibers. Individual fibers to the various cristae range in diameter from 0.5 µm to 8 µm with 80% of fibers being $\leq$ 2 µm and only 5% > 4 µm. Utricular fibers range in size between 0.5 µm and 6 µm. Here again, 80% of fibers are $\leq$ 2 µm in diameter and only 5% are > 4 µm. A pattern of segregation of fibers according to their diameters is found, both in the peripheral cristae and in the superior vestibular root. In the cristae, small-diameter fibers ($\leq$ 2 µm) segregate into bundles at the periphery. At the center, larger-diameter fibers predominate. Large-diameter fibers predominate in the anterior portion of the superior vestibular nerve, while smaller-diameter fibers predominate in the posterior part. In the anterior part, the fibers range in size from 0.5 µm to 12 µm, with 65% of fibers being $\geq$ 4 µm in diameter. In the posterior part, on the other hand, 75% of fibers have diameters $\leq$ 4 µm. In the vestibular nerve root the above pattern of segregation of fibers according to their diameter is not clearly discernible. This is because the primary vestibular fibers divide into smaller secondary branches within the nerve root itself so that there is an intermixture of large- and small-caliber fibers. We counted an average of 17,000 fibers at this level.

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207.13 PROBABLE LOCALIZATION OF GABA AND ACETYLCHOLINE SYNTHESIS IN THE VESTIBULE OF STREPTOMYCIN-TREATED GUINEA PIGS. A.G. Iturbe* and G. Meza (SPON: A.M.López-Colomé) Dept. Neurociencias CIFC, UNAM, Apdo. Postal 70-600 04510 México D.F.

The presence of glutamate decarboxylase (GAD) and choline acetyltransferase (ChAT) the GABA and acetylcholine (Ach) synthesizing enzymes, respectively, in the anfibian and avian vestibule is evidence of their participation as neurotransmitters in these systems. However, in mammalian vestibule this has not investigated, although GABA and Ach have been assumed to be mediators there.

In support to this assumption we measured GAD and ChAT using the vestibule of pigmented guinea pigs as a model.

When both enzymes were measured in homogenates of the whole vestibule as already reported (1), (2) we found GAD activity to be 0.250 μ mol /hr/mg prot. and activity of ChAT 0.550 nmol/min/mg prot.

The precise localization can not be assessed by these experiments, thus in order to localize the cells of origin of these enzymes we used streptomycin sulfate (300 mg/kg daily for 20 days, intramuscular) treated animals, knowing in advance that this treatment causes severe damage in vestibule sensory cells, starting with degeneration, and finally disappearance of the sensory hairs cells, the nerve fibers and nerve endings being however, not affected (3).

After treatment, the animals showed vestibular damage, according to the observation of lossing of the rightening reflex and disappearance of the nystagmus response with caloric stimulation.

When GAD and ChAT were measured in the vestibule of treated animals GAD was found to have decreased about 50% of the vestibular enzyme of control animals injected with normal saline solution. ChAT was not modified in either group.

Although morphological controls are needed to support this assumption, based on the streptomycin effects reported (3) and our results, we can suggest that GAD is localized in at least 50% of the hair cells whereas ChAT is probably in efferent endings, being the GABA and Ach their respective neurotransmitters. (supported in part by Grant PCCBBNA 020897 of CONACyT México)

(1) Meza. G. J of Neurochem 1984 43: 634-639.
(2) Meza. G. G. López. I., Ruiz. M. Neurosci Lett 1984 49: 93-97
(3) A.J. Duvall and J. Wersall Act Oto-laryng (stockh) 1964 57: 581-598.

207.14 POSSIBLE EFFERENT NEUROTRANSMISSION IN THE INNER EAR OF THE FROG G.Meza,I.López*and A.Feria-Velasco.Dept.Neurociencias,CIFC UNAM Apdo.Postal 70-600 04510 México,D.F.(GM and IL),Div.Developmental Biology.Unidad de Investigación Biomed.Occidente,I.M.S.S.Guadalajara,Jal.MEXICO(AFV)

Neurotransmission in the sensory periphery of the vestibular system is chemical in nature; the efferent neurotransmitter is apparently acetylcholine(Ach). In earlier studies we demonstrated choline acetyltransferase(ChAT) activity in homogenates of frog vestibule and choline transport in the whole vestibule of this anfibian(1); however with this type of experiments, the cell of origin of these biochemical parameters can not be assessed. With the purpose of localization of the Ach-utilizing cell by elimination of certain population of terminals, we used Hillman model(2) in which the VIII craneal nerve of the frog was excised and degeneration of efferent nervous fibers was observed. We utilized adults frogs(Rana pipiens) in which denervation of the right VIII craneal nerve was performed leaving the left nerve intact as control. We determined ChAT activity as previously described (3)in the frog vestibule after 3,7 and 15 days of surgery. For morphological studies the right and left vestibules obtained from denervated frogs were fixed "in situ" with cacodylate buffered-1% osmium tetroxide, dehydrated and embedded in epoxy resins. One micrometer thick sections were collected on glass slides and stained with toluidine blue for examination under light microscope.
We observed gradual decreased of the activity with time after denervation until it reached 50% of ChAT present in the control side. Morphological study of vestibular nerves in the vecinity of receptor zones revealed a Wallerian degeneration of some nerve fibers randomly distributed among normal looking fibers. Various button-like nerve endings appeared markedly basophilic at the basal portion of receptor zones. No structural alterations were seen at both, the receptor areas and the vestibular nerve trunks of the non-denervated vestibule of operated frogs, of both vestibules obtained from non-operated animals. Thus apparently the only affected structures are the efferent endings. These biochemicals and morphological results show that ChAT in the inner ear of the frog appears to be localized at least in 50% of efferent terminals which probably use Ach as mediator at the level of the sensory periphery of vestibular system.
1.-Neurosc.Abs,10,1152,1984.
2.-Exp.Brain Res.9,1-15,1969.
3.-Neurosc.Lett,49,93-97,1984.
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207.15 Effects of Utricular and Semi-circular Canal Stimulation on Selected Central Nervous System Reflexes of Children with Severe Developmental Disability. M. A. Boyle, Program in Occupational Therapy, Washington University Medical School, St. Louis, Missouri 63110.

Vestibular stimulation is frequently used in remediation of brain injured for improvement of motor control. Previous reports of vestibular stimulation have indicated short term improvements in motor function of normal and neurologically impaired children. The only study examining the long term treatment effect on developmentally delayed children found no significant improvement in motor performance creditable to vestibular stimulation. This study was developed to evaluate short and long term treatment effects of vestibular stimulation on selected central nervous system postural reflexes.

Institutionalized, severely developmentally disabled children (n=20), aged 6 to 16, with motor disability, spasticity, seizure disorders, and profound mental retardation were evaluated pre-treatment, post-treatment, and at follow-up (six weeks following the last treatment): on a battery of six central nervous system postural reflexes: symmetrical tonic neck reflex, asymmetrical tonic neck reflex, tonic labyrinthine neck reflex (supine and prone), labyrinthine neck righting (vertical), and optical neck righting. Subjects were randomly assigned to a treated and control group. The treated group (n = 10) received five minutes of vigorous utricular and semi-circular canal stimulation daily for six weeks. The control group (n = 10) received five minutes of slow movement with minimal utricular and semi-circular canal stimulation daily for six weeks.

Within-group analysis of the median difference scores demonstrated no significant difference between the median scores of the post-treatment and pre-treatment tests in either the control group or the treated group. Moreover, there was no significant difference between the follow-up and pre-treatment scores of the control group. However, the interval between the pre-treatment and follow-up score of the treated group was significant ($0.005<p<0.01$). Between-group analysis of difference scores also demonstrated a significant change ($p = 0.01$) between the treated group's pre-treatment and follow-up score.

Utricular and semi-circular canal stimulation was found to be effective over a long term period in increasing development level of motor function as indicated by performance on central nervous system reflexes.

207.16 BLIND POINTING TO VISUAL TARGETS DURING BODY ROLL AS POSSIBLE OTOLITH FUNCTION INDICATOR ?
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Vestibular otolith function is presently best being evaluated by measurements of ocular counterrolling during relative changes of the gravitation vector (Diamond & Markham, Neurology,33:1460,1983), although the exact relationship between the ipsi-and contralateral otolith organs and the angle of counterrolling of either eye is still unknown. We report on a sensori-motor task which also depends on the direction of the gravitation vector.

Pointing to visual targets without seeing the pointing hand (blind pointing) was studied in healthy subjects as a function of static body tilt angle α (roll) in a swing chair. Sequences of 42 visual targets, appearing(30 deg left to 30 deg right) on the inner surface of a hemispherical screen(r=23 cm) in the horizontal plane of the tilted head coordinate system were presented monocularly at various roll angles(55 deg left to 55 deg right). Blind pointing positions of the upper hand(e.g. right hand for left ear down tilt) on the outer surface of the screen were measured in two dimensions by means of a modified joy stick device.
1)For a given subject the blind pointing characteristic(see schemes) with significantly different features for the ipsilateral vs. contralateral hemifield was highly reproducible and depended on both the roll angle α and the eye stimulated. 2)The most prominent parameter depending on α was the rotation angle π (see schemes) between α and the angle of the ipsilateral half of the blind pointing characteristic. 3) π reached values up to π =25 deg with increasing α. 4)When the lower eye was stimulated, π was typically larger by at least 5 deg as compared with stimulation of the upper eye.
5)Comparative measurements with a retinal reference paradigm(subjects were fixating a green light dot in the primary position while pointing at the extrafoveally occurring red light dots) yielded qualitatively similar changes for $\pi = f(\alpha)$.

The possible correlation between π and ocular counterrolling will be discussed.

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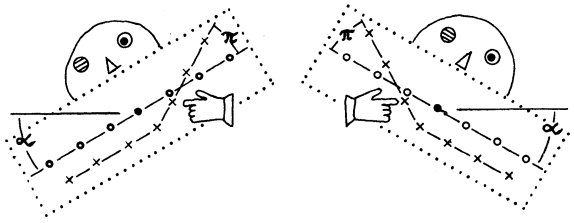

207.17 PROPRIOCEPTIVE INFLUENCES ON VESTIBULAR NYSTAGMUS AND THE DISPLACEMENT COMPONENT OF THE OCULOGYRAL ILLUSION. J. N. Evanoff and J. R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, Massachusetts 02254.

Several studies have demonstrated a potential contribution of proprioceptive information about limb position to visual direction and voluntary oculomotor control. Proprioceptive information specifying the location of an object in relation to the body has been shown to affect the apparent visual direction of the object and to enhance fixation responses (Lackner, J. R. and Levine, M. S., Neuroscience Letters, 7: 207, 1978; Levine, M. S. and Lackner, J. R., Experimental Brain Research, 36: 275, 1979).

We examined the influence of brachial, proprioceptive information on vestibular nystagmus in the dark using six trapezoidal velocity profiles ranging from $2^o/s^2$ to $25^o/s^2$. Nystagmus was induced by exposing subjects to clockwise, angular acceleration with their longitudinal (Z) body axis aligned with the axis of chair rotation. Subjects fixated a target light that was extinguished five seconds prior to the onset of chair acceleration, they then attempted to maintain fixation of the former position of the light for the duration of the trial. These base-line measures of vestibular nystagmus were then compared to conditions in which subjects touched the target light mount with the unseen index finger of their right hand throughout an entire velocity profile.

Brachial proprioceptive information under these conditions was found to be of sufficient fidelity to contribute to a fixation response adequate to suppress both the fast and slow phase of vestibular nystagmus for all acceleration rates, ($p<.01$). Clearly, a sensory input adequate to override vestibular nystagmus need not originate in the visual modality.

This finding allowed us to assess the contribution of reflexive oculomotor signals to perceptual illusions associated with vestibular stimulation, such as the oculogyral illusion (OGI) in which a visual target, stationary in relation to the body, is seen to move and displace relative to the body in the direction of acceleration (Graybiel, A. and Hupp, D., Journal of Aviation Medicine, 17: 1, 1946). Whiteside, Graybiel, and Niven, (Brain, 88: 193, 1965) have suggested that the OGI results from monitoring a fixation command that is necessary to override the latent vestibular nystagmus so as to maintain a retinally stable image. We found that if subjects touch the visual target with their unseen index finger the illusory displacement component of the OGI is attenuated by an amount comparable to the diminution of their nystagmus that occurs when they fixate their finger in the dark, ($p<.05$). Increases in the magnitude of the OGI displacement were observed if the finger was positioned as little as 8 cm. from the target.

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207.18 DIRECTIONAL SENSITIVITY OF CAT FORELIMB AND SHOULDER MUSCLES TO VESTIBULAR AND NECK STIMULATION. R.H. Schor, V.J. Wilson, I. Suzuki* and B.R. Park*. The Rockefeller University, New York, NY 10021.

Combinations of sinusoidal roll and pitch stimuli were used to study the spatial organization of vestibular and tonic neck reflexes acting on forelimb (medial, lateral and long heads of triceps brachii) and shoulder (supraspinatus and infraspinatus) extensor muscles of the decerebrate cat. Neck reflexes were studied in preparations having intact labyrinths as well as those having acute or chronic labyrinthectomies. Reflexes, studied with EMG recording, were described by response vectors whose orientation component is aligned with the optimal excitatory direction of tilt or head rotation. Repeated testing over a period of many hours showed that a muscle's response vector remained reasonably stable, although there was sometimes drift at the beginning or end of an experiment, and sometimes an apparent change of vector during locomotor-like activity. Stimulus frequencies were from 0.05-2 Hz. Over this range, the orientation of muscle response vectors did not change in a systematic fashion with stimulus frequency for either neck or vestibular reflexes. For the latter, this is so despite the fact that reflex dynamics are consistent with convergent input from semicircular canals and otolith organs: the otolith and canal components are apparently aligned. This contrasts with the results of Baker et al. (Soc. Neurosci. Abstr. 10: 162, 1984) on vestibulocollic reflexes. Although there was interexperiment variation, a consistent reflex pattern emerged. For all muscles tested, vestibular reflexes are characterized by response vector orientation near ear-down roll. Neck vector orientation lies in the opposite direction from the vestibular vector, but typically does not lie as close to the roll plane: for the shoulder muscles and the medial and lateral heads of triceps nose up pitch is excitatory, while for the long head of triceps, nose down excites. The pattern of response to neck stimulation generally agrees with the scheme proposed by Roberts (Neurophysiology of Postural Mechanisms, Butterworths, 1978), but the pattern of response to vestibular stimulation does not. Roberts' scheme describes a consistent response to vestibular pitch which we did not see. We suggest that muscle response vectors are produced by vectorial addition of the signals of an array of premotor neurons, each with its own directional sensitivity, and that peripheral and central modulation of this pool of neurons determines which of them are active, and ultimately determines motoneuron vectors. Supported in part by grants from NIH (NS02619) and NASA (NSG2380).

207.19 CONTROL OF LIMB POSITION DURING EXPOSURE TO INCREASED AND DECREASED GRAVITOINERTIAL FORCE LEVELS. John D. Fisk*, J. R. Lackner and P. DiZio* (SPON: Minna Levine). Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

We had 5 subjects reproduce practiced flexion and extension positions of their unseen, unrestricted right forearm while exposed to alterations in the magnitude of the gravitoinertial force vector. Parabolic flight maneuvres in a Boeing KC-135 aircraft generated repeated, alternating periods, each approximately 20 s in duration, of increased G-force and free fall. While seated and restrained by a seat belt, the subjects attempted to move their forearms between 110° and 145° end points (full extension = 180°) during the high force (> 1.7 G) pull-up phase and the free fall (0 G) phase of each parabola.

Pre-flight and in-flight practice of this task was provided, both with and without visual feedback. Two orientations of the arm were studied: a "vertical" orientation requiring rotation of the forearm through the line of action of the G-force vector, and a "horizontal" orientation with the plane of forearm rotation orthogonal to the G-force vector. Two speeds were examined: slow movements (approximately 1 s for flexion or extension), and rapid movements (< 500 ms duration). Forearm position and velocity were measured with a light-weight (200 g) electrogoniometer and recorded on FM tape.

We compared movements made in the high force and free fall phases of flight. Slow movements made during free fall had a significantly lower amplitude than high force phase movements with an average reduction for both arm orientations of approximately 20% of the high force amplitude. The decreased movement amplitude in free fall was accompanied by a 13.5% reduction in the peak velocity of "horizontal" movements and by a 23.5% reduction in peak velocity of "vertical" movements. For rapid movements, no significant differences in movement amplitude were found with changes in the magnitude of the G-force vector. However, a significant reduction in peak velocity of 12.5% was found for "vertical" movements made during free fall.

The data suggest that awareness of limb position is altered by changes in the magnitude of the G-force vector, independent of its orientation with respect to the limb. The rapid movements were less affected by the altered G-force level, perhaps because afferent information is not relied on to the same extent for the determination of end-points as with slow movements. However, the orientation of the moving limb with respect to the G-force vector clearly influenced the velocity of the rapid movements. Together our findings indicate that the position sense representation of the body normally is calibrated to a 1 G terrestrial force level.

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REFLEX FUNCTION II

208.1 QUANTITATION OF ELECTROMYOGRAM (EMG) PATTERNS OF CLONIC-LIKE MUSCLE CONTRACTIONS IN DECEREBRATE CATS. E. K. Stauffer and R. S. Pozos. Department of Physiology, University of Minnesota-Duluth, Duluth, MN 55812.

A feature of clonic-like muscle action is an EMG that exhibits relatively discrete bursts of activity followed by periods of almost complete silence. In spite of its striking qualitative nature, little data exists describing this bursting pattern. In order to gain further insights into the nature of the motor systems' operation during this type of action, the present study was undertaken to examine the quantitative characteristics of this oscillatory activity.

The left hindlimb of decerebrate cats (pre- and midcollicular) was denervated except for the plantaris muscle. Mechanical and EMG activity were recorded during spontaneous contractions. Data included: (1) EMG burst duration; (2) silent period duration; and (3) area (arbitrary units) of the full-wave rectified EMG burst.

Average burst durations ranged (± S.D.) from 17.3 (± 2.7) to 26.9 (± 3.3) ms for the animals with a mean (± S.D.) of 22.0 (± 3.4) ms across animals. Average silent periods ranged (± S.D.) from 17.0 (± 6.8) to 34.0 (± 5.0) ms with a mean (± S.D.) of 26.8 (± 7.9) ms, but they were more variable than the burst durations (mean coefficients of variation [CVs] = 12.1 versus 19.7 respectively, $p < 0.05$). EMG areas were highly variable from burst to burst (CVs ranging from 44.7 to 100.7 with a mean value (± S.D.) of 63.1 (± 20.0). Small extraneous EMGs appeared occasionally throughout the clonic sequences; these were randomly timed with respect to the primary burst.

These data indicate that the muscle's pool of active motor units (MUs) is probably firing only once or twice ("doublets", Zajac & Young, J. Neurophysiol. 43: 1221) per burst, but that this population is brought to firing threshold within a very narrow time "window" for each burst. However, the fact that the burst areas are so variable suggests that the number of active units is not constant from burst to burst. Thus, even though the time of occurrence of discharges within the ensemble of active units is tightly locked into a narrow time frame (but not precisely in-phase with each other), the mechanism(s) responsible for the synchrony may not operate to recruit identically the same motor units from contraction to contraction, and it does not appear to entrain all MUs of the firing zone.

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208.2 TOPICAL ANESTHESIA: EFFECT OF DESENSITIZATION OF THE SKIN OF THE UPPER LIMB ON THE EXCITABILITY OF THE FLEXOR MOTONEURON POOL Mohamed A. Sabbahi, Carolyn R. Mason,* and Peggy B. Gleeson.* Texas Woman's University, School of Physical Therapy, Houston, TX.

Desensitization of the skin of the lower limb, with topical anesthesia, resulted in modulation of the excitability of the extensor motoneuron pool (MNP) (Sabbahi & DeLuca, 1982). This previously reported modulation was dependent on the desensitized skin area. This report investigates the effect of desensitization of the skin on the excitability of the flexor α-MNP in the upper limb.

The H-reflex of the flexor carpi radialis (FCR) was recorded, using surface electrodes, after stimulation of the median nerve at the arm. Electrical stimulation parameters were 1 msec. duration pulses, 0.2 pps at maximum H-reflex threshold. H-reflexes were monitored by the M-response throughout the experiments. Topical anesthetic (10% Lidocaine) or a placebo was applied on the appropriate skin area in normal adult subjects, and the H-reflexes were recorded at intervals up to 40 minutes post application. The following skin areas were desensitized and sprayed with placebo at separate recording sessions: Anterior arm (AA), Anterior forearm (AF), Posterior arm (PA), Posterior forearm (PF). The percent change in the peak-to-peak amplitude of the H-reflex post anesthesia and placebo were compared.

Results showed that the peak-to-peak amplitude of the H-reflex was slightly decreased immediately post anesthetic application to all skin areas except PF. The changes appear to be due to cold receptors stimulation by the applied anesthetic. The H-reflex was significantly increased in amplitude 10 minutes after application of the anesthesia to AA, AF and PA skin areas. Significant reduction in the H-reflex amplitude was recorded post anesthesia to PF skin area. These changes continued to increase 40 minutes post anesthesia. The degree of reflex facilitation or inhibition varied between subjects. No measurable changes were recorded in the M-response or in the H-reflex post placebo.

These results indicate that the skin receptors of the upper limb modulate the excitability of the flexor α-MNP. All skin areas of the upper limb except that over the antagonist muscles (PF) supply inhibitory inputs to the α-MNs of the FCR. Skin overlying the antagonist muscles appear to supply excitatory inputs to the FCR α-MNP.

208.3 SHORT LATENCY REFLEXES FROM CUTANEOUS AFFERENTS IN THE CHRONIC SPINAL CAT. D. McCrea, S. Hochman*, L. LaBella*, & J. Kehler*. Dept. of Physiology, Univ. of Manitoba, Winnipeg, Canada R3E-0W3.

The present study reports changes in short latency reflexes following single shock stimulation of hindlimb cutaneous nerves recorded in lumbar motoneurons after chronic (six week) total transection of the upper lumbar spinal cord in adult cats. Cats were spinalized by blunt dissection with fine forceps under barbiturate anesthesia. Nursing care consisted of twice daily manual emptying of the bladder. For the acute experiment, dissection was carried out under halothane anesthesia: hindlimb nerves were dissected and the animal prepared for intracellular recording. All recordings were obtained under chloralose anesthesia using electrodes filled with potassium citrate. Compared to animals with intact spinal cords, the earliest latencies of EPSPs and IPSPs from cutaneous afferents tended to decrease in the chronic spinal preparation. This probably reflects increased efficiency of synaptic transmission in these pathways and not a reduction of the minimum number of interneurons interposed between cutaneous afferents and motoneurons. The incidence of short latency (<5mS) EPSPs was increased for stimulation of sural, superficial peroneal, cutaneous caudo-femoralis and tibial nerves in the chronic spinal cats. In addition, chronic spinalization results in the appearance of short latency, low threshold (1.1 threshold) excitation of hamstrings motoneurons when the Tibial nerve (cutaneous and motor to the foot) was stimulated.

Motoneurons in both chronic spinal and unlesioned animals show a variety of IPSPs and EPSPs when either high or low threshold cutaneous afferents were stimulated. In some cells different cutaneous nerves produced similar PSPs but in other motoneurons, different nerves produced quite different PSPs. The chronic spinal animals tended to display greater variations in the cutaneous PSPs than the unlesioned animals. High threshold stimulation often produced the pattern of PSPs expected from the flexion reflex (Eccles & Lundberg, Arch.Ital.Biol.97:22,1957) but there were many examples of effects opposite to those of the classic flexion reflex(ibid).

In summary, even though there is great variation in cutaneous PSPs in intact cats, chronic spinalization results in increased incidence of cutaneous EPSPs, a tendency to shorter latencies and the appearance of a powerful excitation from tibial nerve to hamstrings motoneurons not seen in the intact animal.

208.4 MEASUREMENT AND CHARACTERIZATION OF RECURRENT IPSPS PRODUCED BY STIMULATION OF SINGLE MOTOR AXONS. C.-S. Yuan*, T.M. Hamm, S.-I. Sasaki*, U. Windhorst*, S. Vanden Noven and D.G. Stuart, Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

The spike-triggered averaging technique has been combined with methods for stimulating single axons to determine the features of recurrent inhibitory postsynaptic potentials (RIPSPs) produced in spinal motoneurons by activation of single motor axons. Motoneurons innervating the medial gastrocnemius (MG) muscle were impaled with glass microelectrodes in cats anesthetized with chloralose-urethane (C-U) or allowed to become unanesthetized following ischemic decapitation under halothane anesthesia. Simultaneously, single MG motor axons were activated at rates of 6-8 Hz either by intraaxonal/intramyelin stimulation using glass microelectrodes (Yuan et al, Soc. Neurosci. Abstr. 9:863, 1983), or by intramuscular stimulation using fine bipolar EMG electrodes (Taylor and Stephens, Brain Res. 117:331-335, 1976). The RIPSPs so evoked were collected in a signal averager for measurement. In our sample to date, RIPSPs were recorded in 43% (12/28) of the cells in the C-U preparations and in 65% (17/26) of the ischemic decapitate preparations. These single axon RIPSPs are generally characterized by small amplitude and a prolonged time course similar to that of composite RIPSPs. In some instances, RIPSPs were recorded which consisted of two components, a brief hyperpolarization followed by a second, more prolonged hyperpolarization. The characteristics of the RIPSPs are summarized in the following table:

Characteristics of Single-Axon RIPSPs - Mean ± SD (Range)

Preparation	Amplitude (μV)	Latency (ms)	Rise-time (ms)	Half-width (ms)
C-U	12.0 ± 6.7 (3.1-27.7)	3.8 ± 2.2 (1.0-7.3)	5.9 ± 3.3 (1.0-9.9)	24.1 ± 12.4 (8.6-41.8)
Ischemic Decapitate	35.3 ± 28.0 (13.3-134.3)	3.2 ± 2.1 (0.7-9.2)	5.7 ± 2.4 (3.6-10.5)	23.1 ± 14.7 (7.6-51.2)

Not included in these data are two exceptionally large RIPSPs recorded in cells adjacent to the motoneuron whose axon was being stimulated (see accompanying abstract by S.-I. Sasaki). These RIPSPs were of similar time course to the rest in the sample. In addition to providing the characteristics of single-axon RIPSPs, these results demonstrate the feasibility of determining the distribution of recurrent inhibition within a motor nucleus using such measurements.

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208.5 TOPOGRAPHIC DISTRIBUTION OF RECURRENT IPSPS TO MOTONEURONS SUPPLYING THE MEDIAL GASTROCNEMIUS MUSCLE OF THE CAT. S.-I. Sasaki*, C.-S. Yuan*, T.M. Hamm, U. Windhorst*, S. Vanden Noven and D.G. Stuart, Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

We have tested the possibility that recurrent inhibitory postsynaptic potentials (RIPSPs) to motoneurons are distributed according to topographic factors within a motoneuron pool. Single motor axons supplying the medial gastrocnemius (MG) muscle were stimulated while recording intracellularly from MG motoneurons, as described in the accompanying abstract (C.-S. Yuan et al, this volume). The locations of motoneurons and motor axons were approximated to the rostral or caudal part of the L7 segment or the S1 segment of the spinal cord by recording their actions potentials extracellularly with electrodes on the respective ventral root divisions or by referring the site of motoneuron penetration to the boundaries of the ventral-root segments. Motoneuron-axon pairs were classified as "proximate" or "distant" according to whether the two axons were contained in the same or different ventral root divisions.

We have collected a total of 42 such pairs to date (27 proximate, 15 distant). RIPSPs were obtained in 20 pairs, while no response could be discerned in the other 22 cases. These RIPSPs and non-responses were distributed as follows:

	Proximate Pairs	Distant Pairs
RIPSPs	N=17	N=3
Non-responses	N=10	N=12

These results demonstrate a significant dependence of the occurrence of RIPSPs upon the proximity of the motoneuron-axon pair (χ^2=5.52; p<.025).

There were no clear cut differences between RIPSP obtained from proximate and distant pairs. However, we have excluded from this sample two RIPSPs which were of clearly different amplitude than the rest. Both RIPSPs were recorded in response to stimulating a motor axon whose cell body had been located by penetration with the recording microelectrode. Stimulating the axon of this motoneuron produced RIPSPs of 420 μV and 202 μV in motoneurons in the same microelectrode tract as this cell and in a tract 200 μm away, respectively. Recordings from two other motoneurons 1 mm away from this site yielded RIPSPs (included in the present sample) of 41 and 38 μV in response to stimulating the same motor axon. This anecdotal observation suggests that the amplitudes of single-axon RIPSPs as well as their incidence may be dependent upon topographic factors.

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208.6 DIFFERENTIAL CONTRIBUTIONS OF RECTUS FEMORIS AND VASTUS MEDIALIS TO CROSSED EXTENSION REFLEX: PROLONGED CENTRAL SUMMATION. J. A. McMillan, P. R. Hannon* and L. M. Stevenson*, Biol. Dept., Montana State Univ., Bozeman, MT 59717.

Several lines of evidence have suggested a differential, and somewhat independent, contribution of fast and slow motor units to voluntary and reflex motor activities. The differences have (i) been consistent with the size principle of recruitment of motor units and (ii) have supported a model of separate reflex pathways projecting to fast and slow motor unit pools.

We examined prolonged central summation (PCS) of the crossed extension reflex (CER) recorded from the vastus medialis (VM) and rectus femoris (RF), which are relatively fast and slow heads of the quadriceps femoris group respectively. PCS is a feature of nocifensive reflexes associated with repetitive stimulation (f > 0.2 - 0.3Hz) and may be mediated by nociceptive dorsal horn interneurons. It has also been proposed that these interneurons may simultaneously contribute to nociception and to segmental nocifensive reflexes.

Reflexes were monitored in acute decerebrate cats. The left sciatic nerve was stimulated (1 ms, 2-4mA) and isometric tension was recorded from the right VM and RF. VM typically exhibited more pronounced PCS than did RF. The differences were especially evident when the cat was placed with the left body side on the table. First, VM usually began to summate at lower frequencies (down to 0.17 Hz) than did RF. Second, VM typically continued to summate after RF had reached plateau tension. In most cases the long-latency contraction evoked by each shock summated much more than did the short-latency contraction.

Our results do demonstrate that motor neuron pools supplying relatively fast and slow muscles receive differential inputs mediating what has classically been considered to be a singular reflex mechanism. However, the results presented here are not consistent with the size principle of recruitment. Since RF is slower than VM, and since slower muscles have been reported to be recruited at slower rates of rhythmic voluntary contractions, it would be reasonable to predict that RF would summate at lower frequencies of stimulation than would VM. Just the opposite happened. The discrepancy might suggest that responses monitored by recording muscle tension, which is the appropriate index in terms of motor behavior, are different than those monitored by recording electrical activity of individual motor neurons.

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208.7 **DIFFERENTIAL CONTRIBUTIONS OF VASTUS MEDIALIS AND RECTUS FEMORIS TO CROSSED EXTENSION REFLEX: EFFECTS OF BODY POSITION.** P. R. Hannon*, J.A. McMillan and L. Stevenson* (SPON: D. Phillips) Biol. Dept., Montana State Univ., Bozeman, MT 59717

In another paper presented at this meeting (McMillan, Hannon & Stevenson) we reported that vastus medialis (VM) and rectus femoris(RF), relatively fast and slow heads of quadriceps femoris respectively, do not always act in concert in mediating the crossed extension reflex (CER). In the present study we examined effects of body position on two characteristics of the CER recorded from the two muscles: threshold and response to supramaximal stimulation. Body position has been shown to strongly affect excitability of the CER recorded from intact quadriceps femoris (McMillan and Koebbe, (Exp'l. Neurol. 73,233- 242,1981). We report here that body position affects VM differently than it does RF.

Reflexes were monitored in acute decerebrate cats. The left sciatic nerve was stimulated with indwelling electrodes (1 ms, 2-4mA) and isometric tension was recorded from the right vastus medialis (VM) and rectus femoris (RF).

Threshold for CER from VM was higher than from RF when single shocks were given. However, with high-frequency (20 Hz) stimulation, especially when the cat was placed with the left body side on the table surface, there was usually little, if any, difference in threshold intensity. The only difference was, in some preparations, a longer latency for VM. In one instance threshold for VM was, in fact, slightly lower than for RF. Recruitment of additional motor units in RF near threshold intensity was often accompanied by simultaneous recruitment of additional motor units in VM. These results suggest that the motor neuron pools supplying the two muscles do share some common integrative substrates.

Sustained reflexes, evoked by high frequency trains of supramaximal shocks (10-20 Hz, 2-4 mA), from RF were much more sensitive to changing body position than were those from VM. Both muscles exhibited strong responses when the cat was placed on the right body side. However, when the cat was placed on the left body side the response from RF was strongly inhibited whereas little change was usually seen in the response from VM.

These results are in agreement with other observations that fast and slow muscles can contribute differentially to motor behavior. In this case, the contribution of VM was quite consistent, whereas that of RF was much more variable, with different proprioceptive and/or mechanoreceptive inputs.

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208.8 EFFECTS OF A CONDITIONING PATELLAR TENDON REFLEX ON CONTRALATERAL REFLEX CHARACTERISTICS. D.M. Koceja*, G. Kamen and H.H. Morris*. Motor Control Lab, Indiana University, Bloomington, IN 47405.

In an attempt to determine the effects of a conditioning tendon reflex stimulus on the contralateral reflex arc, bilateral patellar tendon reflexes (PTR) were elicited in 5 college-age subjects (SS). The SS were tested over a two day period. On each day the S was seated with each limb independently secured to restrict movement. Two electromagnetic solenoid-driven hammers were positioned so as to deliver a tap to each patellar tendon with a force of approximately 24.5 N. The forces of these hammers were monitored using a digital storage oscilloscope. Peak Force (PF) and Time to Peak Force (TPF) were recorded using two strain gauges positioned at each ankle and the force was recorded using a pen recorder operating at a speed of 200 mm/sec. For the control (C) condition three left limb (LL) and three right limb (RL) PTR were separately elicited. Three bilateral reflex trials were elicited using intertap intervals of 0, 50, 75, 100, 125 and 150 msec. In all bilateral (B) conditions the LL PTR was elicited first. An intraclass reliability ANOVA model incorporating SS, days and trials revealed reliability coefficients ranging from .79-.98 for PF and .42-.96 for TPF. A 2 X 3 (Days X Trials) ANOVA revealed no significant differences between days, trials or days by trials for PF and TPF; therefore data from Day 2 was analyzed. For the C condition, PF revealed no significant differences between the LL and the RL ($t=1.97$, $p > .05$), but a significant difference existed for TPF between the two limbs ($t = 2.11$, $p < .05$). Both PF and TPF analyses (Subj X Cond) revealed no significant changes for the LL across conditions. A similar analysis revealed significant differences among these conditions for the RL (test leg), as shown in the Table.

		Intertap Interval (msec)						
		control	0	50	75	100	125	150
LL:	PF(N)	19.92	17.64	17.47	19.11	18.45	16.82	17.47
LL:	TPF(msec)	72.50	74.58	73.33	74.58	73.75	75.41	73.91
RL:	PF(N)	24.50	22.97	21.31	29.29	30.70	29.18	31.03
RL:	TPF(msec)	65.83	67.08	65.41	64.16	58.33	59.58	57.08

These results suggest a distinct period of depression of PF and a delay in TPF for the second reflex extending until approximately 50-75 msec, after which both PF and TPF were markedly enhanced. Post hoc power analyses revealed that a sample size of approximately 10-12 SS would be sufficient to detect significant differences with power greater than .85.

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208.9 REFLEX AND KINEMATIC ADAPTATION IN HUMANS DURING THE LEARNING OF A PERTURBED ARM MOVEMENT. C. Dugas*, J.S. Frank* and A. Nativ*. (Spon: A. Baylor) Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, N2L 3G1, CANADA

Few attempts have been made to examine reflex and kinematic adaptation as a function of learning (i.e., Christakos et al., Neuroscience Letters, 41:295-300, 1983). These have been confined to the study of monkeys over extended periods of learning. The general finding from these studies was that the M2 response decreased while the M1 response to perturbation increased with learning. The purpose of this study was to examine the adaptation of the reflex behavior in humans during learning of a perturbed goal-directed movement.

Two subjects were required to perform a 65 deg forearm extension movement in 900 ms to a visual target. A perturbation in the flexion direction (90 ms, 23 N.m peak torque) was introduced 500 ms after the onset of movement on every training trial. The subjects performed 100 trials per day for four consecutive days. The position of the manipulandum and the EMG signals from triceps and biceps were recorded for the first, middle and last ten trials of each day.

Results demonstrated both changes in subject strategy and reflex adjustment to the perturbation. The kinematic data indicated that with learning, both subjects increased the slope of their displacement-time function in the period preceding the perturbation. This was accompanied by a greater deflection of the limb following the perturbation. This strategy permitted the limb displacement to approach the correct trajectory resulting in an improvement in performance accuracy with learning.

The reflex component was analyzed first by looking at the mean level of EMG for the M1 (20-30 ms) and the M2 (60-90 ms) components. This analysis demonstrated a reduction of the reflex activity (M1 and M2) with learning in both subjects. This is consistent with the greater limb deflection following perturbation. However, in one subject, this reduction was related to the background level of EMG activity, for both the M1 and M2 response. In the other subject there was a reduction in the M1 response during the course of learning which was independent of the background decrease in EMG activity. The M2 response showed a sharp decrease over days one and two, followed by a gradual increase over days three and four.

Compared to previous animal studies, the results suggest a more complex relationship between learning and reflex adjustment. (Supported by NSERC scholarship to C.D.)

208.10 REFLEX MODIFICATION OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE FOLLOWING HIPPOCAMPECTOMY. A. G. Romano, R. L. Port and M. M. Patterson. Dept. of Psychology and Coll. of Osteopathic Med., Ohio Univ., Athens, OH 45701.

The topography of the rabbit's nictitating membrane (NM) response can be modified by preceding the reflex-eliciting stimulus with an auditory stimulus (Ison & Leonard, 1971; Young, Cegavske, & Thompson, 1976). In general, reflex amplitude tends to increase with increases in the interstimulus interval (ISI) and yields a function which closely parallels the ISI-conditioning function. Two lines of evidence suggest that the latter function is mediated at least in part by the hippocampus. Hippocampectomy has been shown to alter conditioned response topography as well as the ISI-conditioning function (Port, Mikhail, & Patterson, 1985). Moreover, conditioned increases in hippocampal activity have been shown to be sensitive to manipulations of the ISI (Hoehler & Thompson, 1980). Because unit activity precedes the behavioral response and conditioned increases occur very early in training, it has been suggested that hippocampal activity might modulate lower brain structures and alter reflex excitability. Thus, the present study sought to determine if the reflex modification function would be altered by hippocampal damage.

Surgical procedures were carried out on nine rabbits anesthetized with halothane. Bilateral hippocampal ablations were performed on four of the animals. In the remaining animals, only the cortex overlying the hippocampus was removed. An additional five animals served as unoperated controls. Behavioral testing began 3-4 weeks post-surgery. The NM reflex was elicited by a 50 ms, 1 mA ac shock delivered via wound clips to the periorbital region of the eye. The auditory stimulus was a 1250 ms, 90 db, 1 kHz pure tone. Six trial-types were programmed to occur 3 times in each of 5 blocks of trials. Shock-alone trials were used to assess baseline NM amplitude and area. The ISI's on tone-shock trials were 75, 150, 300, 600, and 1200 ms. Response measures on these trials were expressed as absolute measures and as percentages of the measures obtained on shock-alone trials.

Repeated-measures analyses of variance failed to indicate a significant difference among the groups' reflex modification functions. Thus, there is no evidence to suggest that the hippocampus influences reflex excitability. Our reflex modification functions for all three groups are in general agreement with those reported by others (Ison & Leonard, 1971; Young et al., 1976). Both absolute and relative measures of response amplitude indicated reflex inhibition at the shortest ISI and increasing levels of reflex facilitation over the longer ISI's. Absolute and relative measures of response area yielded similar results although response area tended to decline at the 1200 ms ISI.

208.11 REFLEX RESPONSES OF THE HUMAN JAW-CLOSING SYSTEM DEPEND ON THE SITE OF INTRAORAL STIMULATION. A. Smith*, C.A. Moore*, D.H. McFarland*, and C.M. Weber* (SPON: C.A. Pratt). Dept. Audiology and Speech Sciences, Purdue Univ., W. Lafayette, IN 47907.

Our ability to understand the principles underlying control of human oral-motor behaviors such as speech is limited by a lack of knowledge of the basic physiological properties of the human oral-motor system. An important aspect of motor systems is the organization of reflex pathways. We have investigated reflex responses in human jaw-closing muscles. Results of earlier studies have demonstrated that activity in human jaw-closing muscles is typically suppressed by high-level mechanical or electrical stimulation of cutaneous afferent pathways. The reflex effects of innocuous mechanical stimulation of cutaneous receptors, such as that produced during speech, have not been explored. Thus, the aim of the present investigation was to determine if activity in jaw-closing muscles could be significantly modulated by application of brief, innocuous stimuli and to assess whether the spatial parameters of stimulation were important in determining the nature of the responses.

Mechanical stimulation was accomplished by displacement of a smooth metal disk probe (5 mm diameter) that was under servo-control. The probe excursion was a single cosine-shaped pulse of .7 mm amplitude and 10 ms duration. The disk was placed in light contact with the intraoral mucosa, and stimuli were applied to eight sites on the tongue dorsum and palate. During stimulation subjects used feedback to maintain a constant isometric biting force. Reflex responses were measured as changes in biting force and in EMG's recorded from right and left masseter muscles.

The nature of the reflex responses observed strongly depended on the site of stimulation. Stimulation of the palate tended to produce suppressions of ongoing activity, while stimulation of the tongue most often resulted in excitatory EMG responses and increases in jaw-closing force. Stimulation of the right side of the tongue produced asymmetric reflex responses in right and left masseter muscles, while stimulation of the right side of the palate produced bilaterally symmetric responses.

208.12 PROPERTIES OF THE HUMAN ABDOMINAL MONOSYNAPTIC REFLEX. B. Bishop, C. Shaw* and T. Kondo*. Dept. of Physiology. State Univ. of NY at Buffalo, Buffalo, NY 14214.

Occasionally in the past the abdominal monosynaptic reflex (AMSR) has been used to assess the corticospinal tract. Otherwise this reflex has been neglected as a tool for assessing abdominal motoneuron excitability. The purpose of this study was to define standardized conditions for eliciting the AMSR, analyze its intrinsic properties, and identify sources which are facilitatory and inhibitory to it. The EMG of the right external oblique abdominal muscle was recorded from surface electrodes while a subject (S) stood with or without trunk rotation. Taps were delivered by way of a solenoid driven plunger. Tap force was measured with a piezotransducer placed at the tap site on a 12" ruler held against the abdomen in line with the linea alba. A relaxed, standing S had no AMSR unless he stood tall to stretch his abdominal muscles or voluntarily contracted them. To generate and maintain a constant low-level of abdominal activity the S rotated his trunk and monitored a meter displaying his abdominal EMG. Now each tap evoked an AMSR whose latency ranged from 15 to 22 msec among subjects. Once abdominal motoneurons were activated by trunk rotation, the amplitude of the AMSR was independent of the level of background activity. In contrast, the AMSR was proportional to tap force. To test the effects of static lung volume, the AMSR was evoked while S held his breath at different prescribed lung volumes. At lung volumes from one liter below total lung capacity to reserve volume the AMSR was inversely proportional to the static lung volume, showing that vagal-volume feedback from pulmonary receptors modulates abdominal motoneuron excitability. To test the effects of phasic lung volume, the AMSR was evoked at different times in the respiratory cycle. Their probability of occurrence and the amplitudes of their responses evoked at end-expiration were significantly larger than those evoked at any other phase of respiration, suggesting that abdominal motoneurons, like intercostal motoneurons, are recipients of central respiratory drive potentials. In conclusion, abdominal motoneurons resemble those innervating limb muscles in that they receive strong segmental control from somatic proprioceptors and they resemble those innervating intercostal motoneurons in that they receive strong control from pulmonary proprioceptors via central respiratory neurons.

208.13 PUDENDAL NERVE REFLEXES IN THE MALE AND FEMALE RAT. Kevin E. McKenna and Irving Nadelhaft. VA Medical Center and Depts. of Pharmacology and Neurosurgery, Univ. of Pittsburgh, Pittsburgh, PA., 15240.

As part of a study investigating the spinal cord control of pelvic organs in the rat, recordings were made from pudendal nerve efferents while electrically stimulating pudendal nerve afferents. Male and female rats (200-300 g) were anesthetized with Dial-urethane or α-chloralose. The rats were paralyzed and artificially ventilated. Some rats were spinalized at C1, 3 to 5 hours prior to recording.

In the rat, the axons of pudendal nerve afferents and efferents are contained in separate branches (SN Abstracts 264.9, 1984). The sensory and motor branches were dissected bilaterally and placed on hook electrodes. In the sensory branch, conduction velocities of myelinated fibers ranged from 32.1 to 15.3 m/sec (mode 23.7 m/sec) in the male and 29.8 to 10.4 m/sec (mode 17.0 m/sec) in the female. In the motor branch the velocities were 26.7 to 13.0 m/sec (mode 20.0 m/sec) in the male and 26.2 to 8.9 m/sec (mode 18.5 m/sec) in the female. These velocities are consistent with measurements of axon diameters in these nerves. Unmyelinated fibers in both branches had conduction velocities of 1.2 to 0.5 m/sec.

Stimulation of the ipsilateral sensory branch elicited a reflex in the motor branch. It consisted primarily of a single myelinated volley with an onset latency of 10-12 msec and duration of 3.5-5.0 msec. A smaller, more variable component, probably representing unmyelinated axons, occurred with an onset latency of 80-100 msec and a duration of 25-30 msec. Both components were elicited by stimulation of myelinated afferents. The reflex was not different in intact or spinalized rats. Reflexes in males and females differed only in a slightly shorter onset latency in males. The reflex amplitude was maximal at stimulus repetition rates of 0.5/sec and below and was virtually absent above 10/sec. Calculated central delays were 5-7 msec. It is concluded that this reflex is polysynaptic and organized at spinal levels.

Stimulation of the contralateral sensory nerve elicited a reflex virtually identical to the ipsilateral reflex except that its onset latency was 1-1.5 msec longer. The contralateral reflex was also maximal at low stimulus repetition rates and abolished above 10/sec. When the ipsilateral and contralateral sensory branches were stimulated simultaneously at maximal intensity, the resulting reflex was indistinguishable from the reflex evoked by stimulation of either branch alone. This implies that the reflex evoked from either side involves a common pool of interneurons. This conclusion is supported by HRP experiments which showed that a major projection of pudendal afferents in the spinal cord is a midline terminal field in the dorsal gray commissure.

209.1 KINEMATIC PROPERTIES OF HUMAN LOCOMOTOR MOVEMENTS. J.R. Flanagan*, D.J. Ostry*, T.B. Hoshizaki*, and M.P. Gelinas* (SPON: F. Wilkinson). McGill University, Montreal, Quebec.

Kinematic invariances have been reported by several researchers interested in human gait. These include invariances in the relative timing of different phases in walking and running and invariances in joint angle patterns. We have pursued the problem of regularities in human locomotion by examining kinematic characteristics of different locomotor gaits over a wide range of velocities. This included an evaluation of the differences in gait in terms of the form of the joint angular velocity profile. The analysis of velocity profiles has proven to be productive in studying both voluntary arm movements and speech motor control.

Cinematographic and metabolic records were obtained from subjects locomoting on a treadmill. Measurements were taken at walking speeds which ranged from 4 to 11 km/hr and for running speeds from 4 to 26 km/hr. Performance was sampled at 1 km/hr intervals. The kinematic data were obtained for movements about the elbow, knee and hip.

Gait cycles were divided into knee angle flexion and extension for both support and recovery phases. Unimodal velocity profiles characterized each flexion and extension movement. The form of the velocity profiles was examined as a function of gait and speed. In both support and recovery phases of locomotion the geometric form of the flexion and extension velocity profiles was found to be invariant across changes in speed but differed for walking and running gaits.

In addition, we observed a number of systematic changes across the range of velocities. As reported previously, in both walking and running, stride rate and stride length increased with velocity. The contribution of stride length to velocity was greater at lower speeds whereas the contribution of stride rate was greater at higher speeds. In running, the duration of the support phase decreased more with increases in velocity than the duration of the recovery phase. In walking, the duration of the support phase and the recovery phase decreased proportionately.

We also found that the ratio of peak knee flexion during recovery to peak knee flexion during absorption (support phase) decreased as walking velocity increased. However, in running, this ratio increased with velocity.

The contribution of these kinematic patterns to transitions between gaits will be discussed.

209.2 ELECTRICAL STIMULATION OF "LOCOMOTOR" SITES IN THE NUCLEUS PEDUNCULOPONTIS AND ZONA INCERTA ACTIVATED LIMBIC PALLIDAL NEURONS ANTIDROMICALLY. M. Wu* and G. J. Mogenson, Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Neural projections have been demonstrated from the limbic pallidal area to the nucleus pedunculopontis, the site of the mesencephalic locomotor region (MLR), which may mediate the locomotor component of adaptive behaviors involving the forebrain limbic system (Swanson, Mogenson, Gerfen and Robinson, Brain Research, 295:161-178, 1984). Blocking these neural projections by injecting procaine into the zona incerta reduced locomotor activity initiated by injections of picrotoxin into the limbic pallidal area or by injections of dopamine into the nucleus accumbens. In the present study, the possible functional significance of this limbic pallidal-MLR projection was investigated using behavioral and electrophysiological recording techniques. Rats with chronic bipolar electrodes in the MLR or zona incerta were tested in an open-field apparatus equipped with photo-cells and counters. Locomotor activity was increased by electrical stimulation of the MLR (from 10.8 ± 2.9 to 128.8 ± 26.1 in 10 min, $p<0.01$) and of the zona incerta (from 24.8 ± 5.5 to 92.2 ± 9.6 in 10 min, $p<0.01$). Subsequently, these rats were anesthetized with urethane and standard electrophysiological recording techniques used to record action potentials from single neurons of the substantia innominata of the limbic pallidal area. In a sample of 212 limbic pallidal neurons, 65 (30.7%) were activated antidromically by single pulse stimulation of the MLR. In another sample of 111 limbic pallidal neurons, 27 (24.8%) were activated antidromically by single pulse stimulation of the MLR and 19 (17.1%) were activated antidromically by single pulse stimulation of the zona incerta. Nine of these neurons responded to the stimulation of both zona incerta and MLR. The mean latency of antidromic responses of limbic pallidal neurons was 17.6 ± 1.8 ms in response to MLR stimulation and 10.8 ± 1.1 ms in response to zona incerta stimulation. These observations provide evidence that neural projections from the limbic pallidal area to the MLR via the zona incerta may contribute to the initiation of locomotor activity.

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209.3 EMG PATTERNS IN RESPONSE TO POSTURAL PERTURBATIONS AND THEIR INTERACTIONS WITH VOLUNTARY MOVEMENT IN DEAF SUBJECTS. C.S. Layne*, L.D. Abraham and D. Brunt*. Dept. of Physical Education, Univ. Texas, Austin, TX. 78712. Univ. of Otago, New Zealand.

Normal standing subjects display a distal-to-proximal pattern of muscle activation in response to horizontal displacement (Nashner, Exp. Br. Res.,30,1977). Patterns of activity in the lower limb muscles have also been described prior to the activation of the prime mover in arm raising tasks (e.g. Lee, J. Mtr. Beh.,12,1980). We examined these patterns in deaf subjects of varying motor ability. In addition, we examined the interaction between reflexly initiated activity and voluntary movement. In these analyses we compared "highly skilled" (HS) subjects with "low skilled" (LS) subjects (as assessed by the Barrow motor ability test) to reveal possible neuromuscular correlates of the wide range in motor ability of deaf individuals. Nine deaf males (aged 15-19) were selected as subjects; four were classified as HS, five were classified as LS. Three experimental conditions were employed: (A) postural perturbations, (B) voluntary arm movement, and (C) a combination of A and B. Backward horizontal displacement of the standing subjects served as the postural perturbation, while reaching forward for a ball in response to a stimulus light served as the voluntary movement. In Condition C, the perturbation occured 100 ms after the stimulus. Surface electrodes were used to record EMG activity bilaterally from the gastrocnemius, biceps femoris, and anterior deltoid muscles. Ten trials of data in each condition were averaged for each subject. Latencies of EMG onset and peak activity were analyzed to identify response patterns. Reaction time (RT), movement time (MT), and total response time (TRT) for the reaching task were also measured. In Condition A both groups displayed a distal-to-proximal pattern of activation with the LS subjects responding with earlier bursts of activity than the HS subjects. In Condition B both groups displayed a proximal-to-distal pattern. However, although the HS subjects displayed tonic activity in the lower limbs, no bursts of activity were seen prior to deltoid onset, while the LS subjects had clear bursts of activity in addition to tonic activity prior to deltoid activation. Although there were no differences in the latency of the deltoid onset between the two groups, the HS group had faster RTs, MTs, and TRTs in this condition. In Condition C, 3 of 4 HS and 2 of 5 LS subjects displayed a muscle activation pattern which appeared unaffected by the perturbation. Within each group the behavioral measures were the same as in Condition B, but longer latency bursts of muscle activity in the lower limbs were faster with the imposed perturbation, especially in the HS subjects. Thus, HS subjects seemed better able to adapt their responses to the perturbation imposed during voluntary movement.

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209.4 **SYNCHRONIZATION OF MOTOR UNIT FIRING IN DIFFERENT MUSCLES RECORDED DURING VOLUNTARY ISOMETRIC CONTRACTIONS IN MAN.** **A.K. Datta, Jean R. Fleming*, T. Hortobagyi*, and J.A. Stephens, Dept. Physiology, Middlesex Hosp. Med. Sch. London W1P 6DB.**

Experiments were performed on 18 healthy volunteers aged 18-33 years. Recordings were made from intrinsic hand muscles, particularly first dorsal interosseous muscle (1DI) and from large limb muscles including Medial Gastrocnemius (MG), Soleus and Tibialis Anterior (TA). The muscles under study were immobilised by placing the subject's hand or leg in a frame. Two needle electrodes were inserted in to the muscle for recording the activity of two motor units A and B. The subject made a gentle steady isometric contraction such that both units fired continuously, with one, A, firing at 10 impulses/s.

Histograms (bin width 390 μsec) were constructed of the times of firing of unit A relative to the times of firing of unit B. The strength of synchrony was defined as those extra discharges of unit A for each discharge of unit B.

Typically histograms showed a narrow central peak (duration 4-12 msec) centred about the time of firing of unit B superimposed on a slow rising base. Synchrony was always observed and was strongest between pairs of units in intrinsic hand muscles, and in 1DI varied from 0.040 to 0.331 (mean 0.095, SD 0.059, n=25). In the large limb muscles synchrony was sometimes absent, and when observed was weaker than in 1DI, e.g. TA (range 0.016 to 0.094, mean 0.056 SD 0.022, n=18) MG (range 0 to 0.043, mean 0.016 SD 0.013, n=29, absent in 7 pairs). The discharge of a reference unit B in one muscle was correlated with units A from other muscles. Synchrony was greater between units in adjacent intrinsic hand muscles (1DI and 2DI) than between more widely separated hand muscles (1DI and 3DI) which was greater than that between an intrinsic hand muscle and a forearm muscle (1DI and Flexor Digitorum Superficialis, 1DI and Extensor Digitorum Communis) or between adjacent synergist forearm muscles (Ext Carpi Ulnaris and Ext Carpi Radialis Longus).

Significant synchrony was observed between a reference unit B in one muscle and units A in antagonist muscles, in the hand (1DI and first palmar interosseus) and in the leg (MG/soleus and TA).

The close relationship between the distribution of synchrony in different muscles and the function of those muscles in different tasks, taken together with the presence of synchrony between agonist/antagonist pairs is consistent with our hypothesis of the central rather than peripheral origin of short term synchronization in human muscles, probably involving activity of long descending pathways and possibly from branched fibres of the corticospinal tract (Datta et al. 1985, J. Physiol. proc in Press).

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209.5 THE CONTRALATERAL LIMB RESPONSE TO PERTURBATIONS DURING HUMAN LOCOMOTION. M. Belanger and A.E. Patla, Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

The response to stimulation (20 ms train, 10-1 ms pulses, 4-5 X threshold), applied to the right (ipsilateral) foot at five points in the stepcycle, was examined for six muscles of the contralateral limb. The area under the EMG curves for both the normal and perturbed cycles were determined for 150 ms, starting 20 ms after the onset of the stimulus. Five trials, each containing a normal and a perturbed stride, of each stimulation point were averaged together. The averaged normal EMG activity was then subtracted from the averaged perturbed EMG activity, yielding the reflex response from which the latency could be digitized. The ipsilateral limb response has been reported elsewhere (Belanger & Patla, Neuroscience Lettr, 49:291-295, 1984). The functional significance of the contralateral limb responses are discussed with respect to stimulation points in the ipsilateral limb.

At heel contact, knee collapse was prevented by an increase in knee extensor response. In addition, an increased plantarflexor response provided quick push-off for the forward progression. An increase in rectus femoris (RF) and vastus lateralis (VL) at early stance allowed for hip flexion and knee extension while an increased tibialis anterior (TA) response allowed for better ground clearance by the contralateral foot. Shortly thereafter, the knee and ankle were prepared for landing by an enhanced biceps femoris (BF) and plantarflexor response respectively. The late stance response was similar to the ES response, with the leg being brought through at a faster rate by enhanced hip flexor and knee extensor activities and increased TA for ground clearance. At toe-off, there was an enhanced TA and plantarflexor response which acted to stabilize the ankle while an increase in the BF and VL activity acted to stabilize the leg and support the body. During midswing, the activity of the knee extensor was enhanced in order to prevent knee collapse. The activity of the plantarflexors was also increased to provide push-off. The activity of the flexors (TA & BF) was also increased, priming up for the upcoming swing phase.

Thus, the contralateral limb response is dependent on the phase of the stepcycle, and is well coordinated with ipsilateral limb response to provide support and stability as well as maintain the ongoing task of locomotion.

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209.6 MODIFICATION OF THE AMPLITUDE OF ESSENTIAL TREMOR BY TOPICAL ANESTHETIC. R.S. Pozos, J. Beaty*, and T. Long*. Dept. of Physiology, Univ. of Minn., and Dept. of Physical Therapy, College of St. Scholastica, Duluth, MN 55812.

Essential tremor is an overt tremor characterized by a) being absent at rest, b) present on maintaining a posture particularly of the outstretched arms, c) not made strikingly worse by movement, d) not associated with parkinsonian or cerebellar tremor (Marsden, C. D. Movement Disorders: Tremor, edited by Findley & Capildeo, Oxford University Press, New York, 1984, Chapter 5). The mechanism for essential tremor is uncertain; however, it has been reported that peripheral input can reset essential tremor (Lee, R. G. & Stein, R. B. Ann. Neurol. 10: 523-531, 1981). The purpose of this paper is to study the differences between essential tremor and physiological tremor by the addition of weights or the application of topical anesthetic. Previously we have reported that the amplitude of ankle clonus can be decreased by application of local anesthetic (Pozos, R. S., Mills, W., & Iaizzo, P. Neuroscience Abstract 14, #213.7, 1984).

Six control subjects and four essential tremor patients who had the characteristics mentioned above plus a familial history of the disease were used. All signed human subject consent forms prior to the experiments. Surface electrodes were placed on extensor and flexor muscles of the wrist and an accelerometer was placed on the hand. Signals were recorded on a FM tape recorder and later analyzed on a Micro PDP II.

For the weight experiments 100, 200, 300, 500 gms. were placed sequentially on the dominant hand after initial measurements were made of their rest tremor. Topical anesthetic was applied at the end of the weight experiments to the forearm for a period of 20 minutes, after which measurements were taken. Frequency and amplitude analysis were done of these tremors recorded during these experiments. Essential tremor had a range of frequencies from 6-12 Hz. The physiological tremor range was at 6-7 Hz. The addition of weights caused an insignificant change in amplitude in essential tremor whereas 300 and 500 gm on normal subjects caused a significant increase. Topical anesthetic caused a significant decrease in amplitude of both essential tremor and physiological tremor.

The topical anesthetic studies indicate that sensory feedback is used to influence the amplitude of both essential tremor and physiologic tremor.

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209.7 OCCURRENCE AND TIMING OF MOVEMENT-RELATED EMG ACTIVITY DEPENDS ON MOVEMENT PROFILE. S.H. Brown and J.D. Cooke. Department of Physiology, Univ. of Western Ontario, London, Canada.

A characteristic pattern of EMG activity is associated with voluntary step-tracking movements. An initial burst of activity in the agonist muscle (Ag1) is followed sequentially by a burst in the antagonist muscle (Ant1) and a second agonist burst (Ag2). Ag1 begins 40-50 msec before movement onset and is complete before peak velocity is reached. Ant1 occurs at or near the time of peak velocity and, as movement speed increases, occurs earlier in the movement. Recently, we have shown that the duration of Ag1 increases in multiples of 70 msec as movement amplitude increases. In general, such temporal relationships have been described only for movements with symmetrical profiles. The present study investigated the dependence of phasic EMG activity on movement profile, in particular, the relative durations of acceleration and deceleration phases.

Subjects grasped a manipulandum handle pivoted above the right elbow and performed flexion-extension movements in the horizontal plane. The tracking task was presented to the subject as an audio signal (target tone) which varied in frequency with target position. Movement of the handle also produced modulation of the audio signal. Subjects were instructed to move in such a way so as to reproduce the target tone. Changes in movement profile were accomplished by changing the ratio of acceleration duration to deceleration duration which, in turn, produced a change in the target tone. For a given movement amplitude, duration of the target tone (movement duration) remained constant.

Typical triphasic EMG patterns were associated with symmetrical movements. As acceleration duration shortened and the subject was required to accelerate more rapidly, Ag1 duration decreased and Ant1 occurred earlier. In some cases Ag1 and Ant1 were coactive. Ag2 was not present in these movements. In short deceleration movements Ag1 was replaced by a gradual increase in tonic activity. The deceleration phase was associated with contraction of both muscles with Ag2 occurring shortly after Ant1. Coactivation of both Ant1 and Ag2 in these movements paralleled the activation of Ag1 and Ant1 seen in short acceleration movements.

These findings indicate that the occurrence and timing of phasic EMG activity are intimately related to the movement profile. Ag1 duration depends upon the duration of the acceleration phase and, similarly, the timing of Ant1 depends not on movement speed per se, but rather on the timing of peak velocity. In addition, the coactivation of Ag2 with Ant1 in short deceleration movements suggests that Ag2 may function to increase limb stiffness and thus assist in terminating the movement.

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209.8 ACTIVATION PATTERNS OF ANKLE MUSCLES DURING ONTOGENY OF HUMAN GAIT. F. Dumas*, C.L. Richards* and M. Filion. (SPON: C. HARNOIS). Lab. of Neurobiology, Fac. Med., Laval Univ., Québec, Can., G1K 7P4.

During the ontogeny of human gait, the movement of the ankle changes from a digitigrade to a plantigrade pattern (heel contact followed by plantarflexion at the beginning of the gait cycle). To examine changes in muscle activation that could be related to this more mature pattern, we studied ankle movements and EMG activity of the triceps surae (TS) and tibialis anterior (TA) during gait in 33 children aged 1 to 4 years. The children were separated into three groups on the basis of the frequency of the plantarflexion movement during 5 gait cycles. In children lacking the plantarflexion, the TS is activated in early stance and has a lower peak which declines slowly in late stance and also is activated in late swing phase. In contrast, children with predominantly plantarflexion had lower TS activation in early stance followed by a higher peak ($p<.01$) with a more rapid decline in late stance and suppression of the activation in late swing. In the third group with occasional plantarflexion, the shape and the amplitude of the EMG curve of the TS was intermediate. The TA EMG activity at foot contact ($p<.05$) and the stance peak activation ($p<.025$) were higher in the group with predominant plantarflexion. In these children, the TA amplitude decreased less in mid-swing ($p<.025$), relative to the stance peak, and increased earlier in late swing. The changes in TS and TA activation in swing, in the children with predominant plantarflexion were related to more dorsiflexion in late swing to permit foot contact with the heel at the beginning of the gait cycle.

These results provide evidence of changes in TS and TA activation related to the progressive changes from a digitigrade to a plantigrade pattern. Such changes in muscle activation most likely reflect increased supraspinal modulation of the "spinal locomotor set" during the ontogeny of human gait.

(This work was supported by Association de Paralysie Cérébrale du Québec and Fondation Cardinal Villeneuve).

209.9 THE COORDINATION OF LIMB MOVEMENTS WITH DIFFERENT KINEMATIC PATTERNS. S.P. Swinnen*, C.B. Walter* and D.C. Shapiro. Motor Control Lab, Department of Kinesiology, UCLA, Los Angeles, CA 90024 and Katholic Univ. of Leuven, Belgium.

Interlimb coordination was investigated to ascertain the behavioral coupling between the limbs as a function of practice. In the first session, subjects performed unidirectional movements with the same spatial and temporal requirements. In the second session, subjects were required to perform reversal movements in one limb while the other limb performed the unidirectional movement practiced in the first session. Changes in the unidirectional movement as a result of performing a movement with different kinematic properties in the contralateral limb were examined, as was the nature of the coupling between the limbs.

Two sessions consisting of 75 trials each were performed. In the first session, subjects were trained to produce horizontal flexion movements of 72° with both forearms in 500 ms. In the second session, subjects were required to perform the same movement in the left limb while the right limb produced a double reversal task. Both movements were to be performed in the same movement time (500 ms) and to end in a similar position. To examine changes as a function of practice, the initial, middle and final five trials of session two were selected for further analyses. Displacements were indicated by potentiometers connected at the base of the levers, and EMG activity of the biceps and triceps was recorded using surface electrodes.

The results of session two indicated a relationship in final position and movement time between the limbs as they performed together, and these values became more similar to those of session one as a function of practice. The displacement data of the left limb indicated that subjects were successful in performing a unidirectional and not a reversal movement. To examine the relationship between the movements as they were being generated, the acceleration patterns in the right and left limbs were plotted against each other, and cross correlations were computed for the second session. These data demonstrated individual differences in achieving the goal. Some subjects produced high correlations initially, followed by reduced correlations as a function of practice. For others, this trend was reversed. However, regardless of the strategy, there was a tendency for the acceleration patterns to be related. The EMG data demonstrated additional activity in the left limb relative to session one. The results suggest that although the overt behavior of the two limbs appears to be independent, the coupling between the limbs is manifested at the higher time derivatives, indicating that force-producing actions are not independent between the upper limbs.

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209.10 MODELING POSTURAL SWAY IN MAN: EXPERIMENTAL EVIDENCE FOR A RANDOM PROCESS MODEL OF QUIET STANDING. S.H. Roy*, M.T. Bailin*, M.M. Stecker*, and C.J. De Luca. (SPON: J. Kucera). NeuroMuscular Research Center, Boston University, Boston, MA 02215 and Liberty Mutual Research Center, Hopkinton, MA 01748.

Current methods of evaluating postural instability during quiet standing include the use of a force platform which measures the excursion of the center of pressure (COP) over the plane of support. The stabilogram (SBG) is the plot of the COP path over time, and is modulated by intrinsic postural control mechanisms, vestibular, proprioceptive and visual cues. In order to obtain greater insight into postural control mechanisms, we have developed a mathematical model for sway path movement which may explain the sway patterns of normal subjects as well as patients with abnormal postural instability.

The movement of the COP during quiet standing is the result of many neuromuscular interactions. Although the movement of the COP is deterministic, it is well known, in systems with many degrees of freedom, that deterministic processes may appear random on a large scale. In this light, we suggest that the motion of the COP during standing can be modeled as a random process following the laws of diffusion within boundaries specified by postural control centers. In particular, as the diffusing COP approaches the limits of stability, the musculoskeletal conformation will be corrected through the motor functions of the appropriate muscle groups. This returns the COP to the stable zone in which large scale coordinated corrections of posture are not required.

Analysis of SBG in normal subjects demonstrates that the average distance of the COP movement from a specified point varies as the square root of time over short distances. We propose that normal subjects display a characteristic diffusion pattern of the COP, which results from central nervous system influences.

Pilot studies on neurologically impaired subjects yield diffusion pattern plots that are distinctly different from normal subjects. The nature of the deflection process and the geometry of the barrier are being investigated.

(This work was supported by Liberty Mutual Insurance Co.)

209.11 SELECTION OF HUMAN POSTURAL SYNERGIES DIFFERS WITH PERIPHERAL SOMATOSENSORY VERSUS VESTIBULAR LOSS. L. M. Nashner, H. C. Diener, and F. B. Horak. Neurological Sciences Institute of Good Samaritan Hospital and Medical Center, Portland, OR 97209

This study compares human postural movements executed with peripheral vestibular inputs and somatosensory inputs from the feet and ankle joints selectively disrupted. We identify the influence of each type of loss on response latency, muscle contractile patterns or synergies, and organization of the senses in relation to the conditions of the task. Peripheral vestibular losses were studied in 4 neurologically normal patients lacking VOR and caloric responses. Somatosensory inputs from the feet and ankle joints were disrupted in 4 normal subjects by applying ankle pressure cuffs bilaterally.

Postural movements were elicited by brief constant velocity horizontal displacements of the support surface. Movements were measured by recording EMG's in 6 leg and trunk muscles, the forces exerted by each foot on the surface, the antero-posterior (AP) sway of the body center of mass and videotaped joint angle changes. The sensory conditions were altered by rotating the visual surround, the support surface, or both in direct proportion to body sway, termed "sway-referenced" visual and support conditions, respectively.

During standing under normal conditions, the onset latencies and the temporal and spatial structure of postural synergies were unaltered by disruption of either the somatosensory or the vestibular inputs. Loss of sensory inputs, however, caused subjects to select synergies differently: (1) Normals deprived of somatosensory inputs from the feet and ankles used the hip synergy, regardless of the size of the perturbation or the properties of the surface (with intact somatosensory inputs the ankle synergy was used on a normal surface, the hip synergy, on a narrow beam). (2) Patients lacking vestibular inputs used the ankle synergy, even when attempting to stand on a narrow beam.

Under different combinations of sway-referenced visual and support surface conditions, normals performed the same with and without somatosensory inputs from the feet and ankles. Vestibular patients maintained balance when sway-referenced visual or support surface conditions were imposed one at a time, but lost balance with the two conditions combined.

Our results indicate that peripheral sensory losses can alter the selection of postural synergies, while not disturbing the organization of muscle activities within the synergies. Furthermore, somatosensory inputs from the feet and ankles are not required for the normal triggering of postural movements, regardless of the sensory conditions. (Supported by NIH F32 NS06926-03 and R01 NS12661-09.)

209.12 INFLUENCE OF STIMULUS PARAMETERS AND SET ON HUMAN POSTURAL SYNERGIES. F.B. Horak, H.C. Diener, and L.M. Nashner. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon 97209

Automatic postural responses to support surface displacements are partly triggered, central programs and partly determined by the characteristics of the stimulus. We examined the effects of the influence of stimulus size, speed and duration and the influence of set on the parameterization of synergic postural muscle activity.

Ten normal standing subjects were exposed to a series of 120 backward ramp translations of the suppport surface under 12 conditions of varying velocities (constant amplitude) or varying amplitudes (constant velocity). Displacement durations ranging from 34-900 msec had no independent effect on the responses. The effect of perturbation size and speed on body kinematics and on the latencies, patterns, burst durations and integrated areas of surface EMG activity in six trunk and leg muscles were examined. Integrated EMG activity was normalized across subjects and the early (first 75 msec), middle (second 75 msec) and late (last 350 msec) components analyzed separately. The influence of motor set-dependence due to prior experience was examined by comparing responses to practiced and unexpected perturbations.

Platform translations lasting only 75 msec evoked a single burst of muscle activity (75-100 msec duration) in Gastrocnemius, Hamstrings, and Paraspinals (and sometimes Abdominals). Response parameters were unrelated to the size or speed of the perturbations, suggesting a triggered central pattern. For translations lasting longer than 75 msec, the size of the early synergic EMG bursts were best correlated with stimulus velocity (r=.46-.68). The size of later components of the EMGs were best correlated with stimulus amplitude (r=.45-.71). A small, set-dependent influence of ramp amplitude on early Gastrocnemius EMG activity disappeared when perturbation sizes were randomized. Depending upon practice and expectation, very large and fast perturbations resulted in additional activation of antagonist muscles Quadriceps and Tibialis.

The significant gain relations of early burst EMG area with velocity and late, tonic EMG area with amplitude held not only for the muscle stretched by the perturbation but also for proximal muscle components of the synergy. The absolute latencies (98 ± 9 msec), intersegmental latencies (23 ± 12 msec), and burst durations (87 ± 22 msec) were not influenced by the characteristics of the ramp stimulus. These results suggest that centrally organized postural muscle burst patterns are triggered as a synergic unit, fine-tuned by the characteristics of the perturbations and dependent upon prior experience and expectation as well as by concurrent sensory inputs. (NIHF32NS06926,R01NS12661)

209.13 THE EFFECT OF VISION ON NEUROMUSCULAR RESPONSES UNDERLYING POSTURE CONTROL IN CHILDREN. M.H. Woollacott, M. Mowatt*, B. Debû* and E. Keshner. Inst. of Neuroscience and Dept. of P.E. and Human Movement Studies, U. of Oregon, Eugene, OR 97403.

Previous research concerning the influence of vision on body sway in children has indicated that movement of the visual environment, by itself, can cause a newly standing child to compensate for perceived (but nonexistent) sway, and fall in the direction of optical movement. This has led to the hypothesis that vision is the dominant input controlling body sway in the young child (Lee,D. and Aronson, E., Percept. Psychophys.,15:529, 1974). Contrasting studies in adults, which used a moveable platform to cause body sway and analyzed the effects of vision and ankle proprioception on balance control have shown that ankle inputs activate leg muscle responses that are organized into functional synergies specific to the direction of induced sway. Neck muscles have been shown to be activated antagonistically to the leg synergy, possibly via vestibular or neck proprioceptive inputs. Visual inputs have no effect on the latency of these responses in adults. This study aimed 1) to determine the relative influence of visual and ankle proprioceptive inputs on the latencies of postural muscle responses in children, and, within the visual system, 2) to determine the relative influence of peripheral vs. central retinal inputs on posture control.

Postural responses of standing children of 2 age groups (2-3 yrs and 4-6 yrs) were compared to adults using anterior or posterior displacements of a moveable platform. Surface electromyograms were recorded from muscles of the leg, trunk and neck. The relative dominance of postural response activation by proprioceptive vs. visual inputs was determined by comparing response latencies with eyes open vs. closed. In a subset of children, goggles, which blocked either the central or peripheral visual fields, were used to test the roles of the respective visual inputs in posture control.

The youngest group of children tested, the 2-3 yr. olds, showed a consistent reduction in ankle and neck muscle response latencies in the no vision condition. For posterior platform movements causing anterior sway, response latencies were: Vision. gastrocnemius (G): 72+8ms, neck flexors (NF): 126+54ms; No Vision. G:55+16ms, NF: 59+15ms, These changes were not present in the 4-6 yr. olds or adults. No differences were found in the latencies of postural responses for either of the age groups when the peripheral vs. central visual field was preferentially blocked. Thus, it appears that when any portion of the visual field is present, it can be utilized in postural stabilization. The decreased latencies seen in the 2-3 yr. olds with vision removed could imply a shift from the use of longer latency visual inputs with eyes open, to shorter latency proprioceptive inputs with eye closure. It is concluded that 2-3 yr. olds show a visual dominance in the activation of postural responses, which shifts to proprioceptive dominance by the age of 4-6 yrs.

209.14 AN ANALYSIS OF SLOW WALKING IN MAN. R.G. Borkowski*, R.L. Craik, W.F. Freedman, Dept. of Biomedical Engineering, Drexel University, Phila., PA 19104, and Dept. of Physical Therapy, Beaver College, Glenside, PA 19038.

Two modes of ambulation have been described in man, walking and running. The change between the two modes occurs primarily by altering the type of limb movement: in walking,the heel strikes the ground first and knee angle changes during the swing phase are small; in running, the front part of the foot often strikes the ground first and knee flexion is dramatically increased. Although walking and running differ kinematically, a monotonic relationship is maintained between the flexion cycle (swing) and cycle duration and the extension cycle (stance) and cycle duration. At very slow velocities it appears that the relationships between the flexion and extension cycles and cycle duration are no longer linear. The purpose of this study was to determine if the changes observed at very slow walking velocities are indicative of increased variability in performance associated with an unpracticed task or whether the observed changes indicate a third form of ambulation.

Ten men, ranging in age from 16 to 40 years, participated in this study. Pre- and post-training data were collected as subjects walked at the following velocities: 1.7,1.3,1.0,0.7, and 0.3 ms^{-1}. The parameters which were measured included cycle duration, swing and stance duration and support length. A tachometer monitored walking velocity. Four hundred walking cycles at 0.7 and at 0.3 ms^{-1} were practiced over two days; a metronome was used to ensure constant cadence during the practice sessions.

Practice did not alter slow walking performance. No significant difference in pre- and post-test performance was demonstrated by the subjects. Data from all velocities (including 0.3 ms^{-1}) demonstrate a hyperbolic relationship between the flexion phase and cycle duration or the extension phase and cycle duration. Because practice did not change the slow walking performance, it is assumed that the behavior demonstrated at this speed is another mode of bipedal ambulation. This is supported by results of previous study in which changes in the frequency relationship between upper and lower extremity motion were reported with decreasing walking speed. A change in shoulder and hip sequencing was also noted with decreased speed (Craik et al,Adv. Beh. Bio., 1976). Therefore, while the change from walking to running appears to involve a change in limb movement, it appears that the change from walking to "slow walking" is accomplished by a different type of strategy.

209.15 SELECTION OF MOTOR PROGRAMS DURING FUNCTIONAL TASKS IN HUMANS. W. Freedman, L. Kent*. Moss Rehabilitation Hospital, 12th Street and Tabor Road, Philadelphia, PA 19141.

This study was designed to identify factors that determine motor program selection during functional, human tasks. Subjects selected a heel-strike (HS) motor program (as used during level surface walking) or a toe-strike (TS) motor program (as used during stair descent). The task of descending a step of adjustable height followed by level surface walking offered a binary choice of motor program to the subject. Specifically examined was whether the presence or absence of vision or a change in step height influence selection of a TS or a HS motor program for task execution. Ankle angle, muscle activity (tibialis anterior (TA) and triceps surae (TRI)), initial foot contact (HS or TS), and stepdown force of the stepdown limb were measured.

Eleven healthy adult subjects (6M,5F), participated in this study. The experiment for each subject consisted of a set of 50 trials (25 vision, 25 no-vision) with step height randomized at 5 discrete heights (0,2.5,5,10,20 cm). Subjects were informed of step height under all conditions, but were completely naive to the task of interest, i.e., the toe-first or heel-first initial stepdown.

The height of the step did influence whether the subject stepped down toe-first or heel-first, while visual information did not alter the selection of the motor program. Under both visual conditions, all subjects landed heel-first at 0 cm. By 20 cm, the majority of the subjects in this sample had switched solely to toe-first contact. The threshold for switching to the TS program was indistinct, i.e., there were intermediate step heights for which half of the subjects stepped down toe-first or heel-first.

One of two distinct movement patterns was used for any particular trial in this experiment. HS trials were characterized by TA activity and TRI inactivity during swing, with a dorsiflexed ankle at substrate contact. The converse was true for the TS trials, i.e., TRI activity and TA inactivity during swing with a plantarflexed ankle at substrate contact. The selected variables correctly predicted the touchdown motor program by midway through the swing period. The TA and TRI pre-contact EMG bursts and subsequent ankle movement before substrate contact for HS and TS trials appear to be part of pre-programmed movement patterns (i.e., motor programs) which are presumably of central origin.

Movement is controlled through a biological amalgam of voluntary, stereotypic and reflex actions. The particular mixture for any specified movement is based on the intent or functional outcome desired. The switching to the TS motor program as step height increased presumably results in the most efficient and stable movement.

209.16 NEURONAL ACTIVITY CHANGES RELATED TO THE ADVANCE INFORMATION ABOUT BIOMECHANICAL PARAMETERS OF AN INTENDED MOVEMENT. **A.Riehle*, J.Requin.**Dept.of Exp.Psychobiology, Inst.of Neurophysiol.Psychophysiol., C.N.R.S.,F - 13402 Marseille 09, France.

Preparatory processes contributing to the building of the motor program were studied by means of recordings of single-cell activity within motor cortical areas (area 4 and 6) of awake monkeys (**Macaca fascicularis**) during the execution of a wrist flexion-extension movement triggered by a visual stimulus. The animals were trained in a so-called "movement dimension advance information" paradigm the principles of which are as follows: in a choice reaction time (RT) task including a preparatory signal (PS) which provides complete, partial or no information about the biomechanical parameters of the movement, analyses of RT-changes allow one to make inferences about the independence, the timing and the order of processes which underly the preselection of motor program subroutines.

The behavioral results obtained during training and recording sessions show that RT is directly correlated with the type of advance information (complete-direction-extent-none), but movement time is not. This indicates that advance information about movement parameters intervenes at the motor programming and not at the execution level. RT decreases when the amount of advance information is increased. In the situation where the animal is provided with information about only one parameter and is uncertain about the other one, information about direction (flexion or extension of the wrist) shortens RT more markedly than information about the extent of the movement. This result suggests that programming of the movement direction takes longer than programming of the extent.

Various types of neurons were recorded the responses of which could be related to the preparatory processes: (1) neurons responding direction-specifically to the PS which are possibly involved in a process responsible for specifying the activity of the particular muscles by modifying the excitability of the preceding neuronal elements; (2) neurons with slowly developing activity during the preparatory period (PP) (which is partly related to the advance information) that may preset the responsiveness to the response signal; (3) neurons showing sustained activity changes during the PP irrespective of the informative value of the PS, which could play a role in a cortical mechanism underlying the reinforcement of the postural stability of the moving limb; (4) neurons showing activity changes which are strongly time-locked to the response signal but not to the movement onset, indicating a possible mechanism linking the visual information with the motor output.

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209.17 DIVERGENCE OF PREPARATORY TUNING FROM MOTONEURONAL ACTIVATION AS A FUNCTION OF TASK COMPLEXITY IN MAN. C.W.Y. Chan and M.W. Rogers*. School of Physical and Occupational Therapy, McGill University, Montreal, Quebec, Canada H3G 1Y5

Facilitation of the spinal reflexes prior to rapid limb movements is thought to reflect a preparatory "tuning" of the motoneurons. However, in spite of the well known prolongation of reaction time (RT) as a function of complexity/choice of movement strategies, comparatively little information is available on the preparatory tuning processes preceding different motor tasks. To resolve this issue, we compared the profile of H-reflex excitability prior to an ankle plantarflexion performed under simple ballistic (SRT-B) and complex tracking reaction time (CRT-T) paradigms.

Two groups of nine normal subjects, who were similar in age and mean RT latencies in the ballistic task, participated in the two experimental series. They were instructed to plantarflex the right ankle in response to a visual signal (RS), following a tone burst (WS) at 1000 msec. The ballistic task in series I involved a rapid 20° ramp movement (SRT-B). The tracking task in series II consisted of accurately following either a slow ramp or a bell-shaped oscilloscope trace with (CRT-T) or without choice (SRT-T). EMGs were recorded from the soleus and tibialis anterior simultaneously with ankle movement. For a comparison of the preparatory process between the SRT-B and CRT-T tasks, changes in the excitability of the spinal reflex arc were measured at predetermined intervals following the WS by means of the H-reflex technique.

Three principal findings emerged:

(1) The mean soleus EMG and movement onset latencies were significantly longer ($p<.01$, by some 100 msec) for the SRT-T than the SRT-B task. In contrast to some previous reports, there was no difference in the onset latencies between tracking tasks with (CRT-T) and without choice (SRT-T).

(2) The agonist (soleus) EMG response patterns for the ballistic movements were characterized by a brief, high amplitude burst; whereas those for the tracking tasks tended to be more continuous and slowly graded.

(3) Facilitation of the spinal reflex pathway commenced more than 100 msec earlier relative to the agonist EMG onset, and reached a higher maximum level of excitability for the complex tracking task than that preceding the simple ballistic movement.

These results suggested an earlier AND enhanced tuning of the spinal reflex pathway in association with motor tasks requiring greater complexity. Of particular interest is the demonstrated divergence of preparatory tuning from motoneuronal activating processes, under different task conditions.

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209.18 DIFFERENTIALLY TIMED, PROPRIOCEPTIVE INPUT PRODUCES TONIC CHANGES OR TRIGGERING OF VOLUNTARY TORQUE RESPONSES. P. J. Cordo, M. Flanders* and J. G. Anson, Good Samaritan Hospital & Medical Center, Portland, OR 97210.

Isometric torque production at the human elbow joint has been studied in order to identify visual and proprioceptive control mechanisms. Visual control takes the form of a triggered response followed by a series of adjustments aimed at improving accuracy. We now report possible proprioceptive contributions to voluntary torque responses.

Adult, human subjects produced fast and accurate step increases in horizontal flexion torque (24 and 30 Nm) in response to a visual stimulus. In some trials which were randomly selected, the forearm (screened from the subject's view) was briefly displaced at a velocity of 50 deg/s, at various times between presentation of the visual trigger and response onset, and during the voluntary response. In 10% of the trials the expected visual trigger was not provided, however, the proprioceptive perturbation was still imposed.

Effects of perturbations on voluntary torque responses were strongly dependent on the timing of limb movement onset. When perturbation onset occurred during or up to 40 ms prior to the onset of voluntary responses, the torque production system responded like a spring: tonic increases in agonist EMG and elbow torque to extension displacements and decreases to flexion displacements. Reaction times were prolonged by approximately 10 ms following flexion (unloading) limb displacements. These effects are consistent with the expected responses of muscle spindles to joint displacements such as these. When perturbation onset occurred prior to about 40 ms before voluntary response onset, the limb movement (independent of direction) caused an increase in the amplitude of the initial open-loop component of voluntary responses. Furthermore, limb perturbations imposed in the absence of the expected visual stimulus, nonetheless produced triggering of a typical voluntary torque response. The results are consistent with the idea of a proprioceptive control system that is separate and additive to the visual control system. Depending on the specific timing of an imposed limb movement, it can either produce a spring-like response of the limb or trigger the voluntary motor command. In neither case does the proprioceptive control system appear to function as a error correcting mechanism. (NIH RO1 AM31017-02)

209.19 CYCLIC VOLUNTARY FINGER MOVEMENTS: KINEMATIC AND EMG PATTERNS. D. Hahs* and R. Stiles. Dept. Physiology and Biophysics, Univ. Tenn. Center for the Health Sciences, Memphis, TN 38163.

Because much voluntary movement is rhythmical, the question arises as to the ability of the skeletal motor control system to produce smooth oscillations of a body part. Of particular interest in this study are the electromyographic and kinematic patterns obtained during cyclic voluntary movement of a finger.

Normal human subjects were instructed to make self-paced oscillatory movements of the index finger in a parasaggital vertical plane at about 1 Hz. The movement was detected by an AVR-250 accelerometer mounted on a styrofoam sleeve placed over the entire finger. Velocity and displacement records were obtained by digital integration. Jerk was obtained by digital differentation. Bipolar surface EMGs were detected from extensor digitorum and flexor digitorum superficialis muscles. Each EMG signal was full-wave rectified and digitally smoothed. Movement and EMG data were analyzed in the time as well as frequency domain.

Factors entering into the production of the movement were the mechanical-reflex oscillations (tremor) of the finger and the reciprocal- and co-activation pattern of the control signal. Acceleration-related feedback pulses correlated with the tremor, as well as biphasic AGl-ANTl pulses, could be identified in computer-averaged EMG epochs. These latter pulses were correlated with corresponding pulses in the averaged acceleration traces. Position epochs demonstrated that the agonists were being activated (and antagonists deactivated) at or near the extremes of finger position, e.g., the flexor muscle was activated near peak extension. Power spectral density functions of EMG and acceleration both contained bands at the self-paced frequency and contained prominent bands at the second, third, and fifth harmonics.

209.20 THE COORDINATION OF SIMULTANEOUS KEYPRESSES FOR ALL FINGER PAIRS IN HUMANS. C.L. MacKenzie. Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The preparation and coordination of finger movements are constrained by the structure and functional organization of the motor system. These constraints are dynamic and task specific; that is, they vary with the temporal phase dependence of the finger movements (MacKenzie, 1985). Past research provided a description of the selection and intention gradients when the task required a choice between one of two fingers to make a single, discrete keypress (MacKenzie, Neuroscience Abstracts, 1984).

This research examined coordination processes for a task requiring simultaneous keypresses (chords) with two fingers. Ten, female, right-handed undergraduates performed two replicates of 20 trials for each of 28 finger pairing conditions (excluding thumbs). On each trial, after a constant foreperiod of 2 s, an imperative stimulus (.8 probability) or a no-go stimulus (.2 probability) light indicated to respond with a two finger chord or not to respond respectively. The fingers were resting on eight telegraph keys, and the keypresses were made without vision of the hands. Light-response mappings were counterbalanced across subjects.

The reaction time (RT) analysis revealed significant differences in RT to initiate "simultaneous" keypresses. Further, RT for a given finger changed with the context of the paired finger in the chord. For example, RT for a given finger was longer when paired with a ring finger than when paired with an index finger. Overall, RT for between hand finger chords (239 ms) were faster than within hand finger chords (250 ms). For within hand chords only, the left hand fingers (247 ms) were faster than fingers on the right hand (253 ms). Finger gradients on the left and right hands differed for between and within hand chords. A final gradient in the data revealed a significant departure from simultaneity in the initiation of two keypresses; the keypress for the leftmost finger of the pair was initiated consistently 2-5 ms earlier. This was most pronounced in right hand data and was parallelled by the error analysis. These data and their implications for understanding the activity of the underlying neural networks will be further described.

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209.21 **ACTIVATION LEVELS OF TWO ELBOW FLEXORS UNDER ISOMETRIC CONDITIONS.** J.D. Howard*, J.D. Hoit*, R.M. Enoka, and Z. Hasan. Depts. of Physiology, Exercise & Sport Sciences, and Speech & Hearing Sciences, University of Arizona, Tucson, AZ 85724.

In dynamic conditions with varying angular velocities of elbow flexion, it has been shown that the integrated EMG in brachioradialis (BR) and biceps brachii (BB) is highly correlated. This relationship has given rise to the concept of the Flexor Equivalent (Bouisset et al., Electroenceph. Clin. Neurophysiol. 42: 543, 1977). However, Hasan and Enoka (Soc. Neurosci. Abstr. 10: 334, 1984) have observed that changes in the dynamic versus steady-state EMG in the elbow flexors are independent and can go in opposing directions. In elbow flexion movements, they noted a dynamic increase in BR EMG followed by a steady-state EMG that could be either greater than or less than that of the pre-movement EMG.

In light of these studies, the present investigation examined the isometric steady-state activation patterns of BB and BR over a range of elbow angles from 35 to 170°. Each muscle was held at constant activation levels corresponding to 5, 10, 15, and 20% of maximal-effort EMG. The constant activation of one muscle across angles allowed for comparison of the simultaneous activation level in the other muscle. The rectified and filtered EMG signals of both muscles were sampled by computer. A pair of LED lights provided feedback to the subject to activate the test muscle at the prescribed levels. Subjects were unaware of which muscle was the test muscle, and, in fact, typically assumed the feedback related to the torque exerted. The mean coefficient of variation of the test-muscle EMG about the target levels for the entire data pool was 0.08.

The results revealed three features: (1) When the test-muscle EMG was held constant, the EMG pattern of the non-test muscle varied widely across joint angles. When BR was the test muscle, five subjects showed an increase in BB EMG over an elbow-angle range of 65 to 170°. However, the other five subjects showed either a decrease or no discernable directional trend. (2) The activation patterns of the two muscles were inversely related in 9 of 10 subjects. For example, when BR EMG was held constant across angles, five subjects showed an increasing EMG in BB with increasing joint angle. All five of these subjects then demonstrated a decreasing trend in BR EMG when it was the non-test muscle. (3) The four target levels revealed that the correlation between the EMG's of BR and BB varied widely at different angles. Only rarely were the systematic increases in the test-muscle EMG accompanied by corresponding systematic increases in the non-test muscle EMG.

These results caution against the generalization of the Flexor Equivalent hypothesis to isometric conditions. In particular, investigators should use caution in making inferences regarding the activation levels of all elbow flexors when recording from just one.

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PHYSIOLOGICAL EFFECTS OF PEPTIDES I

210.1 ELECTROPHYSIOLOGICAL EVIDENCE FOR SUBSTANCE P AS A NEUROTRANSMITTER IN THE CILIARY GANGLION. S.E. Dryer and V.A. Chiappinelli. Dept. of Pharmacol., St. Louis Univ., St. Louis, MO 63104.

The chick ciliary ganglion contains two populations of postganglionic cells, called ciliary neurons and choroid neurons. These neurons differ in size, location within the ganglion and location of target organ. Nerve terminals synapsing onto both populations of cells contain substance P, which is colocalized with acetylcholine (Erichsen et al., J. Neurosci. 2:994, 1982). We have used intact ganglia in an in vitro chamber for intracellular recording from ciliary and choroid neurons.

Ciliary neurons are singly-innervated, contain dual electrical-chemical synapses, and can transmit action potentials at frequencies exceeding 100 Hz. Ciliary neurons do not display slow EPSPs, and are not affected by substance P. In contrast, choroid neurons are multiply-innervated by purely chemical synapses which fail at frequencies above 30 Hz. Following repetitive preganglionic stimulation, choroid neurons display a non-cholinergic slow EPSP which is associated with an increase in input resistance. This slow EPSP is closely mimicked by superfusion with 1-3 μM substance P, and the two responses have the same apparent reversal potential (~ -90 mV). Moreover, the response to substance P desensitizes within minutes, at which time the slow EPSP is blocked, suggesting that substance P is the mediator of the slow EPSP.

Intracellular recordings were also made from calyciform nerve terminals synapsing onto ciliary neurons. These units are also depolarized by substance P. The physiological significance of this response is unknown. Thus, in the chick ciliary ganglion, substance P produces presynaptic effects at one type of synapse and postsynaptic effects at another.

Leu-enkephalin is also found in nerve terminals synapsing onto both ciliary and choroid neurons (ibid.). We have therefore examined the effects of this peptide in the ciliary ganglion using intra- and extracellular recording techniques. Intracellular recordings in the presence of 20 μM leu-enkephalin reveal a small reduction in nicotinic EPSP amplitude in both populations of cells. In ciliary neurons, however, this decrease was never sufficient to block action potentials. Superfusion of leu-enkephalin at concentrations up to 100 μM had no effect on the extracellular compound action potential associated with ciliary neurons. In contrast, 20 μM leu-enkephalin produced a 15% reduction in the choroid neuron compound action potential. This effect was blocked by naloxone. Studies are currently underway to determine the mechanism of this effect of leu-enkephalin.

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210.2 THE EFFECTS OF NEUROTENSIN ON TRANSMISSION AT THE FROG NEUROMUSCULAR JUNCTION. K. Alkadhi and R. Clothier*. Department of Pharmacology, University of Houston, University Park, Houston, TX 77004.

Neurotensin is a tridecapeptide unevenly distributed in the central nervous system (CNS) and gastrointestinal tract of various animals including man. Neurotensin is known to have multiple central and peripheral actions such as hypothermia, analgesia and hypotension. On the basis of its presence in the CNS and because of certain characteristics, neurotensin has been suggested as a possible chemical mediator in synaptic transmission. Electrophysiological investigation of the effects of neurotensin on central neurons (J.L. Henry, Ann. N.Y. Acad. Sci. 400: 216-227, 1982) showed variable results. Both depression and excitation were observed in sensory and sympathetic preganglionic neurons. Possible synaptic effect of neurotensin was investigated in the present experiments using the frog neuromuscular junction as a model.

Magnesium-blocked isolated cutaneous pectoris muscles were used for intracellular recording. Neurotensin (0.1, 1, 5μM) had no apparent effect on the resting membrane potential. At 0.1μM neurotensin failed to affect transmission. However, at higher concentrations (1, 5μM) neurotensin decreased the amplitude of the endplate potential (EPP) without affecting that of the miniature EPP (MEPP; 0.61 ±0.07 mV before, 0.59 ±0.06 mV after 5μM neurotensin). The quantal content (m) of the EPP was slightly decreased (m: 86± 3% of control after 5μM of neurotensin, 99±5% of control after 20-40 min wash). Neurotensin slightly but consistently reduced (about 17%, 5μM neurotensin) the frequency of MEPP. This effect was also reversed on prolonged washing. Varying the frequency of nerve stimulation (0.3 - 6 Hz) did not seem to influence the effects of neurotensin on transmission.

These preliminary findings showed that neurotensin affects transmission at the frog neuromuscular junction by acting presynaptically.

210.3 ACTION OF VASOPRESSIN ON NEURONS AND MICROVESSELS IN THE RAT HIPPOCAMPAL SLICE. T. Smock and A. Topple*. Department of Psychology, University of Colorado, Boulder, CO 80309.

A complete analysis of the central action of vasoactive transmitter candidates entails a consideration of possible influences on microvasculature as well as direct effects on neurons. To this end we have used a standard hippocampal slice preparation to evaluate the electrophysiological and microvascular response to substances of this kind. Arginine vasopressin (AVP) was chosen for this study as previous reports show it to be contained in central neurons that make vascular contacts, to be released by depolarization in the presence of calcium and to be capable of producing excitation of central neurons that is fully blocked by a specific structural antagonist for AVP's pressor receptor (Muhlethaler et al, Nature 296:749 and Jojart et al, Neurosci. Lett. 51:259).

For electrophysiology 103 neurons in the CA1 region were impaled and intracellular recordings were obtained (avg.V_m=55+/-2mv, avg. spike size =93+/-2mv). Lack of spontaneous activity indicated that most were pyramidal cells. 55% of the cells responded to AVP (1μM) by displaying an increase in excitability without a major change in V_m or r_m. Confirming previous reports, no effect was obtained in saline media that lacked calcium (Mizuno et al, Brain Res. 309:241). No change in epsp or ipsp frequency was observed.

Inspection and time-lapse microphotography of 62 slices revealed that most slices contained vessels that beat spontaneously, indicated by periodic pulsatile movement ranging in frequency from 0.25 Hz to once a minute or longer. Most vessels within a slice lacked the spontaneous movement, however. Some quiescent vessels could be induced to contract by AVP (1μM). In three slices studied at high magnification (field size 450x700μm) four vessels responded to AVP and ten did not. In seven slices studied at lower magnification only large vessels were resolved. Of these, two responded to AVP and fourteen did not. Intracardial injection of an impermeant dye (Evan's Blue, 0.4g/10ml) before slice preparation labeled many more vessels than were apparent in slices from uninjected animals and permitted an estimate of microvessel density in the slice (one vessel every 29 μm).

Three hypotheses are suggested by the observed action of AVP on neurons and blood vessels. First, the neurons alone could express the pressor receptor for AVP, the blood vessel response being a consequence of increased neuronal activity. Second, the vessels alone could express the receptor, producing a secondary neuronal response by mechanical means. Finally, both neurons and vessels could express pressor receptor populations and respond independently to the applied vasoactive compound.

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210.4 **THE EFFECT OF FMRFAMIDE ON FREQUENCY - DEPENDENT SPIKE BROADENING.** D. Doerner* and L.D. Partridge. Dept. of Physiol., Univ. of New Mexico School of Medicine, Albuquerque, N.M. 87131.

The invertebrate neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH_2) has previously been shown to alter the beat frequency and contractility of cardiac muscle (Gen. Pharmacol. 11:237, 1980), to modulate central pattern generation in stomatogastric neurons (Neurosci. Abst. 45.4, 1984), and to modify adaptive behaviors and possibly mediate behavioral state in Aplysia (Neurosci. Abst. 151.9, 1984). The work reported here suggests that FMRFamide may act as a neuromodulatory agent by producing alterations in frequency - dependent spike broadening.

Superfusion of FMRFamide (10 pM - 1 μ M) over the circumesophageal ganglion complex of Helix aspersa or Lymnaea stagnalis consistantly provoked an increase in the percentage broadening. Spike broadening in the presence of the peptide was an average 53% greater than that recorded in Ringer solution. The effect was shown to be dose - dependent and reversible. Changes in spike broadening were accompanied by small (2 - 10 mV) voltage - dependent shifts in membrane potential. The polarity of these shifts reversed near the expected potassium equilibrium potential. Preliminary data suggest that FMRFamide's effect on spike broadening may be due to a modulation of membrane K^+ currents.

Since the amount of transmitter released by a presynaptic neuron should be a function of spike duration, changes in synaptic efficacy will accompany alterations in frequency - dependent spike broadening. One important mechanism for the neuromodulatory action of FMRFamide may result from the ability of the peptide to mediate the extent of spike broadening.

210.5 CALCITONIN (CT) AND CALCITONIN GENE-RELATED PEPTIDE (CGRP) ENHANCE CALCIUM - DEPENDENT POTENTIALS. M. NOHMI*, P. SHINNICK-GALLAGHER, P.W. GEAN*, J.P. GALLAGHER, and C.W. COOPER* (SPON: D. EATON) Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

The indication that CGRP is produced in central neurons utilizing acetylcholine as a neurotransmitter and the finding of CGRP immunoreactivity in sacral areas of the spinal cord and in nerve terminals of parasympathetic ganglia of the urinary bladder suggests that CGRP or CT may serve as neurotransmitters or neuromodulators in vesical neurons. We used conventional intracellular recording methods to test the effects of synthetic salmon CT and synthetic human CGRP in cat vesical parasympathetic ganglia. CT (5nM-1μM) had no effect on the resting membrane potential or membrane conductance but prolonged the duration of the afterhyperpolarization (a.h.p.) of an action potential (AP; 36.5%; 0.5μM) without affecting the amplitude of the AP. We also analyzed the effects of CT on the Ca-spike recorded in the presence of tetrodotoxin (TTX, 1μM) and tetraethylammonium (TEA, 20mM) to block sodium and potassium channels respectively. CT (1μM) prolonged the duration but did not affect the peak amplitude or amplitude of the a.h.p. of the Ca-spike. The effect of CT on the calcium spike was reversible and concentration-dependent (5nM-1μM). These effects of CT on the Ca-spike were also observed in the presence of other agents known to block potassium conductance (intra- and extracellular Cs_4SO_4 and 4-aminopyridine; 4-AP). Synthetic human CGRP (1μM) also prolonged the calcium spike under both previous conditions (15.7% and 17.2%, respectively) but CGRP was 3-4 fold less potent than salmon CT. Like CT, CGRP (1μM) did not affect the resting membrane potential or membrane conductance. Forskolin (25μM), an activator of adenylate cyclase known to prolong Ca-spikes in other tissues, prolonged the duration of the Ca-spike (16.1%) recorded in TTX and TEA but not in the presence of TTX, TEA, Cs_2SO_4 and 4-AP. These data suggest that forskolin acts on potassium rather than calcium channels and that CT and CGRP action may not involved cyclic AMP. We report here the first evidence that the neural mechanism of CT and CGRP action is to enhance a calcium conductance. With the exception of ATP (Yatani et al. Nature 296: 169), CT or CGRP appear to be the only known endogenous substances thusfar which enhance Ca conductance. The prolongation of the Ca-spike suggests that CT or CGRP may act as neuromodulators by enhancing the release of neurotransmitter. These data suggest that the fundamental effects of CT, both neural and endocrine, appear to have a common mediator, namely calcium. (Supported by NS16228 to PSG and AM32060 to CWC).

210.6 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) PRODUCES A NONCHOLINERGIC DISCHARGE AND ENHANCES SLOW EXCITATORY MECHANISMS IN CHRONICALLY DECENTRALIZED CAT SUPERIOR CERVICAL GANGLIA (SCG). M. Rutigliano*, M. Kawatani, and W.C. de Groat, Dept of Pharmacology and Center for Neuroscience, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261

Previous experiments have shown that vasoactive intestinal polypeptide (VIP) elicits a depolarization of cat sympathetic and parasympathetic ganglia cells in vitro. VIP also facilitates muscarinic excitatory mechanisms in ganglia without altering either nicotinic transmission or the response to nicotinic agonists. VIP does not elicit a discharge in normal SCG. The present experiments were undertaken on chronically decentralized SCG to determine if the effects of VIP would be altered by the elimination of preganglionic nerve terminals.

Experiments were performed on SCG in vivo in dial urethane anesthetized cats. Multiunit firing was recorded on postganglionic nerves in normal ganglia acutely decentralized (2-10 hrs) and in chronically decentralized ganglia where the cervical sympathetic trunk was sectioned 14-21 days prior to experimentation. Drugs were administered by injection (0.1 ml) into the common carotid artery.

VIP (5-50 μg) produced a slow onset (30-60 sec), prolonged (2-5 min) discharge in the chronically decentralized SCG. The threshold for the response was 5 μg and the discharge was reproducible when the peptide was given at 20-30 min intervals. VIP did not produce a discharge in the normal SCG even at doses 10-15 times the threshold for the decentralized ganglia. The discharge was resistant to muscarinic antagonists (atropine, 40-70 μg and pirenzepine, 5-200 μg) as well as the nicotinic antagonist hexamethonium (5 mg). However, the discharge was rapidly and reproducibly blocked by agents with a direct postsynaptic depressant action (GABA, 10-100 μg and L-ENK, 10-50 μg). Low doses of VIP also enhanced slow excitatory mechanisms in chronically decentralized SCG. The late (muscarinic) component of the ACh (2-20 μg) induced discharge as well as the late component of the 5-hydroxytryptamine (2-10 μg) induced discharge were both significantly enhanced by VIP (1-5 μg).

In conclusion, the present results indicate that the effects of VIP on the SCG are entirely attributable to a direct postsynaptic action on noncholinergic receptors. The unmasking of VIP-induced postganglionic firing in ganglia could be related to denervation supersensitivity and an enhanced depolarizing action of VIP following the loss of normal excitatory cholinergic inputs. These findings raise the possibility that VIP may function as both a neuromodulator and a neurotransmitter in autonomic ganglia.

210.7 NEUROPEPTIDES REGULATE GUANOSINE TRIPHOSPHATE CYCLOHYDROLASE ACTIVITY IN CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. R. J. Slepetis*, M. M. Abou-Donia, C. D. Unsworth*, W. Haynes*, C. A. Nichol* and O. H. Viveros. Department of Medicinal Biochemistry, The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

We have previously presented evidence that tyrosine hydroxylase (TH) and guanosine triphosphate cyclohydrolase (GTP-CH) activities and tetrahydrobiopterin (BH_4) levels in chromaffin cells of adrenal medulla are regulated by changes in catecholamine levels and by cAMP-dependent mechanisms mediated through nicotinic cholinergic receptors (Viveros et al., Science, 213:349, 1981; Abou-Donia and Viveros, Proc. Natl. Acad. Sci. U.S.A., 78:2703, 1981; Abou-Donia et al., Chemistry and Biology of Pteridines (J. A. Blair, ed.), de Gruyter, Berlin, p. 777, 1983). Recent evidence indicates that in addition to cholinergic fibers, the adrenal medulla is innervated by fibers that contain vasoactive intestinal peptide (VIP) and substance P. Furthermore, catecholamines, opioid peptides, and a number of other neuropeptides are costored in and cosecreted from the chromaffin cell. In order to determine whether these neuropeptides may also be involved in the regulation of BH_4 synthesis, the effects of VIP, substance P and opiate agonists and antagonists on GTP-CH activity and catecholamine levels were investigated in cultured bovine adrenal chromaffin cells. After 24 to 72 hours of exposure to 0.5-10 μM VIP, a small but significant dose-dependent increase in GTP-CH activity was observed (maximal effects were 20-40% above basal activity), and cellular catecholamine content was unchanged by this treatment when values were expressed per 10^6 cells. However, since VIP treatment also stimulated protein synthesis, GTP-CH activity was unchanged, and catecholamine content significantly declined, when expressed in terms of total cellular protein. Treatment of the cells with substance P (0.5 μM-10 μM) for 72 hours resulted in a significant elevation in GTP-CH activity. The maximal increase (75%) was elicited by 1 μM substance P. Total catecholamine levels and cellular protein content were unaffected by substance P treatment. Treatment of the cells with [D-ala^2]-[D-leu^5]-enkephalin (1 μM) and/or naloxone (5 μM) for 24 to 72 hours had no consistent effect on TH and GTP-CH activity. The alterations in GTP-CH activity observed following VIP and substance P treatment suggest that the peptidergic fibers innervating the adrenal medulla may contribute to the modulation of BH_4 biosynthesis.

210.8 EFFECTS OF SYSTEMIC ADMINISTRATION OF A SYNTHETIC ACTH(4-9)-ANALOGUE (HOE 427) ON ACETYLCHOLINE AND CYCLIC NUCLEOTIDE LEVELS IN RAT BRAIN. G. Wiemer* , H. Gerhards* and R. Geiger*. (SPON: H. Braak). Hoechst AG, 6230 Frankfurt/M. 80, FRG

Increases in hippocampal acetylcholine (ACh)-turnover after intracerebroventricular (1 - 30 mcg) administration of ACTH-related peptides have been described (P.L. WOOD et al., J. Pharmacol. Exp. Ther. 209, 97 - 103 (1979) and L.J. BOTTICELLI & R.J. WURTMANN, J. Neuroscience 2, 1316 - 1321 (1982). Since the various behavioral effects of these peptides described by the deWIED group were observed after much lower systemic doses (0.1 - 1 mcg/kg s.c.), we were interested to see whether changes in ACh-metabolism, 3',5'-cAMP and 3',5'-cGMP levels as well as in glucose metabolism also could be detected after behaviorally active systemic doses of short chain ACTH-analogues.

Hoe 427 is an ACTH(4-9)-analogue which shows an increased potency in behavioral tests and an improved metabolic stability in comparison to other short chain ACTH-analogues. Since in ACTH, 11-Lysine contributes strongly to the biological activity (R. GEIGER & H.G. SCHRÖDER, Hoppe-Seyler's Z. Physiol. Chem. 354, 156 - 162, (1973), a similar group was introduced into an analogous position of the molecule.

Male Wistar rats (120 - 140 g) were given various doses (range: 0.01 - 10 mcg/kg) of Hoe 427 s.c. or i.p. and sacrificed at various times (range: 0.5 - 24 hrs) by a microwave beam (4.8 KWsec) focused on the head. Determinations of ACh, cAMP and cGMP were performed by radioassays. Glucose utilisation was measured by using the glucose analogue ^{3}H-2-deoxyglucose (^{3}H-2-DG).

Systemic administration of Hoe 427 in a dose range from 0.01 to 10 mcg/kg produced decreases in ACh-levels mainly in the striatum. The decrease in ACh was more pronounced and found additionally in the hippocampus and hypothalamus when the animals were pretreated with dexamethasone for 1 week (1 mg/kg i.p., daily). The decrease in ACh levels suggests that Hoe 427 increases the utilisation (release) of ACh. This could be further supported by a potentiation of the ACh-depletion induced by pretreatment with the choline-uptake inhibitor hemicholinium-3 (HC-3). In parallel, the levels of 3',5'-cAMP were increased in the hippocampal and hypothalamic areas; 3',5'-cGMP levels were reduced in the cerebellum and elevated in the striatum. Moreover, Hoe 427 enhanced the accumulation of ^{3}H-2-DG in various brain areas.

These results indicate, that short chain ACTH-analogues like Hoe 427 are able to cause potent modulatory effects presumably on cholinergic pathways in different brain areas after systemic administration.

210.9 SINGLE DOSING OF MIF-1 OR TYR-MIF-1 AFFECTS DOPAMINERGIC FUNCTION IN THE BRAIN. C. Hara* and A.J. Kastin. VA Medical Center and Tulane Univ. Sch. Med., 1601 Perdido St., New Orleans, LA 70146.

MIF-1 (Pro-Leu-Gly-NH_2) and Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), brain peptides, were active in the forced swimming test, a screening method for antidepressants (Kastin et al., Pharm. Biochem. Behav. 21:767, 1984). Accumulating evidence has suggested that MIF-1 has a potentiating effect on central dopaminergic (DA) function and may be effective clinically in some CNS disorders. In addition, it has recently been suggested that both peptides may act as opiate antagonists (Kastin et al., Pharm. Biochem. Behav. 21:937, 1984). However, the mechanisms of actions of these peptides in the CNS are still unclear. The present study examined single doses of MIF-1 and Tyr-MIF-1 on haloperidol (HAL)-induced catalepsy and apomorphine (APM)-induced stereotypy in rats in order to study the effects of both peptides on DA function in the brain. Male albino rats (10-11 weeks old) were tested between 9:00AM-12:00PM. All rats were used only once in an experiment. Catalepsy was tested 60 min after HAL administration (1.0 mg/kg, s.c.) with a standard bar test in which catalepsy was defined by rats keeping the imposed posture for over 30 sec (Hara et al., Pharm. Biochem. Behav. 18:423, 1983). The peptides (0.1-10 mg/kg, s.c.) were administered 30 min before the testing for catalepsy. In the APM experiment, MIF-1 and Tyr-MIF-1 (0.2-5.0 mg/kg, s.c.) were injected 10 min before the APM (0.1-2.0 mg/kg, s.c.). Ten min after the APM, each rat was rated for the degree of stereotypy on a 0-4 scale based on 30 sec observations every 10 min (Nakano et al. Pharm. Biochem. Behav. 12:459, 1980). In the catalepsy test, MIF-1 showed the inverted U-shape response found for this peptide in several other situations. Doses of 1.0 and 2.0 mg of MIF-1 suppressed the catalepsy, but doses of 0.1, 0.5, 5.0 and 10 mg did not exert this effect. Tyr-MIF-1 (0.5 and 1.0 mg) showed a suppressive effect similar to that of MIF-1. In the APM experiment, 0.5 mg of MIF-1 potentiated the stereotypy induced by 0.5 and 1.0 mg APM. The 1.0 and 5.0 mg doses of MIF-1 enhanced the stereotypy induced by 0.5 mg of APM, whereas the same doses suppressed that induced by 2.0 mg of APM. However, MIF-1 did not induce stereotypy in the rats that were sedated as a result of receiving only 0.1 mg of APM. Tyr-MIF-1 also seemed to enhance the stereotypy induced by 0.5 mg of APM and suppressed that by 2.0 mg APM, although these results are preliminary. Thus, MIF-1 and Tyr-MIF-1 appear to exert a biphasic effect on the stereotypy induced by APM. The results suggest that MIF-1 and Tyr-MIF-1 exert different responses depending upon the amount of DA neuronal activity. Therefore, MIF-1 and Tyr-MIF-1 may play a role in the indirect regulation of DA neuronal activity.

210.10 POTENTIATION OF ARGININE VASOPRESSIN'S (AVP) CONVULSIVE-LIKE ACTIONS BY SOMATOSTATIN (SS): POTENTIAL MECHANISMS OF ACTION. D.M. Burnard, Q.J. Pittman and W.L. Veale, Department of Medical Physiology, University of Calgary, Calgary, Alberta T2N 4N1.

AVP and SS produce severe motor disturbances when administered into a lateral cerebral ventricle (icv) of the rat. A single injection of AVP or SS increases the likelihood of a convulsive-like episode upon subsequent administrations of AVP. The present work examined: 1) whether SS would potentiate the convulsive-like actions of AVP in rats unable to synthesize AVP (HODI); 2) whether a vasopressor antagonist would block cross-sensitization between SS and AVP; and 3) whether SS/AVP cross-sensitization would occur in the ventral septal area (VSA); a site where AVP is thought to elicit these motor disturbances.

For these experiments, behaviors were recorded and scored over a 10 min period according to a predetermined behavioral code. When male HODI rats (n=6) and the parent Long Evans (LE) strain (n=6) received 2.5 micrograms SS icv on day 1, the behavioral scores did not differ significantly between the two groups. Following 1.0 microgram AVP icv on day 3, both HODI and LE rats displayed barrel rotations (spinning along the long axis of the body) and/or myoclonic/myotonic convulsive-like movements. These behaviors are normally seen following a second icv injection of AVP. When male LE rats received either 5.0 microlitre vehicle (n=8) or 1.0 microgram vasopressor antagonist [d(CH$_2$)$_5$Tyr(Me) AVP] (n=10) icv 5 min prior to 2.5 microgram SS icv on day 1, behavioral responses to SS were not significantly different. In contrast, most rats pretreated with vehicle on day 1 displayed barrel rotations and convulsive-like movements following AVP (1.0 microgram icv) on day 3, whereas antagonist-treated animals showed only minor behavioral anomalies. When male LE rats (n=5) were microinjected bilaterally into the VSA with 250 ng SS on day 1 and 100 ng AVP on day 3, a sensitization process similar to that following icv injections was observed.

The present work demonstrates that SS is able to potentiate the convulsive-like actions of AVP in animals which cannot synthesize AVP, thus sensitization does not appear to rely on endogenous AVP release. Furthermore, SS/AVP cross-sensitization can occur in the VSA, where AVP is thought to trigger these motor disturbances. Since SS-induced potentiation of AVP's convulsive-like effects can be prevented by pretreatment with a potent antagonist of the AVP vasopressor receptor, it is conceivable that SS may interact with the AVP receptor in some unknown manner to elicit this effect.

This work was supported by MRC of Canada. DMB is an MRC student and QJP is an MRC scientist.

210.11 CHOLECYSTOKININ: POTENTIAL MODULATOR OF CLIMBING AND MOSSY FIBER INPUT TO CEREBELLAR PURKINJE CELLS. D.J. Steel*, P.L. Wood and B. Petrack (SPON: B.S. Barbaz). Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ 07901.

Zetler reported that cholecystokinin (CCK) octapeptide (CCK8) blocks seizure activity in response to tremorogenic agents such as harmaline (Zetler, Neuropharmacology 22:757, 1983). Harmaline-induced tremors are associated with marked increases in cerebellar cGMP levels (Wood, Neuropharmacology 21:1235, 1982). We, therefore, explored the possibility that CCK8 also might reduce the harmaline-elevated cGMP.

Mice were injected with CCK8 (.02, .2 and 1 mg/kg s.c.), followed by harmaline (40 mg/kg i.p.). The mice were killed by microwave irradiation; the cerebella were removed, extracted with 1 N HCl, and assayed for cGMP via RIA (New England Nuclear Kits).

We confirmed the observation that CCK8 blocks harmaline-induced tremors. Furthermore, CCK8 dose-dependently reduced the harmaline-elevated cGMP levels. The CCK8 effect was maximal at 10 minutes but still significant at 20 minutes. CCK8 also suppressed basal levels of cerebellar cGMP in a dose-dependent manner. The effects of CCK8 on cGMP levels were not blocked by proglumide (160 mg/kg i.p.), naloxone (50 mg/kg i.p.) or benzotript (150 mg/kg i.p.). Cyclic AMP levels were not affected.

In addition to harmaline, cerebellar cGMP levels are elevated by amphetamine, apomorphine and pentylenetetrazol. We found that CCK8 suppressed the rise in cGMP caused by each of these agents except pentylenetetrazol, indicating that the antagonist actions are not directly at the level of the cerebellum. These results suggest a role for CCK8 in modulation of afferent inputs to the cerebellar Purkinje cells.

210.12 A NEUROPEPTIDE AUTORECEPTOR MEDIATES CHANGES IN NEURONAL EXCITABILITY. J.A. Kauer and L.K. Kaczmarek. Yale University School of Medicine, Departments of Pharmacology and Physiology, New Haven, CT 06510.

Although the bag cell neurons of Aplysia are ordinarily silent, brief electrical stimulation or elevated intracellular cAMP produces a long-lasting afterdischarge. In addition, agents which elevate cAMP levels in the bag cell neurons significantly increase the afterdischarge duration. We now demonstrate that ∝BCP (Rothman et al. PNAS 80:5753-5757, 1983), one of the neuropeptides released during the afterdischarge, alters both the electrical and biochemical properties of the neurons which release it.

We have found that a brief pressure ejection of ∝BCP [1-7] over intact clusters of bag cell neurons at the beginning of an electrically stimulated afterdischarge prematurely terminates the afterdischarge. Two different effects of ∝BCP which may explain this termination have been observed: 1) an alteration in bag cell cAMP metabolism and 2) an autoreceptor-mediated electrical inhibition.

Brief exposure to ∝BCP (1 uM) attenuates the ability of the adenylate cyclase activator forskolin to elevate bag cell neuron cAMP levels. A similar attenuation of forskolin stimulated cAMP levels is seen at the end of a normal electrically stimulated afterdischarge. Measurements of adenylate cyclase activity in bag cell membranes suggest that ∝BCP does not produce its effects directly on this enzyme.

Using isolated cells maintained in primary culture we have shown that ∝BCP also has a direct electrical effect on the bag cell neurons. Pressure ejection of luM ∝BCP near the cell body of a cultured bag cell neuron caused a hyperpolarization (as large as 35 mV in some neurons), accompanied by a drop in input resistance. When the cell was hyperpolarized below E_K, the response to ∝BCP reversed, now appearing as a depolarization. The hyperpolarization in response to ∝BCP ejection was unaffected by 100 mM TEA, while 5 mM CsCl completely abolished it. These observations suggest that ∝BCP produces an autoreceptor activated increase in a potassium conductance.

Our data demonstrate that ∝BCP modulates the excitability of the bag cell neurons which release it, and suggest that release of this peptide during a normal afterdischarge is likely to play a role in determining the firing pattern and duration of the afterdischarge.

210.13 A VOLTAGE-INSENSITIVE POTASSIUM CONDUCTANCE IN APLYSIA NEURONS IS ENHANCED BY FMRFamide AND DEPRESSED BY ELEVATION OF cAMP. C. Erxleben*, V. Brezina* and R. Eckert. Dept. of Biology, UCLA, Los Angeles, CA 90024.

Application of the endogenous neuropeptide FMRFamide or its analogue YGG-FMRFamide to certain Aplysia neurons suppresses the Ca current activated by depolarization, and hence indirectly depresses the Ca-activated K current (Brezina et al. and Erxleben et al., 1985; Biophys. J. 47:435a). Additionally, these peptides have been reported to hyperpolarize Helix (Cottrell et al., 1984, J. Physiol. 356:315) and Aplysia (Ruben et al., 1984, Soc. Neurosci. Abstr. 10:1116) neurons by an increase in K conductance. Using two-electrode voltage clamp and puffed application of peptide dissolved in a solution identical to that in the bath, we have attempted to identify this K conductance, which in the Aplysia abdominal ganglion is enhanced by these peptides in cells L2, L3, L4, L6 and R2, but not in cells L7, L11, R14 and R15. Puffed application of 1-50 μM FMRFamide or YGG-FMRFamide (but not FMRF) to one of the sensitive cells clamped close to the resting potential (-30 to -50 mV) produced a slow outward current that reversed at -70 to -75 mV. The reversal potential shifted with altered K_o(but not Na_o, Cl_o or Ca_o) in the manner predicted for a K-selective conductance. The FMRFamide-induced current was blocked weakly by external TEA or 4-aminopyridine. Apart from constant-field rectification, the conductance was insensitive to membrane voltage. This behavior is inconsistent with the conductances responsible for either the delayed rectifier, inward rectifier, 'A' current, or the Ca-dependent K current. In addition, the increase in K conductance seen in response to FMRFamide and YGG-FMRFamide applications was diminished significantly (by up to 75%) by treatments that elevate intracellular cAMP, such as superfusion with the adenylate cyclase activator forskolin or the phosphodiesterase inhibitor RO 20-1724, and by direct injection of cAMP. All the characteristics of the FMRFamide-sensitive conductance we have determined thus far are consistent with those reported for the 'S' current conductance that is diminished by action of serotonin, elevated cAMP and certain endogenous peptides (SCP's) in Aplysia sensory neurons (Siegelbaum et al., 1982, Nature 299:413; Abrams et al., 1984, PNAS 81:7956). Thus, it appears that the 'S' current conductance may be subject to both 'up' and 'down' modulation mediated by peptidergic mechanisms. The effect of serotonin and SCP in closing the 'S' channel appears to be mediated by a cAMP-dependent protein kinase. Our results suggest that FMRFamide stimulates a mechanism that counteracts this at some level. Supported by NSF BNS 83-16417 and USPHS NRSA GM07185.

210.14 EFFECTS OF NATURAL AND SYNTHETIC PEPTIDES ON APLYSIA NEURONS. M.K.Rock, S.B.Shope, J.E.Blankenship and D.H.Schlesinger. Marine Biomedical Inst., Univ. Tx. Med. Br., Galveston, TX. 77550 and Dept. Medicine, NYU Med. Center, NY 10016.

The atrial gland (AG) and bag cells of A. californica contain peptides capable of inducing egg laying. We have examined the effects of the following peptides on identified neurons of the abdominal ganglion: those released from the bag cells during an afterdischarge, the synthetic 8-amino acid alpha-bag cell peptide (α-BCP), those extracted from the AG, and the synthetic amidated 9-amino acid C-terminal portion of AG peptides A/B/ERH (B_{26-34}). The two synthetic peptides differ in primary structure in only 2 of 8 residues. Peptides were applied by superfusion, arterial perfusion, or pressure ejection from micropipettes placed over specific neurons or by inducing a bag cell afterdischarge. Protease inhibitors were used to prevent peptide degradation.

Inhibition of the left upper quadrant (LUQ, L2-L6) cells follows a bag cell afterdischarge, and this effect is duplicated by application of α-BCP at 10^{-7} to 10^{-4} M and by B_{26-34} at $5x10^{-4}$ M. B_{26-34} at 10^{-6} to 10^{-4} M has either no effect or a slight inhibitory effect on LUQ cells. Inhibition of R2 follows a bag cell afterdischarge, and this effect is duplicated by application of α-BCP at 10^{-7} to 10^{-4} M and by B_{26-34} at 10^{-6} to $5x10^{-4}$ M. AG extract has a powerful excitatory effect on R2. Inhibition of the white cells (R3-R14) follows a bag cell afterdischarge, and this effect is duplicated by α-BCP at 10^{-4} M and B_{26-34} at $5x10^{-4}$ M. AG extract has a powerful excitatory influence on R3-R14. α-BCP and B_{26-34} applied to the soma of LUQ cells, R2, and white cells increase membrane conductance. L10 undergoes a biphasic response (inhibition followed by prolonged excitation) following a bag cell afterdischarge, α-BCP duplicates the inhibitory but not the excitatory response. AG extract has an excitatory effect on L10.

Thus, peptides released by the bag cells inhibit LUQ cells, R2, and R3-R14 and produce an inhibition followed by excitation on L10. AG extract has a mild to powerful excitatory effect on these cells. All inhibitory effects are mimicked by α-BCP. The excitatory effects of the AG extract can not be duplicated by B_{26-34}, suggesting that the C-terminal portion of the AG peptides A/B/ERH are not responsible for these effects. Rather, B_{26-34} at comparatively high concentrations seem to mimick α-BCP (with which it shares homologies) and inhibit these neurons. Preliminary data indicates L10 is initially inhibited by α-BCP while the prolonged excitation may be due to ELH. Supported by NIH: NS-11255, NS-07185 (SBS), NS-18109 (DHS); NSF: PCM 82-15185 (JEB).

210.15 THE SUPPRESSIVE EFFECTS OF SCP_B ON THE ISOLATED GILL OF APLYSIA CALIFORNICA MAY BE MEDIATED BY A CHOLINERGIC MECHANISM. D.R.L. Cawthorpe*, W.F. Colmers and K. Lukowiak* (SPON: W.L. Veale). Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The endogenous neuropeptide, SCP_B, when perfused at concentrations as low as 10^{-13}M through the isolated gill of Aplysia californica, is a potent, reversible inhibitor of the gill withdrawal reflex (GWR) evoked by a mechanical stimulus to the gill. The classical neurotransmitter acetylcholine (ACh), also has inhibitory, as well as excitatory, effects on the GWR. The suppressive effects of ACh, which can be mimicked by the ACh agonist carbachol, are blocked by the nicotinic blocker curare. In this study, we examined whether the suppressive effects of SCP_B on the GWR could be mediated via a cholinergic mechanism by examining whether these effects could be blocked by co-perfusion of the peptide and curare.

Preparations consisted of the siphon, mantle and gill of 150-300 g Aplysia californica, with the abdominal ganglion and opaline and gametolytic glands removed. The preparation was pinned to the Sylgard base of a lucite dish, filled with artificial seawater (ASW; Instant Ocean) maintained at 15°C. The perfusate, ASW, was delivered to the afferent branchial gill vein via a glass cannula. A suture thread, tied to a single gill pinnule, was attached to an isotonic force transducer (Grass FT03C), connected to a Grass polygraph. The GWR was evoked by a mechanical stimulus to the gill by a mechanical trapper.

Perfusion of 10^{-13} to 10^{-9}M SCP_B through the isolated gill induced a suppression of the evoked GWR. After washout and recovery, SCP_B at the same concentration was co-perfused with curare (10^{-9} to 10^{-5}M). Under these conditions, SCP_B did not cause a suppression of the GWR (n=8). Curare concentrations above 10^{-5}M appeared to be equally effective in blocking the SCP_B suppression for all peptide concentrations tested. After complete washout with ASW, perfusion of SCP_B alone again caused suppression of the GWR.

The results show that curare at concentrations of 10^{-9} to 10^{-5}M completely blocks the suppressive effects of SCP_B on the isolated Aplysia gill. Even concentrations of 10^{-9}M SCP_B, which cause a profound, long-lasting suppression of the GWR in this preparation, were without effect in the presence of curare. The suppressive effects of ACh are blocked by curare, indicating their mediation by a nicotinic cholinergic receptor. Thus, it appears that the observed SCP_B effects on the GWR are mediated via this cholinergic mechanism. Experiments are in progress to test the interaction of the central excitatory and peripheral inhibitory effects of SCP_B on this preparation with the CNS attached.

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210.16 PHYSIOLOGICAL ACTIONS OF PROCTOLIN IN THE VENTILATORY SYSTEM OF CRAYFISH AND LOBSTER. V.M. Pasztor, R. Katz*, S. Weizner* and B.M.H. Bush. Biology Department, McGill University, Montréal, Canada, and Physiology Department, Bristol University, England

Proctolin-like immunoreactivity has been demonstrated in both motor and sensory neurons of the lobster ventilatory appendage, (collaborative work with K.K. Sewicki). Several motor axons to ventilatory muscles stain for proctolin as do afferent fibers and the peripheral dendritic arbor of the oval organ, a mechanoreceptor subserving ventilation. This finding suggests that the 75-90 nm dense cored vesicles seen earlier in electron micrographs of neuromuscular synapses on ventilatory muscles (Moody-Corbett and Pasztor. J. Neurobiol. 11, 1980) and of oval organ sensory endings (Pasztor, Zoomorphol. 193, 1979) contain proctolin.

Motor effects. Both central and peripheral motor effects resulted from proctolin application. When injected into intact crayfish it caused acceleration in ventilatory beat frequency and increased beat amplitude. These excitatory effects were confirmed by recording from ventilatory motor roots of isolated perfused subesophageal ganglia. Proctolin initiated rhythmical motor output in quiescent preparations and caused both decreased burst cycle periods and recruitment of motorneurons in active ganglia. Bath applied proctolin (10^{-7}M) produced 20-40% potentiation of contractions from semi-isolated coxal levator muscles in response to electrical stimuli delivered to the motor nerve in a simulated ventilatory pattern. No increase in the duration of contraction was observed nor any change in amplitude or shape of junctional potentials. Bishop et al. (Soc. Neurosci. Abst. 10, 151, 1984) report similar findings for crayfish abdominal flexor neuromuscular preparations.

Sensory effects. Afferents from the oval organ carry both impulses and graded potentials to the neuropil (Pasztor and Bush, Science 215, 1982). Responses to stretch stimuli were enhanced after 10^{-8}M Proctolin application, involving changes in receptor potential amplitude and spiking threshold.

210.17 GAMMA-AMINOBUTYRIC ACID INHIBITS THE POTASSIUM-STIMULATED RELEASE OF SOMATOSTATIN-LIKE IMMUNOREACTIVE SUBSTANCE FROM RAT SPINAL CORD SLICES. M.R. Vasko, S. Cartwright*, and V. Harris*. VA Med.Ctr., Univ. Texas Hlth. Sci. Center, Dallas, Texas 75216

Although somatostatin is localized, in part, in primary afferent neurons that synapse in the dorsal horn of the spinal cord, few studies have been performed to determine the regulation of somatostatin release from the spinal cord. Since gamma-aminobutyric acid (GABA) receptors have been localized on afferent nerve terminals in the dorsal horn, we studied whether GABA would affect the potassium (K+)-stimulated release of somatostatin-like immunoreactive substance (SOLI) from rat spinal cord slices.

Male Sprague-Dawley rats (250-400g) were decapitated and a 2 cm segment of lumbosacral spinal cord was removed, chopped into 0.5x0.5 mm pieces, weighed, placed in a perfusion chamber at 37°C and perfused with oxygenated Krebs-bicarbonate solution (0.5ml/min) containing BSA (0.5%), bacitracin (20μM) and dithiothreitol (6μM). To evoke SOLI release, 50mM KCl was substituted for equimolar NaCl for 8 mins during the perfusion. Drugs were administered starting 4 to 6 mins prior to 50mM KCl and throughout the high K+ period. Perfusates were collected every two minutes into glass test tubes, extracted through octadecylsilylsilica cartridges (Sep-Pak), lyophilized and assayed for SOLI using radioimmunoassay. The anti-serum for somatostatin did not cross react with numerous other peptides and the assay was not affected by any drug manipulations. Serial dilution of spinal perfusate resulted in a linear decrease in assayable substance suggesting that a non-specific contaminant was not released from the spinal cord tissue.

Exposure of spinal tissue to 50mM KCl resulted in a significant increase in release of SOLI from a basal level of 0.15 pg/min/mg tissue to 0.43 pg/min/mg tissue (n=14, $p<.001$). This evoked release (2.8 pg/mg tissue/10 min; defined as released during 10 min period after exposure to 50mM KCl minus the 10min period prior to exposure to 50mM KCl) was calcium dependent since exposure of tissue to high extracellular K+ in perfusate containing 0mM calcium and 3mM EGTA did not increase release of SOLI. GABA (10^{-5}M) did not alter basal release but significantly ($p<0.01$) reduced evoked release from 2.84 ± 0.38 pg/mg tissue/10 min to 1.07± 0.15 pg/mg tissue/10 min(n=10). A lower concentration of GABA (10^{-6}M) also reduced evoked release (1.92± 0.63 pg/mg/10min;n=4)but this reduction was not statistically significant. Bicuculline(10^{-5}M)did not antagonize the GABA induced inhibition of SOLI release. Evoked release in tissues exposed to bicuculline and GABA was 0.78 ± 0.12 pg/mg tissue/10 min (n=6).

These results suggest that GABA-containing neurons in the spinal cord may be important in regulating somatostatin release. Furthermore, since the GABA induced inhibition of SOLI release appears to be bicuculline insensitive, the GABA effect may be mediated by GABA-B receptors. (Supported by the Veterans Administration)

210.18 HORMONAL ACTIONS OF BAG CELLS AND NEUROPEPTIDES ON THE ARTERIAL SYSTEM OF APLYSIA. S.H. Ligman*, E.D. Tripp* and P.H. Brownell (SPON: M.I. Schimerlik). Dept. of Zoology, Oregon State University, Corvallis, OR 97331.

One component of the complex physiological response associated with egg-laying behavior in Aplysia californica may be a prolonged change in the pattern of blood circulation. In innervated, semi-intact preparations of the pericardial organs and major arteries, we found that stimulated bursts of bag cell activity increased periodic contractions of the gastroesophageal (GE) and anterior (Ant) arteries without affecting the abdominal artery. Arterial vasoconstriction lasted 30 to 90 min and appeared to be mediated by hormonal action of a transmitter(s) released from bag cell neurons. Since bag cells discharge just prior to ovulation in Aplysia, the induced pattern of vasoconstriction may function to alter circulation in accordance with tissue-specific metabolism during egg-laying.

The bag cells synthesize and release several peptides which may mediate the vasocontractile response of the GE and Ant arteries. To identify the transmitters involved, we attached 2-4 cm sections of the arteries to a tension transducer and immersed the tissue in mineral oil, thereby reducing the perfusion volume to a thin aqueous layer surrounding the artery. The artery was then perfused by a continuous stream (170 ul/min) of aerated, buffered seawater into which small volumes (80 ul) of transmitters could be introduced without interrupting flow. Our preliminary results indicate that oil-immersed arteries respond to acetylcholine (10 uM) and serotonin (100 nM) in the same manner observed by others. Additionally, we observed that brief (20-30 sec) exposures of the arteries to alpha bag cell peptide (10-30 uM threshold) had a stimulatory effect on contractile activity, while another endogenous peptide, FMRF-amide (10-300 nM threshold), inhibited contractions. These pharmacological studies of the isolated arteries suggest that some neuropeptides act directly on vascular muscle, and that artery-specific actions of peptide transmitters may regulate the pattern of blood flow during egg-laying. Supported by NIH grant NS18681.

210.19 EFFECTS OF ∝-BCP AND FMRF-NH_2 ON RESPIRATORY AND VASCULAR MOTOR SYSTEMS IN APLYSIA. J.L.M. Morgan*, S.H. Ligman*, and P.H. Brownell. Dept. of Zoology, Oregon State University, Corvallis, Oregon 97331.

Neurons in the abdominal ganglion of Aplysia californica synthesize and release several neuropeptides including the cardioactive peptide FMRF-NH_2 and bag cell peptides ELH and ∝-BCP. These putative transmitters affect the activities of identified neurons in the ganglion but the behavioral significance of these neuronal actions is undetermined. To investigate possible behavioral functions, we used a semi-intact preparation of branchial (gill and siphon) and pericardial (heart, kidney and major arteries) organs innervated by the abdominal ganglion. With this preparation, it is possible to record the responses of motor neurons and the organs they regulate while infusing small quantities of peptides into the ganglionic vasculature.

Preliminary studies using this system indicate that ∝-BCP (0.7-10.0 uM) strongly inhibits most of the motor neurons innervating the gill (L7, L9gl,2, LDgl,2, RDg) and siphon (LDsl,2,3, LBsl,2,3), but does not affect siphon motor neuron RDs. These inhibitory effects are similar to the inhibition observed following afterdischarge of the bag cell system suggesting that -BCP is the transmitter that mediates this component of the response. Inhibition of gill and siphon motor neurons by ∝-BCP was correlated with changes in the pattern of spontaneous gill and siphon contractions (respiratory pumping) mediated by these cells. In contrast to these inhibitory actions, ganglionic infusion of FMRF-NH_2 (0.1-10.0 uM) uniformly excited all of the gill motor neurons and caused tonic contraction of the gill.

The arterial vasoconstrictor motor neurons, LBvcl,2,3, were strongly inhibited by FMRF-NH_2 and ∝-BCP, but this neuronal response was not correlated with changes in arterial tonus. Other studies (Ligman et al., this volume) suggest these transmitters also act directly on vascular muscle. Together, these studies indicate that motor neurons are common targets of neuropeptide transmitters in the abdominal ganglion, but that the behavior of organs innervated by these cells are not always directly related to these central actions. Supported by NIH grant NS18681.

CATECHOLAMINES: DOPAMINE RECEPTORS II

211.1 A NEUROCHEMICAL ASSAY FOR DOPAMINE AUTORECEPTOR AGONIST ACTIVITY USING RESERPINIZED MICE. C.B. Davis*, R.P. Shank, G.E. Martin (SPON: P.E. Setler) Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477-0776.

The concept of dopamine autoreceptors has been confirmed by numerous laboratories. There are receptors on dopaminergic neurons from the nigro-striatal pathway which, when activated, function to decrease the synthesis and release of dopamine. It is possible that selective dopamine autoreceptor agonists may serve a therapeutic value as antipsychotics. We have developed a neurochemical procedure to test for dopamine autoreceptor agonist activity using a reserpinized mouse model. Our assay is based on the ability of dopamine autoreceptor agonists to inhibit the synthesis of DOPA in striatal tissue. Reserpine (5.0 mg/kg i.p.) is administered 18 hours prior to administration of the test compound. Thirty minutes after the test compound a DOPA decarboxylase inhibitor, NSD-1015 (100.0 mg/kg i.p.) is administered. In between the test compound and the NSD-1015 injections, the mice are observed for behavioral effects that are indicative of potential postsynaptic effects. The mouse is sacrificed by cervical dislocation 45 minutes post NSD-1015. The striatum is excised and homogenized in 2% perchloric acid. Analysis of DOPA is performed by HPLC using electrochemical detection.

The content of DOPA in striatum of control mice (which received reserpine, saline and NSD-1015) was 13.9 ±2.69 pmol/mg tissue (n=48) which is approximately 100 times the amount of DOPA in untreated mice. The maximum inhibition of DOPA accumulation by dopamine autoreceptor agonists was 80-90%. The ED_{50} value is the dose at which 50% inhibition of DOPA accumulation is achieved. ED_{50} values were generated for seven compounds known to be active at dopamine autoreceptors. These compounds and their ED_{50} values are: apomorphine (0.102 mg/kg i.p.); n-propyl norapomorphine (0.0028 mg/kg i.p.); EMD 23448 (0.227 mg/kg i.p.); (+)-3PPP (2.68 mg/kg i.p.); (+)-3PPP (5.63 mg/kg i.p.); (-)-3PPP (4.85 mg/kg i.p.); and (+)-PHNO (0.0066 mg/kg i.p.). Pentobarbital and phenobarbital, compounds which are not known to be dopamine autoreceptor agonists, were active in this test. The compounds that were inactive in our assay include haloperidol (0.2 mg/kg i.p.); proglumide (10.0, 40.0 mg/kg i.p.); d-amphetamine (4.0 mg/kg i.p.); morphine (16.0 mg/kg i.p.); clonidine (1.0 mg/kg i.p.); SKF 38393A (1.0, 3.0, 10.0 mg/kg i.p.); THIP (10.0 mg/kg i.p.); quipazine (1.0 mg/kg i.p.); and SCH 23390(0.3,1.0,3.0,10.0 mg/kg i.p.). Dopamine autoreceptor agonist activity for compounds that are active can be confirmed by using a D-2 antagonist such as haloperidol to reverse inhibition of DOPA accumulation. The apparent activity of pentobarbital and phenobarbital was not reversed by haloperidol indicating that these compounds are not direct-acting autoreceptor agonists.

211.2 ROBUST MODULATION OF ^{3}H-DOPAMINE RELEASE FROM RAT STRIATAL SLICES BY D-2 DOPAMINE RECEPTORS. Linda P. Dwoskin* and Nancy R. Zahniser (SPON: N. Weiner). Dept. Pharmacol., Univ. Co. Hlth. Sci. Ctr., Denver, CO 80262.

It has been difficult to demonstrate D-2 dopamine (DA) receptors that modulate stimulated release of ^{3}H-DA in rat striatal slices. The effect of a selective D-2 receptor agonist, pergolide (PERG), and antagonist, S-sulpiride (SUL), were systematically studied at different rates and durations of electrical stimulation both in the absence and presence of the DA uptake inhibitor nomifensine (NOMI). We now report that robust modulation of the stimulated release of ^{3}H-DA by D-2 DA receptors from rat striatal slices depends on the stimulation parameters used as well as whether or not an uptake blocker is included.

Electrical stimulation of rat striatal slices prelabeled with ^{3}H-DA produced a calcium-dependent increase in transmitter overflow. The amount of ^{3}H-DA released by 15 pulses of electrical stimulation was not dependent on the frequency of stimulation (0.25-3.0 Hz). In contrast, highly significant positive correlations were obtained between the total number of pulses (6-300) applied and the amount of tritium released. NOMI (10 uM) increased ^{3}H-DA release at each level of stimulation without affecting spontaneous release. PERG produced a dose-dependent inhibition of stimulation-evoked ^{3}H-DA release and was maximally effective at 30 nM. The inhibition produced by 30 nM PERG was greater when low numbers of pulses were delivered and in the absence of NOMI. PERG (30 nM) in the absence of NOMI completely blocked tritium overflow evoked by 15 pulses and inhibited overflow evoked by 60 pulses to 6.5±5% of control and that evoked by 300 pulses to 34±5%. In the presence of NOMI, overflow was inhibited to 47±6%, 80±4% and 76±10% of control at 15, 60 and 300 pulses, respectively. SUL produced a dose-dependent potentiation of stimulated ^{3}H-DA release and again was most efficacious at low levels of stimulation but, in contrast to the agonist, in the presence of NOMI. At a maximally effective concentration of SUL (1 uM) in the presence of NOMI, ^{3}H-DA release evoked by 15 pulses was stimulated by 370±50% over control while that evoked by 300 pulses was stimulated by 210±4%. In the absence of NOMI, increases of 200±60% and 150±10% were observed at 15 and 300 pulses, respectively. Interpretations are (1) higher synaptic concentrations of DA achieved at higher stimulation frequencies and in the presence of NOMI account for the reduced efficacy of the agonist, but that a low level of DA must be present in the synaptic cleft for the antagonist to be most efficacious; (2) D-2 receptors are most effective during periods of low stimulation. The results underscore the importance of the selection of the experimental conditions for studying D-2 receptor modulation of DA release from rat striatal slices. (Supported by grants AM 07391 and NS 09199)

211.3 DEPLETION OF ENDOGENOUS DOPAMINE CAN ENHANCE THE INHIBITION OF STIMULATION-EVOKED RELEASE OF ^{3}H-DOPAMINE FROM RAT STRIATAL SLICES BY D-2 DOPAMINE RECEPTOR AGONISTS. Nancy R. Zahniser and Linda P. Dwoskin*. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

We have observed that pergolide, a D-2 dopamine (DA) receptor agonist, causes a greater inhibition of electrically-stimulated release of ^{3}H-DA from rat striatal slices when low levels of stimulation and no uptake blockers are used. One explanation of these data is that D-2 receptor agonists appear more efficacious if the concentration of endogenous DA in the synapse is low. Thus, it was expected that if endogenous DA were depleted, pergolide would produce an inhibition of ^{3}H-DA release of greater magnitude as compared with control tissue for any given stimulation. In order to test this hypothesis, rats were treated 16 hr before sacrifice with alpha-methyl-p-tyrosine methyl ester HCl (AMPT; 300 mg/kg, i.p.) to deplete endogenous DA levels. This treatment produced an 85% decrease in the striatal DA concentration (control: 8.5 ± 0.43 ug/g tissue; treated: 1.3 ± 0.10). Modulation of electrically-stimulated ^{3}H-DA release by a maximally effective concentration of pergolide (30 nM) was measured in the absence and presence of nomifensine (10 uM) in striatal slices from control and treated animals. Despite the profound decrease in DA levels following AMPT treatment, ^{3}H-DA was taken up and released in response to stimulation to essentially the same extent by the slices from both groups of animals. When 60 pulses (1 Hz) were used to stimulate ^{3}H-DA release in the absence of nomifensine, the inhibition of release produced by pergolide was close to maximal in slices from both control (6.6 ± 4.7% of stimulated release, N = 4) and AMPT-treated animals (17 ± 5.7%, N = 3). In contrast, in the presence of nomifensine, the maximal inhibition produced by pergolide following AMPT treatment was twice that produced in control slices (control: 80 ± 4.2% of stimulated release, N = 4; AMPT: 43 ± 2%, N = 3). With 300 pulses (5 Hz) there was approximately 3-fold more ^{3}H-DA released during the stimulation period. In this case, however, no significant differences were observed in the control versus the AMPT-treated slices whether nomifensine was absent (control: 35 ± 3.7% of stimulated release, N = 4; AMPT: 45 ± 4.2%, N = 5) or present (control: 80 ± 8.2%, N = 4; AMPT: 73 ± 12%, N = 5). The greater inhibition of release by pergolide in the AMPT treated animals at 60 pulses in the presence of nomifensine suggests that lower levels of endogenous DA remained in the synapse in the slices from the AMPT-treated animals. This is consistent with the idea that D-2 receptor agonists produce a greater inhibition of ^{3}H-DA release if the concentration of DA in the synaptic cleft is low. (Supported by grants NS 09199 and AM 07391)

211.4 EFFECTS OF SELECTIVE DOPAMINE AGONISTS ON SUBSTANTIA NIGRA PARS RETICULATA ACTIVITY ARE INCONSISTENT WITH EXCLUSIVE MEDIATION OF DOPAMINE POSTSYNAPTIC EFFECTS BY D-2 RECEPTORS. B.G. Weick and J.R. Walters. NINCDS, NIH, Bethesda, MD 20205.

The substantia nigra pars reticulata (SNpr) is one of the major output nuclei of the basal ganglia complex. Dopamine (DA) neurons are in a position to influence SNpr neurons by indirect effects mediated through the striatum and direct effects mediated through release of DA from DA dendrites which extend into the SNpr. In earlier investigations, the mechanism of the modulation of basal ganglia output by DA and drugs which interact with DA receptors has been explored by observing the changes in tonic activity in the SNpr produced by DA receptor stimulation[1,2,3]. In normal rats, systemically administered apomorphine (APO), presumably acting through both direct and indirect mechanisms, induces variable changes in the tonic activity of SNpr neurons[1]. In rats with supersensitive DA receptors due to 6-hydroxydopamine (6-OHDA) induced lesions of the substantia nigra pars compacta (SNpc) DA neurons, systemic APO consistently induces a decrease in SNpr activity[2]. Iontophoresed DA, on the other hand, increases the activity of about 50% of the SNpr neurons and very consistently attenuates the inhibitory actions of GABA on these cells in normal rats[3]. Most of these effects have been presumed to be mediated by postsynaptic D-2 receptors. In the present study, the nature of the DA receptors mediating these effects has been investigated using DA agonists selective for the D-1 and D-2 DA receptor subtypes.

Extracellular, single unit activity of SNpr neurons was monitored in rats which were either anesthetized with chloral hydrate or paralyzed, artificially respired and locally anesthetized. Although APO and the D-2 agonist, LY 171555, have been shown equipotent at inhibiting the activity of the SNpc DA neurons[4], neither LY 171555 (1.0 umol/kg, i.v., n=11) nor the selective D-1 agonist, SKF 38393 (35.0 umol/kg, i.v., n=11), produced, in normal rats, the magnitude of variability of SNpr responses comparable to that induced by APO (1.0 umol/kg). As seen in behavioral studies, a high dose of SKF 38393 (35 umol/kg, n=11) did induce changes in SNpr activity like those of APO (1 umol/kg) in 6-OHDA lesioned rats (10-12 weeks). Activity was reduced 40 and 57% 5 to 10 min after SKF 38393 and APO, respectively. However, in the DA supersensitive rat, neither 1.0 umol/kg (n=12) nor 4.0 umol/kg (n=7) LY 171555 induced decreases in SNpr activity.

When iontophoretically applied, LY 171555 was considerably more potent than SKF 38393 at inhibiting the activity of SNpc DA cells, and, like DA[3], frequently increased activity and consistently attenuated the effects of iontophoretically applied GABA on SNpr cells. However, SKF 38393 also increased rate and modulated the effects of GABA on some SNpr cells. The results indicate that classic D-2 receptors do not exclusively mediate the effects of systemic APO and iontophoretic DA on SNpr cells.

[1]Waszczak, B.L. et al., Brain Res., 306: 307, 1984.
[2]Waszczak, B.L. et al., J. Neurosci., 4: 2369, 1984.
[3]Waszczak, B.L. and J.R. Walters, Science, 220: 218, 1983.
[4]Carlson, J.H. et al., Soc. Neurosci. Abst., 1985.

211.5 INVESTIGATION OF PHARMACOLOGICAL DIFFERENCES BETWEEN RAT SUBSTANTIA NIGRA DOPAMINE AUTORECEPTORS AND POSTSYNAPTIC D-2 DOPAMINE RECEPTORS. J.H. Carlson, D.A. Bergstrom and J.R. Walters. NINCDS, Bethesda, MD 20205 and George Washington University Dept. of Pharmacology, Washington, D.C. 20037.

Dopamine (DA) agonists inhibit substantia nigra pars compacta (SNpc) DA neuronal firing and stimulate globus pallidus (GP) activity. These effects are believed mediated by pre- and postsynaptic DA receptors, respectively. Both these receptor types have been postulated to be of the D-2 subtype, as the D-1 agonist, SK&F 38393, does not alter tonic SNpc or GP activity. To compare the pharmacological characteristics of pre- and postsynaptic D-2 receptors, the effects of the DA agonists, apomorphine (APO) and pergolide, and the selective D-2 agonists, LY 171555 and RU 24926, on SNpc and GP activity were examined utilizing extracellular, single unit recording techniques.

The 4 DA agonists were comparably efficacious and potent in inhibiting SNpc DA neuronal firing in chloral hydrate anesthetized rats. Each agonist caused a dose-dependent, haloperidol-reversible, complete inhibition of cell firing. The ED_{50}'s for i.v. APO, pergolide, LY 171555 and RU 24926 were 22±6, 25±4, 25±5 and 28±4 nmol/kg, respectively (n=8-12).

As an index of postsynaptic DA receptor stimulation, GP neuronal activity was recorded in gallamine-paralyzed, locally anesthetized, artificially respired rats. We have previously shown that APO and d-amphetamine induce dose-dependent, haloperidol-reversible increases in GP activity, while drugs such as l-amphetamine, clonidine, LSD and SK&F 38393 induce minimal changes. Like APO; pergolide, LY 171555 and RU 24926 caused dose-dependent, haloperidol-reversible excitation of GP activity. However, in the GP, the dose-response curve slopes and maximal efficacies of APO and pergolide were significantly greater than those of the selective D-2 agonists. APO and pergolide were of similar efficacy, inducing maximal rate increases of 113 and 165% (n=6-18), respectively; the D-2 agonists LY 171555 and RU 24926 induced significantly lower maximal rate increases of only 62 and 54% (n=6-14), respectively.

Since APO and pergolide interact with both D-1 and D-2 receptors, it seemed possible that their greater effects on pallidal activity could be due to their stimulation of both receptor subtypes, even though D-1 receptor stimulation alone is without effect on GP activity. However, coadministering SK&F 38393 with LY 171555 did not significantly enhance the excitatory effects of LY 171555. Moreover, coadministration of LY 171555 and APO did not attenuate the effects of APO, indicating that LY 171555 is not acting as a partial agonist at postsynaptic sites. In addition, pretreatment with 0.3 μmol/kg LY 171555 did not attenuate the effects of 1.0 μmol/kg APO as low doses of APO have been shown to do.

These results suggest that the effects of APO and pergolide (and d-amphetamine) on GP activity may involve, in part, interactions with haloperidol-sensitive receptor sites which may be pharmacologically different from the receptors which mediate the effects of the D-2 agonists. These sites appear dissimilar to the D-2 autoreceptors mediating the inhibitory effects of DA agonists on SNpc neurons.

211.6 DOPAMINE AUTORECEPTOR REGULATION OF STRIATAL TYROSINE HYDROXYLASE. K.S. Strait* and R. Kuczenski (Spon: D. Schmidt) Tennessee Neuropsychiatric Institute and Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Tyrosine hydroxylase (TH), isolated from striatal synaptosomes, exhibits biphasic Lineweaver-Burke kinetics for its tetrahydrobiopterin cofactor (BH_4), consistent with multiple K_m forms of the enzyme. Incubation of striatal synaptosomes with forskolin (EC_{50} 0.47 μM), or db cAMP (EC_{50} 1.3 mM), results in activation of TH, isolated from these synaptosomes, via conversion of the enzyme to a single low K_m form (K_m 40 μM). The activation of synaptosomal TH by forskolin or db cAMP is not additive and is similar to activation seen with cAMP dependent protein kinase phosphorylation of purified TH (Vulliet et al. PNAS 77, 92, 1980). The addition of dopamine (IC_{50} 1.0 μM) (with nomifensine and pargyline), or apomorphine (IC_{50} 30 nM), to the synaptosomal incubation medium blocks the activation of TH by forskolin. This effect of dopamine and apomorphine can in turn be blocked by preincubation of the synaptosomes with the dopamine receptor antagonists haloperidol (IC_{50} 4.5 nM), or chlorpromazine (IC_{50} 50 nM). In contrast to the forskolin data above, dopamine failed to block the activation of TH by db cAMP. Similarly, addition of dopamine to the TH assay, in amounts equivalent (as measured by HPLC) to that carried over from the synaptosomal incubation with the TH, had no effect on forskolin activated enzyme. The observations that dopamine and apomorphine can block forskolin activation of TH, that this blockade can in turn be prevented by preincubation with haloperidol or chlorpromazine, and that the amount of dopamine required for blockade of forskolin activation in synaptosomes has no effect on TH when added to the enzyme assay, supports the existance of a presynaptic dopamine receptor (autoreceptor), which regulates the activity of TH by altering the enzyme's K_m for its BH_4 cofactor, probably through a decrease in the phosphorylation state of the enzyme. Failure of dopamine to block db cAMP activation of TH suggests that, if forskolin and db cAMP activate TH through identical changes in phosphorylation state, then autoreceptor regulation of TH must occur through a decrease in cAMP levels. The autoreceptor could accomplish this decrease in cAMP through negative coupling to adenylate cyclase, or stimulation of phosphodiesterase activity, or both. (This work is supported by USPHS Grant DA 02676 and K.A.S. is supported by NIH Training Grant GM 07628.)

211.7 INTERACTIVE EFFECTS OF D-1 AND D-2 DOPAMINE RECEPTOR BLOCKADE IN VIVO. C. F. Saller and A. I. Salama. Dept. of Pharmacology, Stuart Pharmaceuticals, Div. of ICI Americas Inc., Wilmington, DE 19897.

The possible interactive effects of D-1 and D-2 dopamine (DA) receptor blockade in vivo were assessed by measuring the concentrations of the two major DA metabolites: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in rat striatum. We had previously found that the D-1 agonist SKF 38393 attenuates the decrease in DOPAC and HVA elicited by D-2 agonists and potentiates the increase in these two DA metabolites produced by D-2 receptor blockade (Saller and Salama, Eur. J. Pharmacol., 109:297-300). We report here that the D-1 antagonist SCH 23390 can attenuate the effects of D-2 blockade on striatal DOPAC and HVA concentrations. Thus, very low doses of SCH 23390 (< 1 µg/kg, i.p.), injected 30 min. prior to sacrifice, attenuated the increase in DOPAC and HVA produced by either haloperidol or spiperone (100 µg/kg, i.p.), injected 60 min. before SCH 23390. SCH 23390 also attenuated the increase in plasma prolactin concentrations produced by haloperidol. Therefore, these observations, along with other in vitro data (Salama and Saller, this meeting), suggests that a functionally important interaction exists between D-1 and D-2 DA receptors.

211.8 INTERACTIVE EFFECTS ON D-1 AND D-2 DOPAMINE RECEPTOR BLOCKADE IN VITRO. A. I. Salama and C. F. Saller. Dept. of Pharmacology, Stuart Pharmaceuticals, Div. of ICI Americas Inc., Wilmington, DE 19897.

Several studies have suggested that a functional interaction exists between striatal D-1 and D-2 dopamine (DA) receptors. For example, D-2 receptor stimulation by LY 141865 can antagonize the effects of D-1 receptor stimulation (SKF 38393) on adenylate cyclase activity (Stoof and Kebabian, Nature, 294:366, 1981). These observations have been extended to demonstrate that low concentrations of haloperidol (< 25 µM), a D-2 DA receptor antagonist, enhanced the stimulation of adenylate cyclase activity produced by SKF 38393 in striatal membrane preparations. No enhancement was observed when DA, which stimulates both D-1 and D-2 receptors, was used to stimulate adenylate cyclase activity. Haloperidol (< 10 µM) also potentiated the K^+-evoked release of ^{3}H-Ach from superfused striatal tissue slices. Both of these effects of haloperidol were blocked by low (nM) concentrations of the D-1 antagonist SCH 23390. In addition, SCH 23390 reduced the ability of haloperidol to antagonize the inhibition of ^{3}H-Ach release produced by the DA agonist apomorphine. By itself, SCH 23390 did not affect either basal adenylate cyclase activity or K^+-evoked release of Ach. These findings suggest that D-1 receptor blockade may attenuate the response to D-2 DA receptor blockade (refer to abstract by Saller and Salama, this meeting, for in vivo effects).

211.9 A NEW D-1 SELECTIVE RADIOLIGAND FOR AUTORADIOGRAPHY: SKF-83566. Sally J. Boyson[1,2], Kathryn E. Flaim[3*], and Perry B. Molinoff[1]. Departments of Pharmacology[1] and Neurology[2], University of Pennsylvania School of Medicine, Philadelphia, PA 19104; and Smith Kline and French Labs[3], Philadelphia, PA 19101.

Binding characteristics of (N-methyl-^{3}H)-R-7-bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF-83566), including its high affinity and selectivity for the dopamine-1 (D-1) receptor, were described by Flaim et al. (FASEB Abstract 8305, 1985). We now report the use of (^{3}H)-SKF-83566, with a specific activity of 85 Ci/mmol, in quantitative autoradiographic studies of the distribution of the D-1 receptor in rat brain. Autoradiographic images were analyzed on an IBM-based image processing system. Kinetic parameters of the binding of (^{3}H)-SKF-83566 to 32 µ slices of a mash made by homogenization of the striata from several rats and to 32 µ coronal sections through the striatum of individual rats were similar. Slices of striatal mash were therefore used to define pharmacologic parameters. The binding of (^{3}H)-SKF-83566 (0.1-2.0 nM) to slices incubated in buffer (50 mM Tris, 10 mM $MgSO_4$, 2 mM EDTA, 154 mM NaCl; pH 7.4) at 37°C reached equilibrium by 80 minutes. A wash in buffer at 4°C for 20 minutes reduced nonspecific binding to less than 3% of total binding. Scatchard analysis of saturation curves determined in both mash slices and coronal sections yielded a K_d of 1.0 nM and a B_{max} of approximately 4,000 fmol/mg protein in the striatum. K_d values calculated by the method of Cheng and Prusoff from IC_{50} values determined in the presence of 0.2-0.3 nM (^{3}H)-SKF-83566 were as follows: SCH-23390, 0.80 nM; spiroperidol, 500 nM; (+)-butaclamol, 15 nM; (-)-butaclamol, 45 µM; sulpiride, 70 µM; ketanserin, 300 nM; and propranolol, 250 µM. Serial coronal sctions of rat brain were incubated in 1.7 nM (^{3}H)-SKF-83566, washed, and apposed to LKB ultrofilm for one week to determine the distribution and densities of D-1 receptors. The highest densities of the D-1 receptor were found in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra, and structures in the third and fourth ventricles. Moderate densities were found in several nuclei of the septum and amygdala, the subthalamic nucleus, caudal hippocampus, zona incerta, endopeduncular nucleus, claustrum, and superior colliculus. D-1 receptors were present in all layers and regions of the cerebral cortex, but were most prominent in the auditory and entorhinal cortices, and layer 6 of the prefrontal and parietal cortices. There were no D-1 receptors detectable in the cerebellum or brainstem. (Sponsored by The Huntington's Disease Foundation and GM 34781)

211.10 COMPARISON OF THE DISTRIBUTION OF D-1 AND D-2 DOPAMINE RECEPTOR SUBTYPES IN RAT BRAIN USING QUANTITATIVE AUTORADIOGRAPHY. Paul McGonigle, Sally J. Boyson and Perry B. Molinoff. (SPON: Rodney B. Murray). Departments of Pharmacology and Neurology, University of Pennsylvania, Phila., Pa. 19104.

To help elucidate the different roles of D-1 and D-2 dopamine receptor subtypes in the regulation of movement and behavior, the anatomical distribution of these receptor subtypes in the same animals was measured. D-1 receptors were visualized with (^{3}H)-SKF-83566 (85 Ci/mmol) according to the method of Boyson et al. (Neurosci. Abst., 1985) and D-2 receptors were labelled with (^{3}H)-spiroperidol (77-100 Ci/mmol). Uniform sections of caudate-putamen mash were used to optimize the assay conditions for labelling D-2 receptors. Caudate-putamen mash provided a virtually unlimited number of homogeneous sections that contained a high density of D-2 receptors. These mash sections were incubated with the appropriate concentration of radioligand and drugs; washed, and then wiped off the glass slide for direct counting in a scintillation counter. Nonspecific binding was measured in the presence of 100 µM sulpiride. At 25°C, the binding of 0.3 nM (^{3}H)-spiroperidol reached equilibrium by 70 min. Washing the sections for 80 min. at 4°C following incubation reduced nonspecific binding to 30% of total binding. Scatchard analysis of the equilibrium binding of (^{3}H)-spiroperidol (0.05-3 nM) resulted in linear plots and yielded a K_d of 1 nM. At 0.3 nM (^{3}H)-spiroperidol, there was no detectable labelling of serotonin (5-HT2) receptors since the selective 5-HT2 antagonist ketanserin had a uniformly low affinity (IC_{50} = 3.5 µM) for these binding sites.

The distribution of D-1 and D-2 receptors was measured in adjacent 32 micron thick coronal and horizontal sections from a total of six rats. For the coronal maps, 38 sections corresponding to Paxinos and Watson (The Rat Brain in Stereotaxic Coordinates, Academic Press, 1982) plates 4 to 42 were examined. Similarly, horizontal sections corresponding to Paxinos and Watson plates 54 to 65 were studied. Sections labelled with 1.7 nM (^{3}H)-SKF-83566 or 0.3 nM (^{3}H)-spiroperidol were apposed to LKB ultrofilm for one to two weeks and then developed. Quantitative autoradiography with computer assisted densitometry was used to determine the densities of binding sites labelled with (^{3}H)-SKF 83566 or (^{3}H)-spiroperidol. The highest densities of both D-1 and D-2 receptors were measured in the caudate-putamen, nucleus accumbens, olfactory tubercle and substantia nigra. In contrast, relatively high densities of D-1 receptors but low densities of D-2 receptors were found in the subthalamic nucleus, the third and fourth ventricles, the superior colliculus, layer VI of the prefrontal cortex and the medial amygdaloid nucleus. Thus, these regions that exhibit markedly different densities of dopamine receptor subtypes represent promising targets for future study. (Supported by USPHS NS 07272 and GM 34781 and The Huntington's Disease Foundation)

211.11 IN VIVO BINDING OF THE NEUROLEPTIC [^{18}F]-N-METHYLSPIROPERIDOL TO HUMAN CAUDATE-PUTAMEN OVER TWELVE HOURS. C.D. Arnett, A.P. Wolf*, C.-Y. Shiue*, J.S. Fowler*, R.R. MacGregor*, and M. Smith*. Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973

As part of a program to evaluate positron-emitting radioligands for use in studying neurotransmitter receptors by positron emission tomography (PET), we have synthesized and studied several carbon-11 (Biol. Psychiat. 19:1365, 1984) and fluorine-18 (J. Neurochem. 44:835, 1985) labeled radioligands. The most promising of these compounds for imaging dopamine receptors in humans is [^{18}F]-N-methylspiroperidol (Life Sci. 36:1359, 1985).

This radioligand was synthesized from cyclotron-produced [^{18}F]fluoride in 2 h with a radiochemical yield of 10-15% and a specific activity greater than 10 Ci/μmol (EOB). Initial PET studies of this radioligand in normal human volunteers confirm our earlier conclusion as to its suitability for studying dopamine receptors in humans. Following intravenous administration of this compound, decay-corrected radioactivity in the caudate-putamen (a brain region which contains a high concentration of dopamine receptors) increased over a period of more than 4 h and did not decline for the entire 12 h duration of the study. This is consistent with essentially irreversible binding of this radioligand to dopamine receptors in vivo. By comparison, radioactivity declined rapidly in the cerebellum, a brain region which contains virtually no dopamine receptors. At times longer than 2 h after injection, the cerebellum radioactivity curve closely paralleled the blood plasma radioactivity curve. The very long retention of radioactivity in the human caudate-putamen suggests that very little metabolism of this compound takes place in this brain region. Rat studies demonstrated virtually no metabolism of this compound in the rat brain for up to 4 h (Life Sci. 36:1359, 1985).

Because fluorine-18 has a longer physical half-life than carbon-11 (109.8 min vs. 20.4 min), [^{18}F]-N-methylspiroperidol seems superior to N-[^{11}C]methylspiroperidol (Science 221:1264, 1983) for imaging dopamine receptors, since these results indicate that PET studies may need to be carried out for more than 2 h for good definition of in vivo kinetic constants. [^{18}F]-N-Methylspiroperidol thus appears to have fulfilled the basic requirements for a PET neuroreceptor radioligand of: (1) an appropriate radionuclide physical half-life, (2) a rapid radiochemical synthesis and purification to produce a high specific activity product, (3) a significant brain penetration following peripheral administration, (4) little metabolism within the brain during the study, (5) a highly specific distribution to the dopamine receptor, and (6) a slow dissociation from the receptor.

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211.12 IN VIVO [H-3]SPIPERONE BINDING REQUIRES DOPAMINE. Diane C. Chugani*, Robert F. Ackermann and Michael E. Phelps. Department of Pharmacology and the Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, California, 90024, USA

In a previous experiment to determine whether dopamine competes with [H-3]spiperone ([H-3]SP) binding in rat corpus striatum in vivo, we performed unilateral substantia nigra electrical stimulation in order to increase dopamine release. Then left and right striatal [H-3]SP accumulations were compared after intravenous administration. We expected to find decreased [H-3]SP accumulation ipsilateral to the stimulation if dopamine does in fact compete with [H-3]SP. Paradoxically, we found increased, not decreased, ipsilateral [H-3]SP accumulation (Ipsilateral: 324 ±1.4 dpm/mg ±SEM, Contralateral: 286 ±3.2 dpm/mg ±SEM). We hypothesized that this in vivo accumulation of [H-3]SP was due to trapping in endocytotic vesicles following agonist-induced internalization of receptors. Increased dopamine release, according to this scheme, would result in increased [H-3]SP accumulation, as we observed. Depletion of dopamine, according to this scheme, should result in a large decrease in [H-3]SP accumulation in striatum. Therefore, we administered reserpine (5 mg/kg) to rats 24 hours before [H-3]SP injection in order to deplete dopamine in striatum. We sacrificed the rats 1 hour after [H-3]SP injection (250 μCi/kg, i.v.) and rapidly dissected striata and cerebella. The brain regions were homogenized in 10 volumes ethanol, and aliquots were counted. The reserpine treatment resulted in a large, significantly decreased [H-3]SP accumulation in striatum (Control: 271.7 ±5.4 dpm/mg ±SEM, Reserpine pretreatment: 143 ±6.4 dpm/mg ∓SEM, $p<.001$), and in one rat, [H-3]SP concentration in striatum fell to cerebellar levels (Striatum: 105 dpm/mg, Cerebellum: 108 dpm/mg); the cerebellum contains no dopamine receptors. This profound reduction in [H-3]SP binding following reserpine pretreatment supports our hypothesis that, in vivo, [H-3]SP is trapped in regions containing dopamine receptors through dopamine-mediated receptor internalization.

211.13 IN VIVO OBSERVATION OF REGIONAL ACTIVITIES OF FLUORINATED NEUROLEPTICS BY ONE DIMENSIONAL ^{19}F NMR ZEUGMATOGRAPHY. I. L. Kwee*, T. Nakada, and C. B. Conboy* (SPON: M. P. Remler). Neurochem. Res. Lab., VA Med. Ctr., Martinez, CA 94553 and Dept. of Neurology and NMR Facility, Univ. of California, Davis, CA 95616.

Local contents of fluorinated neuroleptics in rat brain were studied noninvasively in vivo using one-dimensional 19-fluorine (^{19}F) nuclear magnetic resonance (NMR) rotating frame zeugmatography. Adult Sprague-Dawley rats (200-300g) were given intraperitoneal injection of fluphenazine (20mg/kg), haloperidol (15mg/kg) or trifluoperazine (20mg/kg) four times at thirty minutes intervals. Subsequently, the animal was lightly anesthetized and placed in the NMR probe which contained a one turn oval (2.5 x 3.5 cm) surface coil centered over the rat calvarium. ^{19}F NMR signals were obtained using a spectrometer operating at 4.69T (Nicolet NMR System NT-200) at 188.2 MHz. Sets of free induction decays (FIDs) were obtained with consecutively incremented pulse width. Standard two dimensional Fourier transfomation of data sets provided sets of spectra representing regional contents of each compound. Activities of these fluorinated neuroleptics showed a similar differential distribution in the brain maximal in the deep structures presumably within the basal ganglia. These observations suggest the feasibility of performing studies of regional pharmacologic activities of fluorinated neuroleptics by means of ^{19}F NMR spectroscopic analysis and hence studies of regional neurotransmitter activities in vivo.

This work was supported by UCD grants D-1921 (ILK) and NMR-8540 (TN).

211.14 INTRASTRIATAL VARIATION IN DOPAMINE RECEPTOR DENSITY AS MEASURED BY IN VIVO BINDING OF N-PROPYLNORAPOMORPHINE. L.D. Loopuijt*, J.B. Sebens*and J. Korf* (SPON: ENA). Division of Biological Psychiatry, Psychiatric Clinic, Oostersingel 59, 9713 EZ Groningen, the Netherlands.

Neuroleptics exert their antipsychotic action by means of blockade of dopamine receptors, but occupy binding sites, that are different from those which are occupied by dopamine and its agonists. Since anatomical localization of receptors determine their function, we studied the localization of the dopamine agonist N-propylnorapomorphine (NPA) to compare our results with those obtained with neuroleptics. So we employed light microscopic autoradiography to localize (3H)NPA, that binds selectively to dopamine receptors under in vivo conditions (van der Werf,J.F. et al.,Eur. J. Pharmacol.,87(1983)259, Köhler, C. et al., Eur.J. Pharmacol.,72(1982)397.

Seven rats were injected into the tail vein with 200 uCi (3H)NPA, were sacrificed 1 h and 10 min later by decapitation. Brains were quickly removed and frozen, cryosections were cut, autoradiograms were prepared using Ilford G5 emulsion. Rats were divided into 3 groups: group 1 received 4 days before (3H)NPA injection reserpine (5mg/kg/day), group 2 received an injection of 6-hydroxydopamine (8ug) into the substantia nigra 3 weeks before (3H)NPA injection, group 3 received an intraperitoneal injection of haloperidol (2mg/kg) 30 minutes before (3H)NPA, and group 4 received only a (3H)NPA injection.

The autoradiograms, obtained in this way show a high silver grain density in the caudate-putamen, a moderate density in the olfactory tubercle and nucleus accumbens and a slight labeling in the frontal cortex. No detectable label was found in the substantia nigra, both compacta and reticulata nor in the hippocampus. The above mentioned labeling was absent after haloperidol treatment (group 3). The caudate-putamen showed a heterogeneous distribution of silver grains: the head of the caudate was most densily labeled dorsally, in more caudal direction the lateral part was heavily labeled, while at the level of the globus pallidus, the highest concentration silver grains was found more ventrally, adjacent to the claustrum. This intrastriatal distribution of label is present in animals with reserpine treatment, with 6-hydroxydopamine lesions as well as with single (3H)NPA injection. So this differential silver grain density does not represent the heterogeneous distribution of presynaptic receptors on nigral afferents nor a selective post-mortem release of endogenous dopamine in separate neuronal pathways. Presumably this striatal pattern is due to differences in postsynaptic dopamine receptor density.

211.15 D-1 AND D-2 DOPAMINE RECEPTOR QUANTITATIVE AUTORADIOGRAPHY USING [^{3}H]-N-PROPYLNORAPOMORPHINE. E. K. Richfield, A. B. Young and J.B. Penney. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

A method of labelling both the D-1 and D-2 dopamine receptors in rat brain for analysis by quantitative autoradiography was developed. The dopamine agonist [^{3}H]-N-propylnorapomorphine (NPA) was selected because of its high affinity for dopamine receptors.

Twenty micron sections of rat brain were thaw mounted onto subbed microscope slides. Sections were pre-washed in 4°C buffer 2x5 min before incubation. Slides were incubated for 120 minutes in varying concentrations of [^{3}H]-NPA from 0.2 to 4.0 nM. The buffer used contained 50 mM sodium phosphate (pH 6.8), NaCl 65 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, ascorbate 0.01%, and pargyline 1 μM. After incubation, slides were given 2x10 min washes in 4°C buffer, a 3 second dip in 4°C distilled water and fan dried.

Saturation studies revealed binding to a single site in striatum with a K_D of 0.62 nM and a B_{max} of 1.52 pmol/mg protein. This K_D value is similar to values seen in homogenate studies. The B_{max} value exceeds the number of sites reported for many homogenate assays using various tritiated dopamine agonists and exceeds the number of sites seen with tissue sections using labeled D-2 antagonists. This suggests that a high percentage of dopamine receptors are in the high affinity state in tissue sections and that [^{3}H]-NPA labels both D-1 and D-2 receptors.

Competition studies using nonselective dopamine agonists including dopamine and NPA, as well as a selective D-1 agonist SKF-38393 and a selective D-2 agonist LY171555 demonstrated curves with a Hill coefficient of one. This indicates that agonists may not be able to distinguish D-1 from D-2 receptors in tissue sections.

Competition studies using the selective dopamine antagonists sulpiride, domperidone, SCH-23390 and cis-flupenthixol demonstrated Hill coefficients less than one and had biphasic curves. Thus, use of appropriate concentrations of domperidone or sulpiride allowed selective studies of the high affinity D-1 receptor, while appropriate concentrations of SCH-22390 allowed the high affinity D-2 receptor to be studied. The approximate ratio of D1:D2 receptors in striatum was 30:70.

The advantage of using quantitative autoradiography to study dopamine receptors is the ability to study the relationship between D-1 and D-2 receptors in multiple brain regions innervated by dopamine and to correlate changes in receptors with other studies using tissue sections such as ^{14}C-deoxyglucose determination of regional metabolic activity. In addition, properties of the high affinity state of both the D-1 and D-2 sites can be studied directly and correlated with other properties of dopamine function.

Supported by a grant from the Tourette Syndrome Association.

211.16 DOPAMINE D_1 AND D_2 RECEPTOR INTERACTIONS.

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Biochemical and electrophysiological studies have shown that dopamine D_1 and D_2 receptors have opposing roles. Although some in vivo studies suggest an interaction between the D_1 and D_2 receptors, it is not known whether different nerve cells or the same neuron is involved in this interaction.

1) We studied the in vitro effects of the D_2 agonist N-propylnorapomorphine (NPA) and the D_2 antagonist spiperone on the binding of the agonist ^{3}H-SKF-38393 to D_1 receptors. Canine caudate nucleus was used. The receptor density (B_{max}) and dissociation constant (K_D) were determined by Scatchard analysis.

The density of the D_1 agonist ^{3}H-SKF-38393 increased by about 60% from 14 to 23 fmoles/mg wet tissue in the presence of 0.1 nM NPA; the K_D was also increased. The B_{max} decreased about 50% in the presence of 0.1 nM spiperone.

2) We also examined in vitro effects of the specific dopamine D_1 agonist SKF-38393 and the antagonist SCH-23390 on the binding of the D_2 agonist ^{3}H-NPA to D_2 receptors. The density of D_2 agonist ^{3}H-NPA sites increased about 20% from 13 to 15 fmoles/mg tissue when incubated in the presence of 0.1 nM SCH-23390; it decreased by about 25% in the presence of SKF-38393.

These results suggest that dopamine D_1 agonists decrease agonist binding to D_2 receptors, and that D_2 agonists increase agonist binding to D_1 receptors. Intact neurons may further enhance this interaction.

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211.17 PERIPHERAL DOPAMINE RECEPTORS ARE INVOLVED IN THE REGULATION OF ALDOSTERONE PRODUCTION. C. Missale, P. Liberini*, M. Pizzi*, M. Memo, M.O. Carruba*, P.F. Spano. Inst. of Pharmacol. Exp. Ther. Sch. of Med., Univ. of Brescia; Dpt. of Pharmacol., Chemiother., Med. Toxicol., Univ. of Milano, Italy.

There is growing evidence for an influence of dopamine on adrenal glomerulosa production of aldosterone. This effect has been suggested to be mediated by peripheral dopamine receptors, namely DA_1. However, in this study we show that two different receptors for dopamine are present in rat adrenal glomerulosa, on the basis of the paradigm that stimulated or inhibited adenylate cyclase are indices of dopamine receptor function.

The cyclic AMP generating system in the adrenal cortex is stimulated by dopamine (EC_{50} = 7.2 μM) as well as by different dopamine agonists such as NPA (EC_{50} = 20 μM) and SKF 38393 (EC_{50} = 2 μM) and these effects are blocked by haloperidol, but not by (-)sulpiride, which slightly potentiates the stimulatory effect of dopamine. On the other hand, when the selective D_1 antagonist SCH 23390 is present in the incubation medium, dopamine induces a concentration-dependent inhibition of adenylate cyclase (IC_{50} = 10 μM); similarly, the dopaminergic ergot derivatives, bromocriptine (IC_{50} = 2 μM), dihydroergotoxine (IC_{50} = 6 μM) and lisuride (IC_{50} = 4 μM) decrease the cyclase activity; (-)sulpiride, in contrast to (+)sulpiride, completely blocks these effects.

In vivo studies in the rat indicate that sulpiride stereospecifically enhances plasma aldosterone levels, haloperidol is less active than (-)sulpiride and SCH 23390 is completely inactive on plasma aldosterone, thus suggesting an involvement of D_2 dopamine receptors in the regulation of aldosterone secreting system.

These data provide direct evidence for the presence of D_1 and D_2 dopamine receptors in the adrenal cortex with opposing roles in the formation of cyclic AMP; the pharmacological profile of this receptor system is identical to that described for D_1 and D_2 dopamine receptors in the corpus striatum. The D_2 receptor, linked to the inhibition of adenylate cyclase, may be functionally involved in the regulation of aldosterone production.

212.1 LACK OF ATTENUATION OF METHYLPHENIDATE-CONDITIONED PLACE PREFERENCES BY HALOPERIDOL. M.T. Martin-Iverson, S. Mithani* and H.C. Fibiger, Div. Neurol. Sci., Dept. Psychiat., University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

It has previously been demonstrated (Martin-Iverson et al., Brain Res. 332:59, 1985) that neither haloperidol (HAL) pretreatment nor catecholamine depletion induced with 6-hydroxydopamine attenuate place preferences conditioned with methylphenidate (MPD), while HAL pretreatment does block place preferences produced with d-amphetamine (AMPH - Spyraki et al., Brain Res. 253:185, 1982). These earlier experiments were conducted in an apparatus in which rats exhibit robust initial preferences for one compartment of a 2 compartment box, and the drugs were associated with the least preferred compartment. As preferences in this apparatus may result from drug effects on processes such as habituation or aversion, the present study examined the effects of HAL (0.2 mg/kg, i.p.) pretreatment on place preferences in rats induced with MPD (5.0 mg/kg, i.p.) or AMPH (1.5 mg/kg, i.p.) in a 2 compartment apparatus in which no initial preferences were apparent. In addition, extinction trials were conducted in a group of rats that had been conditioned with MPD to determine whether or not place preferences were the result of conditioning or some as yet unspecified drug-habituation interaction.

Place preference conditioning was observed following both MPD and AMPH, but not in a group treated only with vehicle (n=10 per group). Repeated testing of rats conditioned with MPD revealed that preferences undergo extinction, suggesting that learning, not habituation, underlies the observed place preferences. HAL signifcantly attenuated place preferences induced with AMPH, but not those produced with MPD. Thus, MPD appears to produce a rewarding action via a mechanism, and possibly a locus of action, that is distinct from that of AMPH.

Other groups of rats were tested for locomotor activity during drug treatments following a protocol identical to that used for place preference conditioning. The results from these experiments, which ascertain if differences in the effects of HAL on stimulant-induced place preferences relate to differences in actions on motor stimulation, will be presented.

Supported by the Medical Research Council of Canada.

212.2 SUPPRESSION OF OPERANT RESPONDING PRODUCED BY BLOCKADE OF DOPAMINE D1 RECEPTORS WITH SCH 23390. S. Nakajima. Department of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada.

Recent experiments in our laboratory demonstrated that a depamine D1 receptor blocking agent, SCH 23390, completely suppressed bar-pressing response for brain stimulation. Sulpiride, a dopamine D2 blocking agent, did not interfere with self-stimulation. A question was raised as to whether a similar receptor specificity exists in other types of reinforcement. The present study shows that the specificity is present in the reinforcement produced by food.

Albino rats were maintained on a food regimen at 85% of free-feeding weight. They were trained to press a bar for food pellets under either continuous reinforcement (CRF), fixed interval of 1 min (FI-1), or fixed interval of 3 min (FI-3). The FI-1 group showed a high level of response rate and maintained the same level for 30 min under a no-drug condition. The performance of this group, however, was highly susceptible to the drug effect. Injection of SCH 23390 maleate (0.05 mg/kg, IP) immediately prior to testing suppressed responding in about 20 min. The animals remained active, showing no signs of sleep or paralysis, but they showed drastic decline in response rate, and many of them stopped responding completely for more than 10 min.

The FI-3 group did not show as high level of performance as in the FI-1 group, and the drug effect was more pronounced. The CRF group was most resistant to the drug effect. Only a few animals stopped responding completely, and others pressed the bar from time to time and ate all of the pellets earned. The fact that the same motor response, bar-pressing, was suppressed under one condition but not under the other indicates that the suppression was not a simple motor interference.

Sulpiride (50 mg/kg, IP) was injected 4 hours prior to testing so that the animals could be tested at the peak of the drug effect. It had no effect on the responding under CRF or FI-1. Performance under FI-3 was slightly reduced with sulpiride.

These results suggest that the D1 receptors are critically involved in producing the rewarding effect of food, as they are in producing brain-stimulation reward. The D2 receptors seem to play only a minor role in this regard.

212.3 EFFECT OF AROUSAL ON THE SPONTANEOUS DISCHARGE AND SENSORY EVOKED ACTIVITY OF NIGRAL DOPAMINERGIC NEURONS. Robert E. Strecker and Barry L. Jacobs. Dept. of Psychology, Princeton University, Princeton, N.J. 08544.

Single dopaminergic (DA) neurons were recorded in freely moving cats by means of low impedance movable microwires. Dopaminergic neurons were identified by their electrophysiological characteristics, their response to the DA agonist drug apomorphine, and by histological localization of the recording site in the substantia nigra. Unit activity was examined during several different behaviors in order to examine DA involvement in arousal/stress, sensory integration, motor/postural control, and selective attention. None of the following tonically presented arousing/stressful conditions had an effect on neuronal discharge rate relative to baseline discharge rate: inaccessible food, feeding, grooming, inaccessible rats, immersion of feet in ice water, tail pinch, white noise, and somatosensory stimulation. Further, no relationship was found between DA unit activity and manipulations involving movement (treadmill locomotion), immobilization, or posture.

In contrast, repeated presentation of phasic auditory or visual stimuli during quiet waking behavior typically produced brief (<200 msec) excitatory neuronal responses, as has been previously reported (Steinfels, et al., Brain Res., 258, 217, 1983). Systematic changes in head orientation, or simultaneous exposure to tonic loud white noise had no effect on these observed neuronal responses to phasic clicks or light flashes. However, simultanous presentation of the other tonic environmental stimuli (e.g., inaccessible food, feeding, grooming, somatosensory stimulation, restraint, and ice bath) dramatically attenuated the DA neuronal responses to phasic clicks and flashes. This contrasts with the inability of these same conditions to influence spontaneous discharge rate.

In conclusion, during the various behaviors tested, DA neurons appear to provide a remarkably constant input to their target neurons in the caudate. However, the presentation of phasic sensory stimuli can change DA discharge dramatically, and this sensory response can be blocked during various active behaviors. This pattern of sensory responsiveness may provide a mechanism for the modulation of sensorimotor processing or attentional mechanisms in the caudate, which in turn influence information processing in the rest of the brain.

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212.4 VENTRAL TEGMENTAL AREA DOPAMINE SYSTEM: LOCOMOTOR AND COGNITIVE PERFORMANCE FOLLOWING 6-HYDROXYDOPAMINE-INDUCED DAMAGE. Brundin*, P., Gage, F.H., Dunnett, S.B., and Björklund, A. Department of Histology, University of Lund, Sweden and Department of Experimental Psychology, University of Cambridge, UK. (SPON: European Neuroscience Association).

Rats with extensive bilateral 6-hydroxydopamine (6-OHDA) lesions in the mesostriatal dopamine (DA) system display a DA-deficiency syndrome which includes akinesia, bilateral sensorimotor neglect and disturbances in feeding and drinking behaviour. In Parkinson's disease there is a reduction of DA-levels in the mesostriatal, but also in cortical and limbic regions. The mesolimbocortical DA-neurons which originate in the ventral tegmental area (VTA, A10 of Dahlström and Fuxe) and project, e.g., to the nucleus accumbens, olfactory tubercle, prefrontal cortex, anteromedial striatum and septum have been implicated in a variety of behavioral functions including regulation of locomotor activity, stress and cognition in rats. Extensive bilateral 6-OHDA lesions of the VTA have been shown to produce a persistent reduction of spontaneous locomotor activity, a blockade of amphetamine-induced locomotor activation, hyperactivity in response to apomorphine, impairments in active avoidance tasks, and disruption of hoarding behavior.

We have studied the effects of large bilateral injections of 6-OHDA in the VTA of rats on locomotor activity and cognitive behaviour in the Morris' watermaze. Rats with extensive lesions showed a prolonged latency to find the escape platform in the watermaze and often exhibited reduced swim speeds. In addition, these animals showed little indication of using spatial cues to find the hidden platform. These findings were reproduced in a subsequent experiment when noradrenergic neurons were partially protected from the 6OHDA effect by administration of desmethylimipramine prior to 6OHDA injection. The extent of DA depletion in different projection areas was assessed neurochemically and correlated with behavioural deficits. It was found that the extent of regional DA depletion was directly related to the magnitude of cognitive deficit. In ongoing experiments we have attempted to alleviate the lesion-induced syndrome by transplantation of a dissociated cell suspension containing fetal DA neurons to VTA projection target areas.

212.5 CONDITIONED PLACE PREFERENCE INDUCED BY INTRACEREBROVENTRICULAR MICROINJECTIONS OF COCAINE: POSSIBLE INVOLVEMENT OF CENTRAL DOPAMINE. M.A. Morency* and R.J. Beninger (SPON: M.W. Donald). Dept. of Neuroscience, McMaster Univ., Hamilton, Ont. L8N 3Z5 and Dept. of Psychology, Queen's Univ., Kingston, Ont. K7L 3N6

Behavioral studies have shown that cocaine is a potent reinforcing stimulus. Although it is often assumed that cocaine's local anesthetic actions are unrelated to its reinforcing properties, Spyraki et al. (Brain Res., 1982, 253: 145) reported that cocaine-induced conditioned place preference (CPP) was unaffected by manipulations which disrupted central dopamine (DA) and norepinephrine function. In addition, systemic injections of procaine, a local anesthetic which does not share cocaine's potent central stimulant effects, produced CPP. The present study was undertaken to evaluate the possible role of DA in cocaine-induced CPP. The apparatus consisted of a shuttle box in which the two end compartments were distinguished by brightness, odor and texture. The neutral central area served as a choice point. In the preconditioning phase, rats were allowed to explore the 3 compartments for 15 minutes; on the 3rd day, the time spent in the end compartments was recorded. During the conditioning phase, rats injected with cocaine were immediately confined for 30 minutes to the initially less prefered end compartment. On alternate days, rats received saline injections and were confined for 30 minutes to the opposite compartment. After 4 cocaine and 4 saline pairings, undrugged amimals were given access to the entire apparatus and time spent in the end compartments was recorded. Experiment 1 consisted of a partial replication of Spyraki et al. (1982). As expected, systemic administration of cocaine (5.0 mg/kg, i.p.) produced a significant CPP ($p<.02$) that was not disrupted in a second group of rats pretreated 4 hours prior to conditioning with pimozide (1.0 mg/kg, i.p.), a specific DA antagonist. In experiment 2, cocaine was microinjected unilaterally into the lateral ventricles to eliminate peripheral local anesthesia. Cocaine (50.0 ug in 1 ul, i.c.v.) produced CPP ($p<.001$). In this case, however, the CPP effect was blocked in rats pretreated with 1.0 mg/kg pimozide ($p>.20$). Results support the involvement of central DA in cocaine reward. (Supported by the Natural Sciences and Engineering Research Council.)

212.6 ELEVATION OF MEDIAL FOREBRAIN SELF-STIMULATION THRESHOLDS FOLLOWING MICROINJECTIONS OF DOPAMINE IN VENTRAL TEGMENTUM. O. Kofman, J. Yeomans and S. Whitfield*. Dept. of Psychology, University of Toronto, Toronto, Ontario, M5S 1A1.

Medial forebrain bundle self-stimulation is due mainly to the direct activation of myelinated fibers which pass from basal forebrain to ventral tegmental area (Bielajew and Shizgal, 1982; Yeomans, 1982). The involvement of ventral tegmental area dopaminergic neurons in medial forebrain bundle self-stimulation has been inferred from experiments which showed blockage of self-stimulation following injections of dopamine antagonists systemically (e.g., Fouriezos and Wise, 1976), or in mesolimbic terminal regions (e.g., Mora, Sanguinetti, Rolls & Shaw, 1975).

The present experiment tested this idea more directly by microinjecting dopamine directly into ventral tegmentum. Stimulation of autoreceptors on dopamine cells inhibit their spontaneous or glutamate-induced firing (e.g., Grace and Bunney, 1984). Rats were implanted with bilateral monopolar electrodes in the lateral hypothalamus and a single 28 gauge injection cannula aimed at the ventral tegmental area. They were trained to bar press for 0.5 sec trains of cathodal pulses (0.1 msec duration). Current was held constant and pulse frequency was varied until 20 min of stable baseline frequency thresholds were obtained. Dopamine or artificial CSF was then injected into the ventral tegmental area, and frequency thresholds for self-stimulation measured for up to one hour following injection (Yeomans, Kofman and McFarlane, 1985). Elevations of thresholds were observed for both ipsilateral and contralateral stimulation sites, peaking at about 10 min following the injection of 2, 5 or 10 ug of dopamine. Motor deficits were not observed, except at doses of 10 ug. No threshold changes were seen after injections of CSF. The duration of these effects was shorter than previously found for atropine (Yeomans, Kofman and McFarlane, 1985).

Testing the animals repeatedly over a period of one week resulted in a decline of the dopamine effect. This effect is consistent with previous studies (Antelman and Chiodo, 1981; Antelman, Chioda & de Giovanni, 1982) which found decrements of the behavioral and physiological effects of dopamine autoreceptor stimulation over several days due to the development of subsensitivity.

212.7 6-OHDA LESION OF THE CAUDATE NUCLEUS BUT NOT NUCLEUS ACCUMBENS PRODUCES DISRUPTION OF COMPLEX MOTOR FUNCTION IN RATS. M. Amalric, R.E. Lintz, and G.F. Koob. Div of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Impairment of the dopaminergic system in the brain induced by neuroleptics or by specific lesion result in motor disturbances in rats. In humans, neuroleptics often produce Parkinson-like neuromuscular side effects which are attributed to dopamine (DA) receptor blockade in specific anatomical loci in the extrapyramidal motor system. Lesions to the terminals of the mesolimbic system (destroyed by 6-hydroxydopamine 6OHDA injections aimed at the nucleus accumbens) but not of the striatal system produce an attenuation of spontaneous locomotor activity. In order to further specify the role of the two main DA pathways in the brain on motor function, we studied the effects of a neuroleptic, α-flupenthixol, administered systemically and specific lesion of DA activity by 6OHDA perfusion in the nucleus accumbens or nucleus caudate in rats performing a motor task for food reinforcement. Rats were trained to press a lever and release it as fast as possible after a light cue (conditioned stimulus CS). Four different time periods (0.25, 0.5, 1.0, 2.0 sec) delivered at random preceded the CS. In order to be rewarded by a food pellet, the rats had to wait long enough for the light to come on and to release the lever within 1 sec after the C.S. Reaction time was measured from the CS to the release of the lever for each trial. Daily sessions ended after 100 trials. Results were expressed in percent of success for the reaction times (successful trials both for the delay period and for the response latency within 1 sec) and for the delay period (whatever the reaction time value). α-flupenthixol (0.1, 0.2 and 0.4 mg/kg) injected intraperitoneally 2 1/2 h before the training session impaired the performance of the rats. Reaction time values were significantly lengthened for the 0.2 mg/kg dose. At the highest dose (0.4 mg/kg) rats stopped the task after 2 to 10 trials. When dopaminergic neurons were destroyed by 6OHDA injection differences in the two systems were observed. While disruption of the dopamine activity in the nucleus accumbens did not affect the performance of the rats, lesions of the dopaminergic neurons in the nigrostriatal pathway significantly affected the reaction time performances. Rats showing 95 to 99% success for the reaction time restriction before lesion decreased to 68-75% success 3 weeks post lesion. Reaction time values increased more than 4 sec. No changes were seen in the delay period. Results support the hypothesis that whereas the nucleus accumbens is involved in locomotor activation the corpus striatum has a role in the initiation of complex goal directed responses.

212.8 AMPHETAMINE-INDUCED REBOUND IN PLAY. J. Cox*, T. Strohe* & J. Panksepp. Dept. Psych., Bowling Green State University, Bowling Green, OH 43403

It is now well established that acute amphetamine (AMPH) administration reduces the play behaviors of young rats, although the mechanism of this effect remains obscure. We now report that repeated AMPH treatment (5mg/kg) produces a "rebound" in pinning behaviors and also markedly increases the average duration of each pin within a play session. In a initial experiment, using much milder AMPH treatment (1mg/kg once daily for 8 days) we did not see AMPH-induced rebound during the regimen or during withdrawal. However, under these conditions we did see a mild amount of tolerance to the play-reducing effects of a test dose of AMPH.

Subsequently, we administered AMPH (5mg/kg) every 8 hours to individually housed animals. We tested them daily, 7 hours following the preceeding AMPH treatment. Rebound emerged following 4 successive injections and persisted at least 16 hours after the final treatment. Rebound emerged concurrently with marked tolerance to a test dose (1mg/kg) of AMPH given before testing and with apparent cross-tolerance to fenfluramine (5mg/kg). Replications have indicated that AMPH-induced rebound emerges regardless of whether or not the rats are tested during the regimen and regardless of whether the injections are given every 8 or every 12 hours.

Repeated AMPH treatment is known to produce subsensitivity of dopamine autoreceptors as well as marked norepinephrine depletion. In preliminary tests of the neurochemical specificity of AMPH-induced rebound, we have found that rebound is attenuated in rats with 6-OHDA lesions of the nucleus accumbens. Since we found that these lesioned rats played normally in baseline tests, mesolimbic DA activity does not appear to be critical for play. Nevertheless, repeated AMPH appears to be acting through a DA mechanism to amplify play.

212.9 COMPARISON OF THE ABILITY OF (+)-AMPHETAMINE AND CAFFEINE TO PRODUCE ENVIRONMENT SPECIFIC CONDITIONING. R.S. Herz* and R.J. Beninger. Dept. Psychology, Queen's University, Kingston, K7L 3N6, Canada

A Pavlovian paradigm was used to assess the ability of two stimulant drugs, (+)-amphetamine or caffeine to produce environment specific conditioning. Based on evidence gathered from previous research dopamine appears to be involved in environment specific conditioning produced by many drugs including (+)-amphetamine, cocaine and morphine. The present experiment attempted to examine the hypothesis that direct activation of dopaminergic neurotransmission is critical to this phenomenon by comparing stimulant drugs with different mechanisms of action. Thus, the stimulant properties of (+)-amphetamine have been shown to be medi ated by dopamine, whereas the stimulant properties of the methylxanthine, caffeine are not thought to be directly medi ated by dopamine. Rather, the primary mechanism of caffeine's stimulant effects is thought to be the inhibition of adenosine receptors. As it was important for comparison that these drugs produce similar stimulant effects, pilot work established that doses of 30.0 mg/kg of caffeine and 2.0 mg/kg of (+)-amphetamine were appropriate. Forty-eight male Wistar rats were assigned to four groups of 12 rats each. Animals experienced four conditioning days with either drug or saline injected intraperitoneally prior to a 60 minute session in the photocell activity monitors and the alternate substance, saline or drug, respectively, injected in the home cage following the session. The fifth day was the test day, when all animals received saline in the activity monitors. This procedure was repeated so that days six to nine were the same as days one to four, and a second test session was conducted on day ten. Results revealed significant group differences on conditioning days between the experimental drug animals and their controls for both locomotor activity and rearing/jumping. It was also consistently found that on test days only the experimental animals previously receiving amphetamine in the activity monitors exhibited significantly higher activity scores than their controls. The hypothesis that environment specific conditioning may require the direct activation of dopaminergic neurotransmission was supported by the results. This does not exclude the possibility that caffeine may be able to produce conditioned effects at different doses or in different conditioning paradigms. However, these results show that the production of increased locomotor activity seen repeatedly with stimulant drugs in a conditioning paradigm is not sufficient to produce conditioning. (Funded by the Natural Sciences and Engineering Research Council.)

212.10 RAT ENDOCRINE RESPONSE TO PHYSOSTIGMINE IN A NOVEL ANIMAL MODEL OF DEPRESSION. N. Downs[1*], K. Thatcher Britton[2], G. Koob[2], D. Gibbs[1] and N. Swerdlow[3]. [1]School of Med., UCSD, San Diego, CA 92093; [2]PCN, Scripps Clinic and Res Fnd., La Jolla, CA 92037; [3]Dept Neurosci. and MST Program, UCSD, San Diego, CA 92093.

Previous workers (Risch et al, J Clin Psychopharm 1981) reported an abnormal endocrine response in clinically depressed patients compared to matched controls following administration of the acetylcholinesterase inhibitor physostigmine (PS). In these studies, depressed patients demonstrated a supersensitive increase in serum ACTH and prolactin (PRL) following PS challenge. Based on these findings, we examined the ACTH and PRL response to PS in rats following destruction of brain monoamine systems which have been implicated in the etiology of depression.

Thirty-three male Wistar rats were handled daily for 7 d and then injected with PS (0 - 0.6 mg/kg sc). After 20 min, animals were decapitated and trunk blood was collected for RIA analysis of plasma ACTH and PRL content. PS produced a dose-dependent increase in plasma ACTH, and both decreased (low doses) and increased (highest dose) plasma PRL levels.

Sixty-eight rats were then divided into 5 groups that received stereotaxic injections of saline or amine-selective neurotoxins so as to deplete brain levels of dopamine (DA), norepinephrine (NE) or serotonin (5HT). After 7 d, most animals were tested for the effect of these lesions on gross motor activity, using a standard pencil catalepsy test. One wk later, all animals were injected with PS (0 or 0.1 mg/kg sc), trunk blood was collected as above for RIA analysis, and brains were dissected for HPLC analysis of regional neurotransmitter content.

Compared to sham-lesioned controls, only DA-lesioned animals exhibited catalepsy. Analysis of plasma PRL levels in saline- and PS-injected animals revealed that 0.1 mg/kg PS increased plasma PRL in all groups with no between-group differences. Analysis of plasma ACTH levels in saline- and PS-injected animals revealed that 0.1 mg/kg PS increased plasma ACTH levels in all groups. ANOVA with repeated measures on lesion group revealed a significant main effect. Newman-Keuls individual means comparison revealed that, compared to sham-lesioned controls, ACTH levels following PS were significantly elevated in DA-lesioned rats, but not NE- or 5HT-lesioned animals. The effect of PS on plasma ACTH levels in DA-lesioned rats corresponded to a 6-fold increase in sensitivity to PS in these animals.

These results suggest intriguing parallels between the supersensitive increase in the plasma ACTH response to PS in clinically depressed patients and that seen in rats following depletion of central DA, but not NE or 5HT levels.

212.11 PROGESTERONE DECREASES 5-HT (SEROTONIN) RELEASE FROM ESTROGEN-PRIMED RAT HIPPOCAMPAL SLICES., Laura Imig and Peter Lipton, Dept. of Physiology, Univ. of Wisconsin, Madison, WI (Sp: H. Karavolas).

Progesterone (P) administration to ovariectomized, estrogen (E)-primed rats increases sexual receptivity. A large body of data implies this may be mediated by decreased activity of the serotonergic system, possibly due to an increase in monoamine oxidase (MAO) activity. Although these effects have been studied largely in the hypothalamus, hippocampal P implants and serotonergic receptor blockers also facilitate lordotic behavior.

We determined (E+P)'s effect on 5HT metabolism and release in hippocampal slices. Rats (210-220g) were ovariectomized. About one week later they were injected with 2.5µg E_2B, for two days, then sacrificed. Slices were depolarized in 60mM or 30mM K^+. When added to buffer, P was at 2µg/ml. Tissue and buffer 5HT and 5HIAA were measured by HPLC and used to calculate release and synthesis (per mg protein) for 50' periods.

P decreased 5-HT release in E-primed rats by 30% at both 30mM and 60mM K. (30 mM: + P, = 1.97 ± 0.18 ng/mg; - P = 3.30 ± 0.14 ng/mg. 60mM K, + P = 3.42 ± 0.14 ng/mg; - P = 4.66 ± 0.24 ng/mg.) 5HIAA release from P-treated slices was decreased by approximately 20% at both 30mM and 60mM K. Progesterone had no significant effect on 5HT or 5HIAA release in non E-primed animals depolarized by 30mM K^+. (5HT: + P = 2.53 ± 0.18 ng/mg; - P, 2.81 ± 0.18 ng/mg.)

5HT synthesis was reduced by 30% in slices incubated with P at 30mM K. However, P did not decrease 5HT synthesis at 60 mM K. (30 mM: + P, = 6.55 ± 0.71 ng/mg; - P = 9.31 ± 0.63 ng/mg. 60mM K: + P = 8.50 ± 0.54 ng/mg; - P = 8.74 ± 0.99 ng/mg.) 5HIAA synthesis was reduced by 13% at 60 mM K and 16% at 30mM K.

Thus, P decreases 5HT and 5HIAA release in E-primed rats, but has no effect on non E-primed rats. This suggests that the hippocampal slice could well be a model for studying the mechanism of action of P on sexual behavior.

The effects of P are most easily explained by a decrease in Ca^{++} entry into serotonergic terminals during depolarization. This would account for decreased release. It would also account for the reduction in synthesis at 30mM but not 60mM K as Ca^{++}-activated synthesis is apparently saturated at low concentrations of Ca^{++} (Auerbach and Lipton, J. Neurosci. 1985). The absence of 5HT cell bodies in the hippocampus suggests two possible modes of P action: 1) direct action on 5HT terminals to decrease Ca^{++} entry, ii) activation of an inhibitory interneuron transmitter (e.g. GABA) which would act presynaptically to inhibit Ca^{++} entry.

The first hypothesis would imply P action independent of the genome while the second would be consistent with an action on the genome (of the inhibitory interneuron).

212.12 GRADED LESIONS OF THE NIGROSTRIATAL PATHWAY. TOPOGRAPHIC DISTRIBUTION OF DOPAMINE DEPLETIONS, AND CORRELATION WITH AMPHETAMINE INDUCED ROTATIONAL BEHAVIOR. G.Pasinetti*, D.G. Morgan and C.E. Finch, (SPON: V. Henderson). Andrus Gerontology Center, and Dept. of Biol. Sciences, Univ. Southern Cal., Los Angeles, 90089-91.

Graded lesions of the nigrostriatal pathway were made in male rats to determine the threshold lesion size for induction of striatal dopamine (DA) supersensitivity. Male rats were pretreated with 25 mg/kg imipramine and a series of graded unilateral lesions were made by injecting 8,4, or 2, ug 6-OHDA (2 µl volume in 0.1% ascorbic acid) through a cannula stereotactically placed in the anterior portion of the right substantia nigra, pars compacta. Three weeks after the lesions, rats were injected with amphetamine sulfate (5.0 mg/kg) and tested for rotational behavior. One week after behavioral testing, animals were sacrificed by decapitation.

To estimate the topographic distribution of DA depletion, one mm punches were collected from anterior, middle and posterior levels of the striatum. At the middle level, punches from the dorsal, ventro-medial, and ventrolateral aspects of the striatum were pooled separately. The concentrations of DA, NE and DOPAC in these punches were measured by reverse-phase HPLC.

The DA depletions in these animals were proportional to the amount of 6-OHDA injected. The overall DA depletions using 8, 4, and 2 ug 6-OHDA were 95%, 89%, and 57% of the contralateral striatum (mean concentration in control side = 155 ± 12 ng/mgprotein; not different than uninjected animals). The range of overall depletions was 13% to 98%. In general, the depletions were less in the ventromedial aspect of the striatum than in the dorsal, or ventrolateral aspects. The depletions at the posterior level of the striatum tended to be slightly greater than those at the anterior or middle levels.

The rotational behavior produced by amphetamine injections correlated significantly with the severity of overall DA depletion (r= -0.67, $p<0.05$). When anterior, middle, or posterior levels of the striatum were considered separately, the depletions at both the anterior and middle levels correlated with the number of turns (r= -0.64, and r= -0.59, respectively; both $p<0.05$). In the posterior striatum, where all but 2 depletions were greater than 90% of control, no significant correlation was found (r= -0.51).

Thus, amphetamine induced rotation can be used as an index of the severity of DA depletion for unilateral depletions of 50-90%. This is of importance for a second experiment, already in progress, examining the regulation of both D-1 and D-2 striatal DA receptors following graded lesions such as these. The amphetamine induced turning can be used to pool animals with similar depletions for neurochemical analyses.

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212.13 INVOLVEMENT OF THE SEPTO-HIPPOCAMPAL PATHWAY IN THE TIMING BEHAVIOR OF RATS: THE EFFECT OF DIFFERENT NEUROTOXINS. J.D. Steketee, P.B. Silverman, C.M. Davis* and C.A. Harrington. Neuropsychopharmacology Section, Texas Research Institute of Mental Sciences and University of Texas Graduate School of Biomedical Sciences at Houston, Texas Medical Center, Houston, Texas 77030 USA

The differential reinforcement of low rates (DRL) operant schedule was used to study the involvement of the septo-hippocampal pathway in the time behavior of rats. Electrolytic lesion of the septum or hippocampus have been shown to disrupt the performance of rats on a DRL schedule. Little has been done to determine the neurochemical basis of the septo-hippocampal involvement in rat DRL performance.

After four days of DRL 1 bar press training, rats received either electrolytic lesions or lesions induced by 6-hydroxydopamine (6-OHDA), 5,7-dihydroxytryptamine (5,7-DHT), ibotenate or ethylcholine aziridinium ion (ECA) in the medial septum or hippocampus. The rats continued DRL 1 bar press training until they had recovered from surgery, at which time they were placed on a DRL 15 schedule for 30 days. Total responses, reinforcements, and efficiency (reinforcements/responses) were recorded. Temporal distribution of responses in 5 second bins was also recorded. Following DRL 15 training, the rats were sacrificed. The septal area and hippocampus were dissected out and used for choline acetyltransferase (CAT) assay or monoamine assay via high performance liquid chromatography (HPLC).

Electrolytic lesions of the medial septum or hippocampus significantly ($p < 0.5$) altered performance on the DRL 15 schedule. Electrolytically lesioned rats responded more frequently and received fewer reinforcements, thus demonstrating decreased efficiency. Of the chemical septal lesions only those made with ibotenic acid significantly affected DRL performance. Ibotenic lesioned rats responded at the same rate as controls, but received fewer reinforcements and exhibited a lower efficiency. Ibotenic acid lesions also had a significant effect in the hippocampus. Rats with ibotenate lesions in the hippocampus responded at a higher rate than controls during the last half of DRL 15 training. They received fewer reinforcements and were less efficient than controls throughout the 30 days of training. 5,7-DHT and 6-OHDA induced hippocampal lesions resulted in a decreased number of reinforcements and decreased efficiency. The effect of 5,7-DHT lesions on reinforcement rate and efficiency occured after 10 days of training, while 6-OHDA affected reinforcements from Day 1 and efficiency from Day 10 of training.

Biochemical data are being used to verify lesion placement and will ultimately be used in an attempt to delineate the role of specific neurotransmitters in DRL deficits resulting from septo-hippocampal damage.

212.14 XYLAZINE EMESIS, YOHIMBINE AND MOTION SICKNESS SUSCEPTIBILITY IN THE CAT. J.B. Lucot and G.H. Crampton. Depts. Pharmacology and Psychology, Wright State Univ., Dayton, OH 45435

The mechanism of emetic action of drugs whose emetic potency correlates with motion sickness susceptibility may suggest the receptor types involved in the systems mediating motion sickness. The emetic potency of xylazine, an alpha-2 adrenoceptor agonist, was tested for correlation with motion sickness susceptibility. The role of alpha-2 adrenoceptors in both emetic stimuli was then evaluated using the alpha-2 antagonist, yohimbine.

Cats were divided into two groups according to motion sickness susceptibility and were observed following subcutaneous injections of xylazine. The incidence of vomiting increased with the dose and at each dose, the high susceptibility group had a greater emetic incidence than the low susceptibility group. In another experiment with cats divided into two groups according to motion sickness susceptibility, s.c. administration of yohimbine effectively antagonized the xylazine-induced emesis in both susceptibility groups. These latter cats were then challenged with a motion sickness stimulus after s.c. pre-treatment with yohimbine. Yohimbine failed to prevent motion sickness, but did occasion an unexplained varibility in response rates. These findings establish that the emetic effect of xylazine is a function of alpha-2 adrenoceptors, but that these receptors are not involved in feline motion sickness. The fact that susceptibilities to xylazine emesis and to motion sickness are correlated suggests a point of interaction other than the area postrema, which, in cat, is known to be essential for xylazine-induced vomiting but not for motion sickness. (Supported by NASA-Ames Cooperative Agreement NCC-2-229.)

212.15 STUDIES ON THE HYPERACTIVITY PRODUCED BY INJECTION OF MORPHINE OR MUSCIMOL INTO THE MEDIAN RAPHE NUCLEUS. M. Klitenick, D. Wirtshafter and K.E. Asin, Dept. Psychology, Univ. Il at Chicago Chicago, IL. 60680.

Recent studies have demonstrated that various manipulations of the median raphe nucleus (MR) are able to produce hyperactivity. For example, large increases in locomotion have been observed following electrolytic lesions of the nucleus, as well as after intra-MR injections of particular compounds.

We report here that rats with indwelling cannulae terminating in the MR display a pronounced increase in photocell cage activity following injections of the GABA agonist muscimol (100 ng/0.5 ul), confirming the results of Sainati & Lorens (1982). In order to examine whether or not serotonergic mechanisms play a role in this response, we examined muscimol-induced hyperactivity in rats pretreated with either 1 or 2 systemic injections of the serotonin synthesis inhibitor PCPA (300 mg/kg). Despite the fact that these injections depleted serotonin in several forebrain structures by 80-90%, they did not alter the hyperactivity induced by intra-MR muscimol.

In other, preliminary, studies we have found that intra-MR injections of 10ug serotonin fail to produce hyperactivity. Since electrophysiological studies have shown that iontophoretic application of serotonin inhibits the firing of serotonergic cells within the MR, these results, like those of the study using PCPA, fail to support a model in which ascending serotonergic fibers mediate the hyperactivity produced by intra-MR injections of muscimol, as suggested by Sainati & Lorens (1982).

Since recent reports have demonstrated the presence of opiate receptors within the MR, we next examined the effects of intra-MR injections of morphine (0.1,0.5,1.0,5.0 & 10.0 ug/0.5 ul) on locomotor activity. Morphine injections were found to increase activity at all of the above-mentioned doseages, although the response was not as large as that seen following muscimol. The response to the 5ug dose of morphine could be blocked in a dose-dependent fashion by naloxone (1,2.5 & 5 mg/kg). These results suggest that opiate receptors within the vicinity of the MR may play a role in the control of locomotor activity.

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212.16 REWARD SUMMATION FUNCTIONS SEPARATE PIMOZIDE'S MOTOR AND HEDONIC EFFECTS IN RATS LEVER-PRESSING FOR SUCROSE. C. S. Bailey, S. Hsiao and J. E. King*. Department of Psychology, University of Arizona, Tucson, AZ 85721.

Dopamine (DA) is implicated in hedonic processing, possibly in the mesolimbic DA system. However, DA's involvement in the nearby nigrostriatal motor system indicates that motor effects must be separated from hedonic effects before accurate interpretation can be made. Attempts to separate these effects have had mixed success, and no attempt has been made to quantify the relative hedonic value of reinforcers.

Reward summation functions (RSF), a plot of rate of behavior versus log concentration of reinforcer, typically are a positive slope ending in a peak rate of responding. RSF's have been used to separate motor and hedonic effects using brain stimulation reinforcement (Edwonds, D. E. & Gallistel, C. R., J. Comp & Physl. Psych., 87:876, 1974). The RSF paradigm has not been used with other reinforcers.

Male Sprague-Dawley rats (n=18) lever-pressed for sucrose solutions of varying molarity in an easy task condition (30 g of weight required to press the lever) and then in a difficult task condition (60 g of weight required to press the lever). The peak of the RSF showed a vertical increase but the slope of the function did not shift. Thus, a motor impairment was reflected in the peak and not the slope of the function. That the rate of responding increased, rather than decreased, may be due to increased frustration which causes an increase in drive.

The same rats then lever-pressed for sucrose molarities laced with 0.05% quinine. The peak of the RSF did not change, but the slope of the function showed a horizontal increase when compared to the RSF for sucrose only. Thus, a hedonic change was reflected in the slope but not the peak of the function.

The rats were then injected with 0.2 mg/kg pimozide, a DA antagonist, and four hours later responded for sucrose solutions. The RSF for pimozide showed no change in the peak, but did show a horizontal increase in the slope, similar to the RSF for quinine-sucrose. A RSF for 3% tartaric acid injection, the pimozide vehicle, was not different from the RSF for sucrose.

Thus, RSF's represent motor effects as a vertical change in peak of the function and hedonic effects as a horizontal change in slope of the function. Pimozide's effects at this dose are hedonic and not motor. RSF slopes are different for brain stimulation, sucrose and sucrose-quinine reinforcers and thus may indicate quantitatively the relative hedonic value of the three reinforcers.

212.17 GENETIC APPROACHES TO STUDY RELATIONSHIPS BETWEEN BRAIN AND BEHAVIOR. C. Vadasz*, I. Sziraki*, L.R. Murthy* and A. Lajtha (SPON: N. Marks). Lab. of Neuro-behavior Genetics, Neurochemistry Div., Nathan Kline Inst., Orangeburg, N.Y. 10962.

One of the central goals in neurobiology is to find and analyse relationships between behavior and its neural substrate. There are several useful genetic methods which are applicable to establish these relationships (Vadasz, Cs. et al. In: I. Lieblich, Ed., Genetics of the Brain, Elsevier, p. 127, 1982).

Recently it has been reported that strain differences in tyrosine hydroxylase (TH) activity in midbrain dopamine (DA) systems are entirely attributable to differences in number of dopamine neurons in the substantia nigra - A10 (SN) area (Ross, R.A. et al. Nature, 264: 654, 1976) and such strain differences in enzyme activity and neuron number can be found in the hypothalamus (HT) also (Baker, H. et al., The J. of Neurosci., 3: 832, 1983). DA mediated spontaneous or amphetamine induced locomotor activities were paralleled with TH activities in SN, corpus striatum (CS) and HT in our experiments on reciprocal F1 hybrids (Vadasz, Cs. et al. J. of Neurogenet. In press). This was consistent with the hypothesis that some of the genes which control central DA systems contribute also to the expression of these motor activities.

In the present work, we studied highly inbred strains, their F1 hybrids and F2 segregating populations to further investigate the genetically based relationships between central DA systems and various types of ostensible behavior.

TH activity was measured in SN, CS and HT and correlated with behavioral variables used to assess emotionality, territorial marking, exploration and spontaneous motor activity. Factor analysis, a multivariate technique, was used to analyze the data. The resulting environmental, genetic and phenotypic factor matrices were significantly different from each other and gave further support to our hypothesis that multivariate genetic factor analysis can be a powerful method in constructing genetic maps of the brain or brain-behavior relationships.

AGING: NEURAL BASIS OF BEHAVIOR

213.1 INCREASED DYE COUPLING IN HIPPOCAMPAL NEURONS OF SENESCENT RATS. G. Rao, C.A. Barnes, B.L. McNaughton and J. Baldwin*. Dept. Psych., Univ. of Colorado, Boulder, CO 80309.

Several studies have examined the electrical characteristics of mammalian neurons in advanced age and a number of rather specific changes have been described. One such change is an apparent increase in the excitability of both pyramidal (Landfield and Pitler, Science, 226, 1089, 1984) and granule cells (Barnes and McNaughton, J. Physiol. (Lond.) 309, 473, 1980) of the hippocampus which appears in the absence of an altered resting potential or input impedance. In granule cells, it was found that this excitability change was more pronounced following synaptic activation than following intracellularly applied depolarizing current. One mechanism that could account for this observation is an increase in the extent of electrotonic coupling. MacVicar and Dudek have shown that both granule and pyramidal cells exhibit clusters of 2-3 units which transfer low molecular weight dyes freely and exhibit high electrical coupling ratios. If the extent of coupling increased with aging, this would result in an apparent decrease in the mean synaptic activation threshold since this would be set by the most excitable cell in an ensemble. This possibility is addressed by the present investigation.

Hippocampal slices were prepared from 6 old (25 mo) and 5 young (10 mo) Fischer-344 retired breeder rats, obtained from the NIA breeding colony. Intracellular recordings were made from pipettes containing a 5% solution of carboxyfluorescein in 0.1 M K^+Ac. Hyperpolarizing current pulses (1 nA, 100 msec, 5 Hz) were passed through the recording pipette to eject the dye. While ejection times varied, there were no age differences in the mean ejection times nor was there a correlation between ejection time and the number of filled cells. After filling, the slices were mounted in a 5% solution of n-propyl-gallate in glycerol (pH 8.0), observed using epi-fluorescence optics, and photographed.

Successful impalements were obtained from 43 cells in the young group and 38 cells in the old group. The old animals showed a statistically significant increase over the young animals in the average number of cell bodies observed per impalement ($\bar{x}$ old=1.67 ± 0.14 S.E.M.; $\bar{x}$ young=1.25 ± 0.08 S.E.M., $p < .05$). As many as four cells per cluster were observed after impalements from old animals. Coupled cells were frequently observed as far apart as 50 to 100 µm with an obvious point of contact only between adjacent dendrites. In addition, field potential recordings from the same slices revealed an increase in the ratio between population spike to field EPSP similar to that found in the fascia dentata.

These data strongly suggest a more extensive electrotonic coupling of hippocampal neurons in old animals which may have profound consequences for the information processing capability of these neurons. Supported by PHS grant AG 03376.

213.2 AGE-RELATED DIFFERENCES IN THE DECAY OF ENVIRONMENTALLY-INDUCED HIPPOCAMPAL PLASTICITY. P.E. Sharp, C.A. Barnes and B.L. McNaughton. Dept. Psych., Univ. of Colorado, Boulder, CO 80309.

Cells in the dentate gyrus (FD) show an activity-dependent long-term enhancement (LTE) of synaptic strength, which has become a promising candidate mechanism for information storage. In a recent study FD responses exhibited a persistent increase in amplitude as a result of exposure to a spatially complex environment. This suggests that an LTE-like phenomenon can be induced by environmental as well as electrical stimulation.

The present study compared this environmentally induced plasticity in young and old rats. Old rats show deficits in learning on spatial tasks and also show faster decay rates of artificially induced LTE in hippocampus (Barnes and McNaughton, D. Stein (Ed.), Psychobiology of Aging: Problems and Perspectives, 1980). If spatial information storage shares a common mechanism with LTE, it was reasoned that the environmentally induced plasticity might also show faster decay rates in old rats.

In this study 8 old (32 mo) and 8 young (14 mo) rats were implanted with electrodes for chronic recording of extracellular field responses in hippocampus. After recovery from surgery, perforant path evoked granule cell responses could be measured in the unanesthetized, freely-moving state. The experiment consisted of three phases. Phase I (lasting 15 days) provided baseline response measures. Animals lived in standard individual colony cages, as they had for many weeks prior to the start of the experiment. During Phase II (11 days) the rats were given daily exposure (from 5:00 PM to 9:00 AM) to a spatially complex enriched environment. Phase III was identical to Phase I; environmental enrichment was discontinued, and the animals spent all time except for recording sessions in the standard colony cages.

While no consistent or significant changes were seen in the synaptic component of the response, the population spike amplitudes for both old and young animals showed significant increases during Phase II (fractional change 1.37 ± .34 S.E.M. and 1.01 ± .48 S.E.M. for the young and old groups respectively). During Phase III, population spike amplitudes slowly decreased toward baseline, reaching fractional changes of .81 ± .25 S.E.M. (Young) and .26 ± .25 S.E.M. (Old) by day 15. The slope of the exponential decay function was significantly greater for the old animals.

These results indicate that a long-lasting increase in perforant path evoked granule cell response can be induced by environmental stimulation. Young and old animals showed asymptotic levels of change which did not differ statistically in magnitude but which decayed at different rates. These results are very similar to those of Barnes and McNaughton (1980) for the synaptic component of artificially-induced LTE. They support the idea that these two forms of plasticity may play a role in memory. Supported by PHS AG 03376.

213.3 AGING AND SELECTIVE ATTENTION: VISUAL EVENT RELATED POTENTIALS
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The performance of the normally aging individual on selective attention tasks is typically slowed, particularly when the number of irrelevant stimuli are increased or when the location of the relevant stimulus is uncertain. The purpose of this study was to investigate the effects of age on two types of selective attention as indicated by event related potentials: the effects of attending a particular location (interlocation) and particular feature (intralocation) of visual stimulation. It was predicted that the ERP indicants of both types of selective attention in older, as compared to younger subjects, would reflect a slowing and reduced selectivity of neural information processing.

Healthy normal subjects, inexperienced in ERP studies, were recruited for the study from the community. The mean age of the younger group was 25 yrs.: the mean age of the older group, 76yrs. The stimuli consisted of two color flashes, (red and blue), presented at two locations, (central and 16 deg. in the right visual field). These four stimulus conditions were presented in random order. For a given relevance condition, subjects were instructed to count one of these four conditions. There were four relevance conditions: red center, blue center, red right, blue right. ERPs from the occipital midline were averaged to 50 red flashes in the central and in the right visual field for all four relevance conditions. The amplitude and latency of the largest delfection in two time windows of the ERP components associated with interlocation and intralocation effects were measured: 200-350 msec. (N200-350), and 350-512 msec. (P350-512).

As predicted, the ERP indicants of selective attention, (N200-350 and P350-512), had a longer latency for the older, as compared to the younger group. The nature of the two types of selective attention, as reflected by P350-512, depended on age. The intralocation attention effect was greater for the younger subjects than for the older subjects: in contrast, there was no significant difference between the two groups in the magnitude of the interlocation effects. For both groups, the interlocation effect occurred earlier in time than the intralocation effect, replicating earlier findings.

The results were discussed in the context of neurophysiological mechanisms that might underlie these two types of selective attention, and how these mechanisms might change with age.

213.4 STIMULUS EXPECTANCY ASSESSMENT: POSSIBLE AGE EFFECTS ON THE EVOKED POTENTIAL.
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Attention may be assessed through comparison of evoked brain activity to predictable versus unpredictable events and may be related to age. The objective of the present research was to examine the effects of stimulus presentation within and between modalities [same vs. random order of visual, auditory, and bimodal (simultaneous visual and auditory)] on groups of individuals differing on age.

The average age for the younger group (N=11) was 19.3 (SD=1) years and for the older group (N=11) was 33.5 (SD=8.3) years. Evoked potentials were obtained from F3, F4, T3, T4, P3, P4, O1, and O2 referenced to nose. Visual stimuli were 17 minute visual angle (VA) checks in a checkerboard pattern subtending 9 degrees VA, 5 ftL. Auditory stimuli were 65dB(A) clicks about 30 ms duration. All stimuli were presented aperiodically with a 2 second interstimulus interval. A 512 ms post stimulus epoch was averaged (N=20 stimuli) and μVrms of the average obtained.

Analysis of variance demonstrated: greater EP amplitudes in the younger group overall; greater amplitudes to randomized than to same mode presentation; greater parietal than frontal amplitude; and a significant presentation-by-site-by-age interaction. There was a greater difference between same and random presentations for the younger group in the parietal, but a greater difference for the older group in the frontal region.

Possible explanations include: (1) greater arousal in the younger group related to test anxiety, (2) age related decline in reactivity, and (3) differential control of attention related to experience or maturity.

213.5 AGE-RELATED DETERIORATION OF LEARNING ABILITY IS ACCELERATED IN AUTOIMMUNE MOUSE STRAINS.
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Deterioration in the abilities of young C57BL/6 mice to acquire an avoidance response occurs following immune system manipulations which produce senescence-like increases in serum brain-reactive antibodies (BRA) (Nandy, K. et al., Neurosci. Abs., 10:721, 1984). Similar learning deficits occur in association with heterochronic formation of BRA over the life spans of C57BL/6 and autoimmune New Zealand Black (NZB) mice (Nandy, K. et al., Life Sci. 33:1499, 1983). Recent findings have indicated that deficient memory for avoidance learning may also be related to BRA formation in those strains (Harris, C.M. et al., Neurosci. Abs., 10:451, 1984). In order to further test the relationship between BRA and avoidance learning or memory, an additional autoimmune mouse strain was tested. Inbred mice of the MRL/Mp-lpr strain are known to exhibit BRA formation just following puberty, whereas genetically related, non-autoimmune mice (MRL/Mp-+ strain) are without this characteristic. Learning/memory abilities of the autoimmune MRL mice were expected to deteriorate beginning after 1.5 months of age, in parallel with the formation of BRA. Male MRL/MpJ-lpr (autoimmune) or MRL/MpJ-+ (non-autoimmune control) mice aged 1.5 or 3 months were tested for acquisition of a one-way active avoidance response until an avoidance had occurred on at least 8 of the last 10 trials. Separate groups of these mice were retrained to the same criterion either 1 or 48 hours following acquisition. Performance of the mice during the 1- and 48-hour retention tests indicated little difference between the strain or age groups. On the other hand, the control MRL mice showed an improvement in avoidance learning between 1.5 and 3 months, whereas the autoimmune MRL mice exhibited a marked deterioration within the same time period. The age-related learning deficits shown by the autoimmune MRL mice were qualitatively similar to those associated with elevated BRA in C57BL/6 and NZB mice. The current findings provide added support for the hypothesis that immunologic mechanisms related to the formation of BRA are involved with senescence-related learning dysfunctions. (Supported by a research grant from Miles Institute for Clinical Pharmacology).

213.6 ACCURATE SPATIAL MEMORY IN EXPERIENCED OLD RATS SURVIVES TEN MONTHS WITHOUT TRAINING.
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If a win-shift food searching strategy is required, young rats rapidly learn to enter each of the arms in a radial maze only once. Imposing a delay of several hours between their 4th and 5th choices does not degrade performance on this working memory (WM) task. Aged rats that receive their first training on the maze when they are already old acquire accurate spatial memory more slowly than young rats although once criterial performance is attained old and young animals perform equally well even at retention intervals of several hours. By contrast, 26 month old rats that had learned the radial maze problem while young and performed the task almost daily until 22 months of age displayed highly accurate WM, even at long delays (e.g., 96% correct at 5 hours), as soon as they were reintroduced to the task after 3 months without training (Beatty, et al., in press).

Bahrick and his colleagues have shown in humans that memory for some kinds of information is maintained with little loss for 30 years or more. To determine whether or not spatial memory in rats might be similarly long-lived we studied the reacquisition of accurate spatial WM at various delay intervals by a group of 21-5 month old rats that had received 225 radial maze tests between 3-11 months of age but received no testing during the intervening 10 months. The old rats regained accurate performance very quickly (Mdn sessions to criterion including 5 criterial sessions = 7, 7 and 5 for 0, 1 hour and 5 hour delays respectively). Their reacquisition was more rapid than the original acquisition of a group of 3 month-old rats tested concurrently (Mdn sessions to criterion = 13, 19, and 22 for the same rentention intervals). The old rats maintained highly accurate WM without employing response strategies. Forcing them to enter a randomly selected set of 4 arms had no effect on retention after a 5 hour delay (89% correct vs 91% under free choice conditions). These results show that the skills required for accurate spatial WM persist with little loss in aged rats. Spatial WM in rats may provide a model system for studying the neurobiology of enduring memory.

213.7 A CRITICAL EVALUATION OF SPATIAL LEARNING DEFICITS IN SENESCENCE. P.R. Rapp, M. Gallagher, and R.A. Rosenberg*. Dept. of Psychology, Univ. of North Carolina at Chapel Hill, Chapel Hill, N.C. 27514

The identification of behavioral testing procedures which are sensitive to age-related cognitive dysfunction has received considerable attention recently. Although a number of investigations have reported that the performance of aged animals is impaired in tasks which young animals solve on the basis of spatial information (e.g., the 8-arm radial maze), the degree to which spatial learning, per se, is compromised in senescence remains undetermined. The following experiments were therefore designed to critically evaluate age-related changes in spatial learning using the Morris water maze task.

In Experiment I, young (6mos., n=11), middle-age (12mos., n=11), and senescent (22-28mos., n=11) male Long-Evans hooded rats were tested for 32 trials, 2 trials per day, in the Morris task. During training an escape platform was submerged in clouded water in a fixed location within the maze for each subject. During the first 32 trials the escape latencies of all groups eventually decreased to equivalent asymptotic levels. Subsequent analyses, however, revealed that acquisition among senescent subjects was significantly impaired during the first half of training relative to young and middle-age animals ($p<.01$). This acquisition deficit was observed to occur in a reliable pattern such that aged subjects were relatively more impaired on the first trial of each day compared to performance on the second trial of the same day.

In Experiment II, naive young (n=13), and senescent (n=11) animals were tested (2 trials/day) for their ability to swim to a visible escape platform placed in a fixed location for individual subjects over 13 trials. This experiment was thereby designed to assess sensory and motoric factors which might contribute to age-related impairments in task performance. Although aged subjects were initially slower to escape in this cued version on the Morris task, both groups rapidly reached equivalent levels of learned performance. All subjects were subsequently observed in the maze for 90secs. with the escape platform removed. During this trial young animals spent significantly more time in the quadrant of the maze which had previously contained the platform compared to any other quadrant of the apparatus ($p<.01$). Aged subjects, conversely, showed no spatial bias. Similarly, other measures of behavior indicate that during cue training young animals acquire a significant amount of spatial information while senescent subjects show no such acquisition. These findings strongly support the interpretation that age-related impairments in acquiring spatial information contribute importantly to senescent learning deficits in the Morris water maze.

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213.8 OLFACTORY, VERBAL AND VISUAL RECOGNITION: AGE-RELATED CHANGES IN SECONDARY MEMORY PERFORMANCE. P.J. Moberg*, G.D. Pearlson, L.J. Speedie*, J.R. Lipsey*. (SPON: K.L. Kubos). Johns Hopkins Med. Inst., Dept. of Psychiatry and Behav. Sci., Baltimore, MD 21205

Perceptual losses appear to accompany the normal aging process. Previous research has documented age decrements in verbal and visual recognition (Schoenfield & Robertson, Can. J. Psych., 20: 228-236, 1966), and in olfactory identification ability (Doty, et al., Science, 226: 1441, 1984); however, declines in olfactory recognition memory have not been similarly examined.

One hundred and ten normal controls were administered three similarly structured recognition tasks. Visual and verbal recognition were measured implementing abbreviated forms of the Rey Auditory Verbal Task (1964) and Kimura's Figures (1963). The third recognition task was an olfactory paradigm of the authors' design (Moberg, Pearlson, et al., Neurosci. Abstr., 10: 318, 1984). All subjects were screened, and performed normally on an odor discrimination task to assess olfactory acuity. They were then presented with a series of 10 target odorants in identical masked bottles. The subjects were asked to remember, without naming, the target odors. After administration of a short cognitive battery, the Mini-Mental State Exam (MMSE) (Folstein, et al., J. Psychiat. Res., 12: 189, 1975), they were presented with 20 odorants including the original 10, plus 5 odors similar to 5 of the target odors, and 5 which were dissimilar. Subjects were asked to indicate which odorants they had been previously presented. Recognition accuracy for all tasks was represented using d' scores.

All participants scored in the normal range (≥ 25) on the MMSE. Results indicate significant age-related declines in all recognition modalities, with olfactory recognition being least affected ($r=-.27$, $p<.01$) compared to verbal ($r=-.43$, $p<.001$) and visual ($r=-.36$, $p<.001$) modalities. Of the three tasks, only the olfactory paradigm was not significantly correlated with educational level. Gender, smoking and prescribed medication effects were insignificant. These data indicate a decline in olfactory recognition with age, but at a lower rate than that for visual or verbal modes. The lack of strong educational, age and gender influences suggests that olfactory recognition may be a modality specific type of memory that is relatively culture-free. Such a test may allow for easier detection of pathological changes while avoiding the bias seen in other memory paradigms.

213.9 THE BEHAVIORAL AND PHYSIOLOGICAL CONSEQUENCES OF RIGHT MIDDLE CEREBRAL ARTERY LIGATION IN RATS FOLLOWING TREATMENT WITH NIMODIPINE, A CALCIUM VOLTAGE-OPERATED CHANNEL BLOCKER. C-A. Hardy and R.L. Isaacson. Dep't of Psychology and the Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, N.Y. 13901.

Preventing calcium influx in ischemic cells of the brain has been demonstrated to limit neuropathological changes associated with the loss of functioning in neural tissue (e.g. edema, EEG changes). The present experiment was designed to test the potential ability of nimodipine (NIM), a calcium voltage-operated channel blocker, to limit cerebral infarct size and aid in behavioral and physiological recovery in rats subjected to ligation of the right middle cerebral artery (MCA). Subjects were 57 adult male Long-Evans rats, who received either: no-surgery (n=20), control-surgery (n=19), or right MCA ligation (n=18). Approximately half of each surgery group was injected with a single systemic dose of NIM (3 mg/kg,i.p.), or a volume equivalent of the saline vehicle. The drug and/or vehicle was administered 10 min after ligation was completed in the MCA animals, 10 min after the control-surgery damage, or 100 min after the anesthesia in the no-surgery animals. Beginning on the day after surgery (i.e. post-surgery day 1= PS1), and on PS7, PS14, and PS21, all animals were tested in (1) a 10 min open field activity/exploration test, (2) a 6 min cool water swim test, (3) a 10 min post-swim grooming observation test in the home cage, and (4) sensory-motor tasks. The behavioral changes shown by the MCA animals were imperceptible in the majority of responses examined, and the observation of hyperactivity in the open field by Robinson (1979) was not replicated. However, the MCA animals did show an increased preference for left-sided grooming across test days in the home cage observation test, as well as a decreased likelihood of removing an alligator clip attached to the fur in one of the sensory-motor tasks. NIM had a pronounced behavioral effect only in the MCA animals. Specifically, the drug tended to decrease the amount of responding on a variety of behaviors only in the MCA rats (e.g. duration of hole-pokes and the number of central hole-pokes in the open field, right-sided flank grooming, orientation to a visual stimulus, & removal of a clip to the fur). The effect of NIM on behavior of MCA rats changed from PS1 to PS21, with certain behaviors becoming more frequent (e.g. right & left sided grooming, removal of clips from the fur), and other behaviors becoming less frequent (e.g. Duration of peripheral hole-pokes, number of central hole-pokes). It is proposed that the abnormal behaviors produced by NIM in the MCA rats is due to the administration of the drug after the ligation of the MCA, since NIM analogues have been previously demonstrated to have beneficial effects on the recovery of physiological functioning when given prior to the onset of ischemia. Future studies should examine the differential effects of calcium voltage-operated channel blockers when given before vs after the onset of ischemia.

213.10 COMPARISON OF DIAZEPAM PHARMACOKINETICS AND PHARMACODYNAMICS IN HEALTHY YOUNG AND ELDERLY ADULTS. A.M. Nikaido*, E.H. Ellinwood, Jr. and D.G. Heatherly*. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

A number of pharmacokinetic and receptor kinetic studies have indicated age-related changes in the activity of benzodiazepines. Previous research has reported no or weak correlations between diazepam plasma levels and psychomotor or cognitive impairment in both young and elderly adults. However, only a few studies have attempted to examine directly the relationship between the pharmacokinectics and pharmacodynamics of diazepam. The present set of studies examines the effects of an acute 10 mg dose of diazepam on the performance of 8 healthy young and 8 healthy elderly men. Prior to the drug sessions all subjects were trained to a plateau level of performance on several behavioral tasks. Each test session started with a predrug battery followed by an oral dose of the drug or placebo and repeated performance testing for 4 to 5 hrs. Blood samples were drawn concurrently with the task batteries.

Both young and elderly volunteers demonstrated significant ($P < .05$) impairment at the 10 mg dose as compared to placebo. On the other hand, significant ($P < .05$) age differences were evident for the two primarily motor coordination tasks of tracking and standing steadiness but not for any of the cognitive tasks, such as digit symbol substitution (DSS) and keyboard reaction (KRT).

The following linear model based on one proposed by Ellinwood et al. (in press) was used to examine the change over time in the relationship between drug serum levels and behavioral scores:

$$E = A + [(FF \times P) \times KATC]$$

where E = performance score, FF = free fraction, P = serum drug concentration, A = constant term for KATC and KATC = linear regression coefficient relating performance to drug concentration over time. In general, the KATC values for tracking (Young: -.464; Old: -.252) and sway (Young: -.569; Old: -.493) were higher than those for DSS (Young: - .258; Old: -.3218). The data indicated that there was a faster decline in impairment relative to serum concentration for the motor than cognitive tasks.

The indication of task specific effects supports hypotheses of physiological tolerance. In summary, the results of the present experiment indicated that the behavioral effects of diazepam in the elderly cannot be accounted for only by the corresponding drug serum concentrations and emphasize the necessity of considering the pharmacodynamics of benzodiazepines when prescribing and designing drugs for this age group.

213.11 3,4-DIAMINOPYRIDINE IMPROVES SPATIAL WORKING MEMORY BUT NOT SPATIAL REFERENCE MEMORY IN AGED RATS. C. Eppich*, C.A. Barnes, and J. Baldwin*, Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

A recent study (Davis, et al., Exp.Ag.Res. 9, 1983) has suggested that an age-related deficit in short-term memory on an 8-arm radial maze may be attenuated by pretreatment with 3,4-diaminopyridine (3,4-DAP). There were two questions raised by this work that the present experiment was designed to address: 1) Is the memory improvement age-specific or will 3,4-DAP improve radial maze performance in young animals as well? 2) Is the memory improvement specific to the spatial working memory problem, or will 3,4-DAP improve spatial reference memory as well?

Two different tasks were used to examine the effects of 3,4-DAP on spatial memory. Spatial working memory was assessed using the 8-arm radial maze, where optimal performance requires that an animal remember which arm it has obtained food from on any given trial. Spatial reference memory was assessed on the circular platform, a task involving escape from a brightly illuminated surface into a concealed goal tunnel.

Fifteen young (10 mo) and 15 old (28 mo) male Fischer rats were used in this study. The animals were further divided into 3 drug groups: saline, low-dose (750 pmole/kg) 3,4-DAP, and high-dose (2750 pmole/kg) 3,4-DAP. For the present analysis the low-dose and high-dose animals were treated as one group. Rats were injected daily, approximately 10 min prior to being placed on the maze. Training took place on the circular platform first. The animals were tested for 16 trials at 24 hr intervals. After the completion of this test, the animals were food-deprived to 85% of their ad libitum weight and were then trained to a criterion of 4 errors or less on 3 consecutive trials on the radial arm maze.

The finding of Davis et al. was confirmed by the present experiment, as 3,4-DAP was found to improve the performance of old rats, relative to saline-treated animals, on the radial maze. This result appears to be a selective improvement of the older animals, however, since the drug did not improve young animals' performance.

The results from the reference memory task were quite different. 3,4-DAP did not change the number of errors made by old rats on the circular platform, and, in fact, it led to an increase in errors made by the young rats. Interestingly the decrement in performance on this task in the young group was not due to repetitive errors in the same incorrect hole but to a less accurate approach to the escape tunnel.

Our results suggest that 3,4-DAP may be specific in its effects on spatial memory. It appears selectively to improve memory of the older animals and, within that age group, improves only working memory and not reference memory. This result may have important implications for the clinical use of 3,4-DAP in the treatment of certain age-related memory impairments. Supported by AG03376.

213.12 PREFERENCE DIFFERENCES FOR SUCROSE SOLUTIONS IN YOUNG AND AGED SQUIRREL MONKEYS. R. R. Michels*, J. E. KING* and S. Hsiao. Department of Psychology, University of Arizona, Tucson, Az. 85721.

We measured licking patterns and molarity preferences of six young and six aged squirrel monkeys in response to two sets of sucrose molarities (0.0,.25,.5,.75,1.0,1.25,1.5 and 1.75 or 1.0, 1.5,2.0,2.5 and 3.0). In addition to conventional measures of lick rate and consumption, several microbehavioral measures of licking performance were recorded. Sucrose preference thresholds were determined for aged and young animals, using four sucrose solutions ranging from a .025 to .1 molar concentration.

Sucrose preference thresholds for aged and young monkeys were virtually identical. The concentration at which 75% of the total test volume consumed was sucrose, was estimated to be .033 molar for the old and .049 molar for the young animals.

In the licking experiment, consumatory activity by both aged and young monkeys increased monotonically with sucrose concentrations below 1.0 M. At molarities higher than 1.0, consumatory activity of the young animals decreased, while that of the aged animals continued to increase until sucrose solutions were above a 1.5 molar concentration. Increases in consumption by both young and aged animals were effected by increasing the number of licking bursts, length of licking bursts and number of licks.

Aged monkeys displayed a greater within animal variability of tongue-on times, a constant and exponentially decreasing rate of licking at high molarities and a constant efficiency decrement in amount drunk per lick and amount drunk per second of licking. These age dependent behaviors opposed, but could not eliminate, the expression of a sucrose molarity preference shift in the aged animals. These results are consistent with the hypothesis that aged animals suffer decrements in perception of hedonic value.

213.13 BEHAVIORAL AND NEUROPHYSIOLOGICAL EFFECTS OF BASAL FOREBRAIN LESIONS. K. Perryman*, J. Fitten and A. Kling (SPON. M.K. Menon). Dept. of Psychiatry, Sepulveda V.A. Med. Cen., Sepulveda, CA 91343.

The magnocellular basal forebrain nuclei are cholinergic cells of origin for widespread neocortical areas and limbic structures. The decline in this cholinergic innervation is reasoned to be instrumental in the development of neuritic plaques and neurofibrillary tangles in the cerebral cortex and hippocampus that has been associated with the cognitive decline of Alzheimer's disease. Our group is presently examining the behavioral-neurophysiological aspects of the cholinergic hypothesis of senile dementia of the Alzheimer type (SDAT) using non-human primates that have kainic acid lesions of the nucleus basalis (nBm) and diagonal band (dB). Preliminary performance data on simultaneous and delayed matching-to-sample tasks suggest that the integrity of the cholinergic basal forebrain is necessary to acquisition and short-term retention. Monkeys with bilateral kainic acid lesions involving nBm and dB required more trials to reach 90 percent criterion on a red/green color color discrimination than unoperated controls and could not master the 3 second delayed comparison of the same stimuli. The operated animal's cortical and hippocampal EEGs were shifted from a predominantly delta-theta (1-7 Hz) activity profile during the performance of the tasks prior to the lesions to a theta-alpha (4-12 Hz) type spectrum. We were also no longer able to elicit visual evoked potentials from the frontal cortex to the onset of the discriminanda following destruction of nBm and dB. Although one of the neurophysiological markers of SDAT in the later stages is a reduction in alpha frequencies, our observations of an early post-lesion upward shift in the EEG spectrum may reflect some sort of compensatory mechanism associated with increased production of cholinetransferase (CAT) by intrinsic cortical neurons. We are taking weekly samples of EEG cortical activity to observe if and when there is a swing back in the predominant EEG frequency toward the delta and theta bands that has been associated with SDAT.

213.14 ASSOCIATION OF SLEEP PARAMETERS AND MEMORY IN INTACT OLD AND NUCLEUS BASALIS LESIONED YOUNG RATS. W. S. Stone[1,2], H.S. Altman[2], D.F. Caldwell[2], R.F. Berman[1], M.M. Kilbey[1]. [1]Dept. of Psychology, Wayne State University, Detroit, MI 48202; [2]Lafayette Clinic, Detroit, MI 48207.

The purpose of the present experiment was to examine whether young rats with nucleus basalis of Meynert (NB) lesions, which are suggested as a model of Alzheimer's Disease (AD) because retrieval of passive avoidance is shown to be impaired, also show sleep disturbances similar to those reported in AD (Prinz, 1982).

In order to test this we examined whether sleep parameters in young rats with NB lesions are similar to those of intact older rats. In addition, we employed a passive avoidance paradigm to determine if sleep parameters were related to retention scores, and whether young lesioned and old intact rats showed similar sleep-retention relationships. Young (6 months) and old (24-26 months) animals were divided into 4 groups. All Ss were implanted with EEG electrodes. One young and one old group did not receive any other surgical treatment while the other two groups, both young, also received bilateral implantation of cannulas aimed at the region of the nucleus basalis. After recovery, 24 hour sleep-wake recordings were obtained from all animals by placing them on activity platforms in sound attenuation chambers. The activity record and EEG were recorded on a polygraph machine and scored in 30 sec. epochs as awake, paradoxical sleep (PS) or non-PS (NPS). One young group then received bilateral injections of ibotenic acid dissolved in artificial rat CSF, while another young group received only vehicle injections. Two weeks later all animals had 24 hr. sleep recordings retaken. The Ss were then trained with one trial on a 2 compartment passive avoidance (PA) apparatus and tested for retention 24 hrs. later.

The results indicate that prior to lesioning, the 3 young groups did not differ from each other on any of the sleep parameters. Among the age differences, young animals slept significantly more than old animals, and had significantly: fewer brief arousals (3 sec. and 15 sec.), higher PS/total sleep ratio, longer PS periods, and larger differences in day-night sleep percentages After the injections, the lesioned animals resembled the old animals on a number of dimensions. As in previous studies PA scores declined in both the old and NB lesioned young rats. Significant sleep-wake cycle disturbances and fragmention of sleep bouts were also seen in the young lesioned group. When sleep parameters were compared with PA scores, the closest association was found between retention and mean length of PS bouts. Shorter length PS bouts which were most common in old and lesioned animals were related to decreased retention, while longer PS periods were more closely related to intact and, mainly, younger animals.

213.15 BRAIN CT AND DST IN ELDERLY DEPRESSIVES. R.C. Young, G.S. Alexopoulos, M. Brown*, Shamoian, C.A., R. Roe*, and M. Deck*. Cornell University Medical College and Westchester Division, The New York Hospital, 21 Bloomingdale Road, White Plains, N.Y. 10605.

Depressive illness is prevalent among the elderly. Brain neurotransmitter dysfunction may be especially prominent in late life depression. For example, hyperactivity of the hypothalamic-pituitary adrenocortical axis, as reflected in an abnormal dexamethasone suppression test (DST), is more common in elderly than in young adult depressed patients. Atrophic changes on brain computerized tomography (CT) can occur with normal aging and are prominent in some depressed patients.

Patients admitted to a geriatric psychiatric service were studied. Subjects were included who were 60 years of age or older and met DSM III criteria for major depression, and who had both pretreatment DST and brain CT performed as part of their evaluation. Plasma samples were obtained at 0800, 1600, and 2300 hours on the day following 1 mg of dexamethasone orally at 2300 hours. Cortisol was determined by radioimmunoassay. Cortical sulcal widening, lateral and third ventricular enlargement were each rated as: 0-absent; 1-minimal or slight; or 2-definite, at least moderate. CTs were evaluated blind to DST results.

A preliminary sample (n=10) had a mean age of 74.5 years $\pm$ 5.1 years; all were Caucasian and nine were female. Their age at the time of their first depressive episode ranged from 28 to 75 years and was not related to index age (r_S=0.05). Seven of ten had abnormal DST by the criteria of Carroll et al.

Rating of ventricular enlargement was strongly positively correlated post-dexamethasone plasma cortisol at 0800 (lateral ventricular: r_S=0.80, $p<.01$, two-tailed; third ventricle: r_S=0.69, $p<.05$) and 1600 (lateral ventricle: r_S=0.64, $p<.10$; third ventricle: r_S=0.36 N.S.), but not at 2300 hours. The relationship with sulcal widening was not as strong (0800: r_S=0.55, N.S.). All three CT measures were strongly related to age (sulci: r_S=0.76, $p<.05$; lateral ventricle: r_S=0.64, $p<.10$; third ventricle: r_S= 0.62, $p<.10$). Their correlation with age of illness onset was weaker but positive (sulci: r_S=0.19; lateral ventricle: r_S=0.34; third ventricle: r_S=0.57, $p<.10$). Except for age and post-dexamethasone plasma cortisol at 0800 hours (r_S=0.45), age and age at illness onset were only weakly related to cortisol levels within this sample.

These preliminary results suggest that further investigation of relationships between measures of brain structure, clinical course, and neuroendocrine function in late life depression is warranted.

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213.16 **INCREASED CHROMOSOME 21 MARKER (SOD-1) IN ALZHEIMER'S DISEASE FIBROBLASTS.** **O.J. Thienhaus*, F.P. Zemlan and H.B. Bosmann* (SPON: J.B. Suszkin). Lab. of Geriatrics Research, Univer. of Cincinnati College of Medicine, Cinti., OH 45267.**

Based on the hypothesis that excessive chromosome 21 material may play a pathogenetic role in Alzheimer's disease (AD), we measured superoxide dismutase-1 (SOD-1) activity in skin fibroblasts from an AD patient and compared them with SOD-1 activities in trisomy 21 fibroblasts (Down's Syndrome) and a normal control.

SOD activity was assayed according to the xanthine oxidase/nitroblue tetrazolium method developed by Beauchamp and Fridovich (Anal. Biochem. 44:276, 1971), i.e. a spectrophotometric assay at 560 nm. After identifying the fibroblast cell concentration range where small changes in protein concentration would not cause appreciable changes in the rate of absorbance, further determinations of SOD-1 activity were made employing protein concentrations thus determined, i.e. 13.33+ 0.36 ug/tube, representing approximately 166,000 cells/tube. Measured SOD-activities were standardized against commercially available, purified SOD. Addition of 2nM sodium cyanide, a specific inhibitor of SOD-1, eliminated all SOD activity, indicating that the activity measured was indeed due to the chromosone 21-coded SOD-1, and not to random superoxide scavanger activity.

We found that SOD-1 activity in AD fibroblasts was approximately 1.3 times greater than in an age-matched normal control ($p<0.01$). SOD-1 activity in trisomy 21 fibroblasts was 1.92 times greater than in the control ($p<0.001$), a finding replicating earlier studies (Feaster, W.W., et al., Am. J. Hum. Genet. 29:563, 1977). All enzyme determinations were done after the same number of cell culture passages.

Analysis of enzymatic activity in the three diagnostic groups (each comprising 42 samples) employed multivariate analysis of variance utilizing a general linear model. Post-hoc individual comparisons used Tukey's Honest Significant Difference Test.

213.17 CEREBROSPINAL FLUID ACETYLCHOLINESTERASE AND CEREBRAL ATROPHY IN ALZHEIMER DEMENTIA. G.D. Pearlson and L.E. Tune. Dementia Clinic, Johns Hopkins School of Med., Baltimore, Maryland 21205.

In 16 patients with the diagnosis of senile dementia of the Alzheimer type (SDAT), computed tomography head scans were rated blind to clinical and laboratory data, and lateral ventricular-to-brain ratios (VBR's) calculated planimetrically. Lumbar cerebrospinal fluid (CSF) acetylcholinesterase (AChE) activity was measured by radioenzymatic assay in all patients (Tune,L.E. et al, Ann. Neurol. 13: 46, 1985). The degree of dementia was quantified using the Mini-Mental Status Examination (Folstein,M.F. et al. J. Psych. Res. 12: 189, 1975). Significant correlations existed between cerebral atrophy as assessed by VBR, and CSF AChE activity (Pearson's r= -0.59, $p<.01$). Cognitive impairment, as quantified by the MMSE score, also correlated significantly with VBR (r=-.46, $p<.05$). These correlations were not accounted for either by age or by duration of illness. This relationship between VBR and AChE activity demonstrates an association between two independently determined clinical measures in senile dementia of the Alzheimer's type.

213.18 DECLINE IN CORTICAL $GABA_A$ AND $GABA_B$ SITES IN ALZHEIMER'S DEMENTIA. D.C.M. Chu, J.B. Penney and A.B. Young. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48109.

Cortical atrophy is one of the pathological hallmarks of senile dementia of the Alzheimer's type (SDAT). GABA is a major inhibitory neurotransmitter of cortical interneurons. Using a quantitative autoradiographic method, we have assayed $GABA_A$ and $GABA_B$ receptors in postmortem human frontal cortex from 4 persons with SDAT and 4 persons without neurologic disease. Blocks of frozen cortex were cut at -18°C in 20um sections, thaw-mounted onto gelatin-coated slides and air-dried. The slides were prewashed for 20 min at 4°C in 50 mM Tris-HCl + 2.5 mM $CaCl_2$ (pH 7.40) and blow-dried. $GABA_A$ receptors were assayed by incubating the tissue sections with ^{3}H-GABA (20 nM, 79 Ci/mmol) in the presence of increasing concentrations of isoguvacine to obtain IC_{50} and K_I values for isoguvacine. $GABA_B$ binding was performed by incubating tissue sections with ^{3}H-GABA in the presence of increasing concentrations of baclofen with 10 uM isoguvacine also present to block binding to $GABA_A$ sites. Assay conditions for both $GABA_A$ and $GABA_B$ receptors involved 30 min of incubation at 4°C with the ligand in 50 mM Tris-HCl + 2.5 mM $CaCl_2$, pH 7.40. Tissue sections were then rinsed once with ice-cold buffer and once with cold 2.5% glutaraldehyde in acetone and immediately blown-dry. Slides were mounted in an X-ray cassette and exposed to LKB Ultrofilm-^{3}H for 3 weeks at 4°C. Film densities were measured in cortical layers 2 and 4 by spot densitometry. Log-logit plots were constructed to yield IC_{50} values for isoguvacine and baclofen.

In SDAT cortex, layer 2 displayed a 43.1% decrease in total isoguvacine-sensitive $GABA_A$ binding (0.281 $\pm$ 0.053 pmol/mg tissue for SDAT vs. 0.494 $\pm$ 0.134 for control, $p<0.05$ by two-tailed, unpaired t-test) and a 60.9% decline in baclofen-sensitive $GABA_B$ binding (0.054 $\pm$ 0.010 for SDAT vs. 0.138 $\pm$ 0.055 for control, $p<0.05$). There was no statistically significant difference in either $GABA_A$ or $GABA_B$ binding in layer 4 between SDATs and controls. No clear trend in IC_{50} values for isoguvacine or baclofen was observed in the two groups. $GABA_A$ binding in human frontal cortex from both groups represented 80% of total ^{3}H-GABA bound whereas $GABA_B$ binding comprised 20%. This differs with our finding in rats in which $GABA_A$ and $GABA_B$ sites appear to be present in equal amounts in the cerebral cortex.

This study represents the first demonstration of a decline in both $GABA_A$ and $GABA_B$ receptors in layer 2 of frontal cortex from SDAT individuals.

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213.19 **EFFECT OF AGE ON GLYCINE RECEPTOR BINDING IN THE RAT.** C. Hunter,* E. Chung and M.H. Van Woert. Graduate Program in Neurobiology. Departments of Neurology and Pharmacology, Mount Sinai School of Medicine, New York, N.Y. 10029

The concentration of the inhibitory neurotransmitter GABA in the CNS is reduced in epilepsy (1) and ^{3}H-GABA receptor binding has been reported to decrease with aging in the rat. (2) Glycine is another major inhibitory transmiter which also may play a role in convulsive disorders, (3) as well as spasticity (4). In the present study, the effect of aging on glycine levels, ^{3}H-Strychnine binding and ^{3}H-GABA binding was investigated in the medulla and spinal cord of 2, 10 and 24 month old Fischer 344 rats.

^{3}H-Strychnine and ^{3}H-GABA receptor binding decreased in both the medulla and spinal cord of 10 and 24 month old rats compared to the two month old animals.

	Specific Binding (fmoles/mg protein)	
	^{3}H-Strychnine	^{3}H-GABA
Medulla		
2 mo	265.3±17.5	32.01±2.7
10 mo	232.8±11.1	29.93±1.7[a]
24 mo	165.5±7.2[ab]	22.36±2.1[a]
Spinal Cord		
2 mo	269.24±10.7	41.46±1.6
10 mo	199.64±7.9[a]	21.51±1.0[a]
24 mo	177.23±5.6[ab]	16.63±1.23[ab]

Each value is the mean ± SE of 8 rats.
a) P< .05 compared to 2 mo old. b) P < .05 compared to 10 mo old.

Scatchard analysis of ^{3}H strychnine binding in the spinal cord of 24 month old rats showed a reduction in B_{max} of 25 percent compared to the 2 month old controls. Glycine, threonine and serine levels in the medulla did not change with age.

The relevance of decreased ^{3}H-strychnine binding in older rats to glycine receptor function and seizure threshhold will be reported. (Supported by USPHS grant NS 12341)

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213.20 AGE-DEPENDENT CHOLINE UPTAKE IN RAT BRAINS. H. Goldman, S. Murphy* and H. Altman . Dept. of Pharmacology and Lafayette Clinic, Wayne State Univ. Detroit, MI 48201.

The blood-to-brain transfer of ^{3}H-choline relative to a ^{14}C-butanol reference indicator was measured by a single capillary-transit technique in conscious, unrestrained rats, as a function of age. Standard caged Sprague-Dawley male rats were studied at 6, 12 and 24 months of age. Indicator dilution of butanol in arterial blood provided the estimate of cardiac output. Regional brain vascular spaces and plasma/red blood cell choline levels were measured in subsets of animals from each age group. The results from these studies indicate that there was a significant reduction in regional cerebral blood-to-brain transfer rates of choline in the aging rat brain. The decline was seen in all regions examined. In seven of these, the decline between 6 and 24 mo was significant at less than the 0.05 level, e.g., pons-medulla-30%, basal ganglia-20%, parietal cortex-18%, pyriform cortex-amygdala-21%, septal region-18%, inferior colliculus-24%, olfactory bulb-24%. Least affected were the hippocampal and frontal cortical areas-7%. The decline in most regions was progressive, with the exception of the pyriform cortex-amygdala, in which a decrease of 20% was observed mainly between middle and old age. These decrements occurred in the absence of significant changes in cardiac outputs, in regional brain vascular spaces, plasma choline or RBC choline levels. Furthermore, the age-dependent regional reductions in choline transport occurred in the absence of changes in the uptake of sucrose, a marker of simple blood-to-brain diffusion. Our data strongly suggest that the transport and/or permeability of choline in most areas of the brain decreases with age. The diminished availability of this substrate for the synthesis of acetylcholine and membrane phospholipids may be important in the reduced cerebral functions associated with aging in the rat and other animals.

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213.21 LOSS OF COMPLEX-SHAPED AXOSPINOUS SYNAPSES IN THE DENTATE GYRUS AS AN ANATOMICAL MARKER OF AGE-DEPENDENT SPATIAL MEMORY DEFICIT IN F344 RATS. Y.Geinisman, L. de Toledo-Morrell* and F.Morrell. Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL. 60611 and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL. 60612.

Most but not all aged F344 rats exhibit a profound spatial memory deficit when tested in the 8-arm radial maze (de Toledo-Morrell, L. et al.,Behav. Neurosci., 98:902, 1984). Accurate performance in this task in young animals depends on the integrity of the hippocampal formation (HIF) or its afferent supply (Olton, D.S. et al.,Brain Res., 233:241, 1982). A possible anatomical substrate of this spatial memory deficit in aged rats was assessed by electron microscopic examination of synapses in the dentate gyrus (DG) of HIF, at the perforant path-granule cell junction; i.e., the termination site of entorhinal afferent input to HIF. Animals were given one trial a day in the maze and trained to a criterion of 3 consecutive trials with no errors (no repeated entries into a given arm) or to a maximum of 30 trials. Three groups of rats (N=6 in each) were examined morphologically: 1) 5 mos. old animals that reached criterion, 2) 27 mos. old rats that reached criterion and 3) 27 mos. old rats that failed to reach criterion (memory-impaired group). The morphologist (Y.G.) was "blinded" with respect to age and behavioral history. The tissue preparation consisted of perfusion fixation with aldehydes, osmication and routine processing. Two tissue blocks from the dorsal HIF (right side) whose rostral face was 1.5 or 3.5 mm caudal to the HIF septal pole were used. From the rostral face of each block, serial ultrathin sections, which included the molecular layer of the DG hidden blade, were prepared and stained with uranyl acetate and lead citrate. Axospinous synapses were quantified in the middle of the molecular layer. The postsynaptic density was employed as the counting and measuring unit. The synaptic numerical density per unit volume of neuropil (N_V) was estimated stereologically, using the method of Fullman (J.Metals, 5:447, 1953). The minimum sample size was evaluated by the technique of progressive means. Axospinous synapses were divided into simple- and complex-shaped ones; the latter included both perforated and nonperforated synapses. No differences in synaptic N_V were found between the groups of young adult and aged rats that reached the criterion. N_V values were lower by 16% (n.s.) for simple-shaped synapses and by 33% ($p<.005$, two-tailed U test) for complex-shaped synapses in memory-impaired aged rats as compared to aged rats without memory deficits. The differential magnitude of changes in N_V of the two synaptic types is indicative of an absolute loss of complex-shaped synapses and cannot be explained by a change in volume. It appears, therefore, that a loss of complex-shaped axospinous synapses in DG may underlie age-related deficits in spatial memory.
Supported by Grant 1 R01 AG03410 from the N.I.A.

213.22 AGING AND A NOVEL NEUROTOXIN, MPTP(1-METHYL-4-PHENYL-1,2,5-6-TETRAHYDROPYRIDINE) ELICIT DEFICITS IN BRAIN DOPAMINE TRACTS AND INCREASES IN DUODENAL ULCERS. J.Z. Fields, Research Service (151), Hines IL 60141

This report describes a new animal model of duodenal ulcer disease that implicates deterioration in a brain dopamine (DA) tract as an etiologic factor. MPTP is a novel neurotoxin that appears to be specific for lesioning only the nigrostriatal (NS) DA tract and induces parkinsonism in man, primates and some rodent species. Although the extensive NS lesions (>80%) required for overt signs of parkinsonism have not yet been elicited in rats, large decreases in levels of nigral and striatal DA and DA metabolites have been observed. Recently, Szabo et al reported (Fed Proc 43 <1984> 945) that MPTP induces duodenal ulcers (DU) in rats. At 2 mg/100g, s.c., X3/day for 4 days, DU were consistently elicited in 80 to 100% of animals (Sprague Dawley females). There was diminished output of gastric acid and duodenal/pancreatic base, little or no mortality and no neurlogical signs. We repeated thse experiments and extended them to an evaluation of the effect of aging on MPTP-induced DU. We confirmed that MPTP (same schedule) can induce severe DU. In the first experimnt (age = 3 mos) we saw a fall in striatal DA (-15%) and DOPAC (-45%, $p<.01$). The diminished DOPAC/DA ratio ($p<.01$) suggests a decrease in DA turnover. In a 2nd experment, nigral DOPAC ($r=-.69, p<.0002$), nigral DA ($r=-.46$, $p<.01$) and striatal DOPAC ($r=-.47$, $p<.02$) decreased as the dose of MPTP (0 to 4 mg/100g) increased. In a third experiment (ages=1,2,3 mos) frequency and severity of MPTP-induced DU increased with age as has been reported for cysteamine-induced DU. Since there is a strong age-related correlation in man between decreases in NS DA and increases in the incidence of DU, both of which change linearly across a large portion of the human lifespan - ages 15 to 75 - these two correlates of human aging may be mechanistically related, presumably through neuroendocrine and/or autonomic changes. MPTP-inuced DU may be a useful model not only for the pathophysioloy of DU but also for the involvement of brain catecholamines in aging.

213.23 DECREASED CEREBRAL BLOOD FLOW AND IMPAIRED LEARNING IN RATS ASSOCIATED WITH AGING. Berman, R. F.[1], Goldman, H.[2], and Altman, H. J.[3] [1]Dept. Psychology, [2]Dept. Pharmacology, Wayne State University, Detroit, MI 48202 [3]Lafayette Clinic, Detroit, MI 48207.

The relationship between reduced cerebral blood flow and impaired learning in aged rats was examined. Male, Sprague-Dawley rats, aged 6, 12, and 24 months were housed either in standard cages or in an enriched environment, and then trained to traverse a 14 arm sequential T-maze (i.e., Stone maze) for food reward. An age-related progressive decline in maze learning was found, with 24 month old rats making significantly more errors and requiring significantly more trials to reach criterion then either 6 or 12 month old rats. After training, regional cerebral blood flow was measured in 14 brain regions, including pons-medulla, hypothalamus, basal ganglia, midbrain, hippocampus, occipital cortex, parietal cortex, frontal cortex, olfactory bulbs, septal region, inferior colliculi, superior colliculi, and amygdala-pyriform area. There was an overall mean decrease in cerebral blood flow of 19.7% in middle-aged rats (12 months) and 17.9% in old rats (24 months). A regional analysis indicated that the decrease was present in all of the brain regions examined at both 12 and 24 months of age. The range of decreases in cerebral blood flow was from approximately 7% to 27%. These data support the possibility of a relationship between impaired learning and reduced cerebrovascular functioning in aged animals. Furthermore, 12 and 24 month old animals exposed to an enriched environment for 2 months prior to training and cerebrovascular measurements, showed better learning (i.e., reduced errors and fewer trials to criterion) and higher regional cerebral blood flows then similar aged standard housed animals. Thus, environmental enrichment appears to minimize some of the effects of aging on both learning and cerebral circulation. These findings emphasize the importance of environmental conditions in behavioral and physiological studies conducted on aged animals.
(Supported by Grant AG03571 from the NIA)

213.24 ACCELERATED ENHANCEMENT OF SENSITIVITY TO ETHANOL IN AGING NEW ZEALAND BLACK (NZB/BlNJ) MICE. K.C. Retz, M.J. Forster, N.J. Ashford* and H. Lal. Dept. of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Relatively young NZB/BlNJ mice exhibit several characteristics of senescence, including deficiencies in learning and memory processes, elevations of brain reactive antibodies, and alterations in CNS-mediated drug effects (Nandy et al., Life Sci. 33:1499, 1983; Retz et al., Fed. Proc. 43:624, 1984; Harris et al., Soc. Neurosci. Abstr. 10:451, 1984; Nandy et al, Soc. Neurosci. Abstr. 10:721, 1984). We now report the effects of ethanol upon motor impairment of these mice. Male NZB/BlNJ and C57BL/6J mice 2-3, 4-6, or 10-14 months old (Jackson Labs; Bar Harbor, ME) were given ethanol 1.25, 1.88, 2.50 g/kg, i.p. prepared in saline vehicle (10 ml/kg). Fifteen min following EtOH the mice were placed on a 3.33 rpm constant velocity rotorod. The mice were permitted to remain on the rotating rod until they fell or 120 seconds had elapsed. The degree of motor impairment was estimated by the following equation: "% Motor Impairment" = [(120 sec. - latency on rotorod) x 100%] / 120 sec. All animals used in the studies were tested before receiving drug and found to have 0% motor impairment. A 3-way ANOVA on the data indicated that, overall, motor impairment varied as a function of: strain $F(1,30)=35.6, p<0.005$; age $F(2,30)=13.3, p<0.005$; and EtOH dose $F(2,60)=78.8$, $p<0.005$. However, the overall effect of strain was primarily a reflection of differential age-related changes in EtOH sensitivity among the strains occurring after 2-3 months of age. Whereas no strain difference in the effect of EtOH was apparent for 2-3-month-olds, the older groups of NZB mice were more sensitive than age-matched C57 mice following 1.25 and 1.88 g/kg EtOH. These age-related sensitivity changes were reflected in significant strain by dose $F(2,60) = 11.0, p<0.005$ and strain by age by dose $F(4,60)=2.6$, $p<0.05$ interactions. These data demonstrate an accelerated age-dependent enhancement of sensitivity to ethanol in NZB/BlNJ relative to C57BL/6 mice, a "reference" mouse strain widely employed in aging and EtOH studies. (Supported by USPHS grant AG03623 to H.L. and Texas College of Osteopathic Medicine grant 34144 to K.C.R.)

DEVELOPMENT AND PLASTICITY I: AGING

214.1 AGE-RELATED CHANGES IN SEROTONIN TURNOVER IN THE MEDIAN EMINENCE (ME) OF OVARIECTOMIZED ESTROGEN-TREATED RATS. I.R. Cohen-Becker and P.M. Wise. Dept. of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

Cyclic reproductive function is intimately timed to the photoperiod and thus is dependent upon the presence of normal circadian rhythmicity. Previous reports have demonstrated an age-related delay in the onset of cyclic release of LH in ovariectomized rats following the initiation of estrogen-treatment (Wise, P.M. Endocrinol., 115:801,1984). Recently, reports have indicated that changes in the normal circadian rhythm of serotonin can alter the timing and amplitude of LH surges (Walker, R.F. Neuroendo., 36:468, 1983). Therefore, we studied the effects of age on ME serotonin turnover rates in estrogen-treated, ovariectomized rats at various times during the day.

Studies were performed in young (3 mo) and middle-aged (8 mo) female Sprague-Dawley rats maintained on a 14 L: 10 D photoperiod with lights on at 0400h. All animals were ovariectomized and 7 days later received Silastic implants containing estradiol. These implants maintained physiological levels of estradiol in the circulation. Two days later animals were given either pargyline (75 mg/ kg body weight, ip) or were untreated at 0800, 1200, 1800 and 2400h. Controls were killed at 0800, 1200, 1800 and 2400h. Pargyline-treated animals were killed at 0810, 1210, 1810 and 2410h. Brains were removed, frozen and sliced at 300 microns. The ME was microdissected and analyzed for serotonin (5HT) and 5-hydroxy-indole-acetic acid (5HIAA) by HPLC. Both a comparison of the ratio of 5HIAA/5HT prior to and following pargyline treatment as well as 5HIAA/5HT ratios by themselves were examined as indicators of changes in serotonin turnover rates.

Age-related differences were found in the diurnal pattern of ME-5HT. Young animals exhibited low 5HT turnover in the morning (0800 and 1200h) compared to the afternoon (1800h) and night (2400h) when turnover was high. This diurnal pattern was apparent regardless of which of the two methods were used to determine turnover. In contrast, no diurnal change in 5HT turnover was seen in middle-aged rats. Turnover rates in these animals remained high at all times examined.

These data indicate that there is no deficit in serotonin turnover in middle-aged animals. Moreover, these animals failed to exhibit a diurnal pattern of serotonin turnover which may be necessary for normal cyclic release of LH. These data also demonstrate that the ratio of 5HIAA/5HT alone is as good an indicator of serotonin turnover rates as a comparison of ratios following pargyline treatment. (Supported in part by NIH AG-05351 to IC-B and NIH AG-00168 and AG-02224 to PMW).

214.2 PERGOLIDE SLOWS AGE-RELATED DETERIORATION OF THE DOPAMINERGIC NIGROSTRIATAL SYSTEM IN FISCHER 344 RATS. D. L. Felten, S. Y. Felten, T. Romano*, D. T. Wong, M. J. Schmidt, R. W. Fuller, and J. A. Clemens. Dept. of Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642, and The Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

Pergolide is a dopamine (DA) agonist with both presynaptic and postsynaptic action. We administered pergolide chronically in the diet (0.5 mg/kg/day) to male Fischer 344 rats to study its effects on nigrostriatal DA neurons. Four groups of rats were studied: (1) Rats fed pergolide from 3 mo. of age to 27 mo. of age; (2) Rats pair-fed from 3 mo. of age to 27 mo. of age, as pair-fed controls for pergolide administration; (3) Rats fed ad lib to 27 mo. of age; and (4) 3 mo. old untreated control rats. Fluorescence histochemical observations showed aging changes in substantia nigra (SN) at 27 mo. of age, including a marked decrease in fluorescent intensity in both perikarya and dendrites, increased accumulation of lipofuscin pigment in perikarya, and a reduced density of neurons in SN pars compacta (3 mo.controls--100% ± 12.6% (mean ± S.D., n=8); 27 mo. pair-fed controls--68.3% ± 14.0%, n=8, compared with 3 mo. old controls). Pergolide treatment enhanced the intensity of DA fluorescence in both perikarya and dendrites, led to increased density of neurons in SN pars compacta (27 mo. old pergolide treatment--85.1% ± 11.4%, n=8, compared 3 mo. old controls), but did not prevent lipofuscin accumulation in neurons. Rank order evaluation demonstrated significant differences in fluorescent intensity in both the SN pars compacta and striatum (3 mo. controls, 27 mo. pergolide, 27 mo. pair-fed, $p < .01$). Uptake of ^{3}H-DA into striatal synaptosomes was increased significantly (178%) in animals receiving pergolide compared with 27 mo. old pair-fed controls ($p < .05$). ^{3}H-Spiperone binding studies with striatal membranes in 21 month old rats fed pergolide for 18 months was no different than binding in pair-fed animals. These findings suggest that chronic dietary administration of pergolide to Fischer 344 rats slows the normal age-related deterioration of DA nigrostriatal neurons, and does not produce chronic down-regulation of DA receptors in striatum. Pergolide, therefore, may offer an important advantage in the treatment of Parkinson's disease by preserving the integrity of remaining DA nigrostriatal neurons.

214.3 BETA NOREPINEPHRINE RECEPTORS IN THE SENSORIMOTOR CORTEX OF THE FISHER 344 RAT: CHANGES AS A FUNCTION OF AGE AND CHRONIC CARDIOVASCULAR EXERCISE. R.E. Wilcox (1), M. Limb* (1), J.A. Severson (2), G. Cartee (3), W.W. Spirduso (1 and 3), J. Fineg*(1), and R.P. Farrar (1 and 3). (1) Dept. of Pharmacology, College of Pharmacy, Univ. of Texas, Austin TX 78712 (UT). (2) Med. Sch. Univ. of Southern California, Los Angeles, CA 90033. (3) Dept. of Health & Phys. Ed., UT 78712.

Age and exercise appear to produce opposite patterns of adaptation of dopamine (DA) receptors in the basal ganglia (P.E. Gilliam et al., Pharmacol. Biochem. Behav., 20, 863-867, 1984; P.E.Gilliam et al., Soc. Neurosci. Abstr., 11, 1985). We were interested in the response of beta norepinephrine (NE) receptors in sensorimotor cortex to age vs. exercise to determine the extent to which the reciprocal pattern shown for DA would apply to another central processing area in the control of movement.

Male Fisher 344 (F344) rats 4 or 18 mo. old were subdivided into untrained and endurance trained (treadmill) groups. All rats maintained their exercise program up to 48 hr before and were fasted 12 hr before sacrifice. At the conclusion of the 6 mo. training program half of the animals from each group were sacrificed immediately while the remaining subjects were given a 30 min treadmill test at 75% of the maximal oxygen consumption immediately prior to sacrifice. Saturability of 3H-dihydroalprenolol (DHA; 6 concentrations from 0.1 to 5nM) and NE displacement of 3H-DHA binding (20 NE concentrations from 1nM to 0.1 mM) in "sensorimotor" cortex (neocortex minus frontal and occipital pole regions) were determined in tissue from individual rats (n = 32) as described in O'Donnell et al. (J. Pharmacol. Exp. Ther., 228, 640-647, 1984). dl-Propranolol (10uM) was used as a blank. Initial analyses of the data by LIGAND (Munson and Rodbard, Anal. Biochem., 107, 220-239, 1980) were supplemented by analysis of variance (ANOVA) and Kruskal-Wallis ANOVA by ranks.

The major results were as follows. (1) NE binding in the cortex of 10 mo. old F344 rats was best described by a 2-site (high and low affinity agonist state) model. (2) By 24 mo. the binding had altered so that a 1-site (a single low affinity agonist state) model provided the best fit. (3) In contrast, neither the overall Kd nor Bmax of 3H-DHA binding were altered by age or exercise from 10 to 24 mo. (4) Chronic exercise increased the affinity of the low affinity agonist state (Kl) in young and old rats and decreased the affinity of the high affinity agonist state (Kh) in the young animals. (5) A 30 min exercise bout was able to alter the NE binding to the same extent in 10 and 24 mo. old animals regardless of training status.

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214.4 THE EFFECTS OF AGE ON REACTIVE CAPACITY AND NIGROSTRIATAL DOPAMINE FUNCTION IN SPRAGUE-DAWLEY RATS. P. Gilliam MacRae[a], R.E. Wilcox[b], W.W. Spirduso[c]. [a]Dept. of Sports Medicine, Pepperdine University, Malibu, CA 90265. [b]Dept. of Pharmacology and [c]Dept. of Physical and Health Education, University of Texas, Austin, TX 78712.

Twenty young, 3 month, and twenty old, 18 month, male Sprague-Dawley rats were shaped in a reactive capacity test to quickly release a lever, in response to auditory and visual stimuli, in order to avoid footshocks. The animals were tested for 50 trials a day for 7-14 days (Session 1). The animals that met the preset performance criterion level were tested again 3 months (Session 2) and 6 months (Session 3) later for consecutive 5 days. The animals were then sacrificed and their striata dissected for biochemical assays. [^{3}H]-Spiperone receptor binding assays were performed to determine the density and affinity of striatal D_2 receptors. Concentrations of dopamine and two of its major metabolites, DOPAC and HVA, were measured using high performance liquid chromatography with an electrochemical detector.

Significant age differences in reactive capacity indicated that the young (9 month) animals were more successful at the task and responded with faster latencies than the old (24 month) animals. Further analysis however indicated that the age differences in reactive capacity were significant only on the first two days of each testing session. The young animals demonstrated a greater retention of reactive capacity performance across sessions than the old animals. In this well-learned task, retention of performance across sessions, and not the capacity to react, was most affected by the aging process.

Receptor binding results showed that the young animals had a significantly greater number of dopamine D_2 receptors than the old animals. No significant age differences were found in receptor affinity (1/Kd) or concentrations of dopamine, DOPAC, or HVA. When the concentrations of dopamine, DOPAC, and HVA were expressed as a ratio of DOPAC+HVA/dopamine the old animals had a significantly higher ratio than the young animals.

Further support for the relatonship between reactive capacity and nigrostriatal dopamine function was found in that significant positive correlations were found between reactive capacity performance and receptor density (r=.67) as well as between reactive capacity and the ratio of DOPAC+HVA/DA (r=.47).

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214.5 Alterations of neurotransmitters with aging in coho salmon (Oncorhynchus kisutch, Walbaum). S.O.E. Ebbesson, J.E. Smith, G.T. Bazer*, R.P. Bailey*, and J.B. Reynolds*. Depts. of Anatomy and Psychiatry, Louisiana State Univer. Sch. of Med., Shreveport, LA 71130 and Dept. of Med. Sci. and Alaska Cooperative Fishery Unit, University of Alaska, Fairbanks 99701.

Whole brain content of several biogenic amines and amino acids was determined in two age groups of coho salmon using HPLC techniques. Brains of presmolt salmon (about 4-5 months old) were compared to brains of homeward migrants (about 4-5 years old). Levels of dopamine and serotonin were significantly higher in the homeward migrants than in presmolts, while glutamine and GABA were significantly lower in the older age group. Levels of other amino acids, such as aspartate, glutamate, serine, glycine and taurine were the same in the two age groups.

Biogenic Amine Content (^{pmoles}/mg protein)

	Presmolts n=5		Adult's n=6		
*Dopamine	4.8	(±1.5)	14.8	(±4.9)	***
*Serotonin	4.5	(±1.2)	15.2	(±3.9)	**

Amino Acid Content (^{nmoles}/mg protein)

GLN	61.46	(±7.64)	43.26	(±4.52)	**
*GABA	38.17	(±2.34)	28.18	(±3.87)	**

*Known neurotransmitter

Values are means ± standard deviations.
The significance of the differences determined with Student's t-tests were: ** p<0.001; *** p<0.01

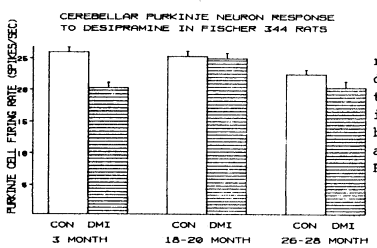
These findings point to selective changes in neurotransmitters with aging and perhaps with changes in behavior, but whether this relates to qualitative alterations in circuitry remains to be seen.

The brains were collected October 4, 1984, at the Crooked Creek Hatchery, Kenai Peninsula, Alaska.

214.6 **AGE-RELATED DECREASE IN CEREBELLAR PURKINJE NEURON RESPONSE TO DESIPRAMINE IN FISCHER 344 RATS** P.C. Bickford[1,2,3], K. Parfitt[1,*], B.J. Hoffer[1,2,], and R. Freedman[1,2,3,*]. [1]Departments of Pharmacology and [2]Psychiatry, University of Colorado Health Sciences Center, and [3]VAMC, Denver, CO 80262.

Depression is a significant problem in the elderly population and antidepressant drugs are frequently administered to alleviate the symptoms. Enhancement of noradrenergic (NE) neurotransmission is thought to be important in the mechanism of action of tricyclic antidepressants. In aged rats the effects of locally applied NE on neuronal discharge are markedly diminished. We therefore have investigated whether the tricyclic antidepressant desipramine (DMI) can augment neurotransmission in these animals. DMI (10 mg/kg/day) was administered for 3 weeks via Alzet minipumps implanted subcutaneously in young (3 month), and old (18-20 month or 26-28 month) Fischer 344 rats. Effects on noradrenergic neurotransmission were monitered by measuring cerebellar Purkinje neuron firing rate which is regulated primarily by NE (Hoffer et al, Brain Res. 30:425-430, 1971). Increases in NE neurotransmission slow Purkinje cell firing rates, while decreases result in increased firing rates.

No effect of DMI was observed with a single acute dose of 20 or 40 mg/kg i.p. in young rats. However, chronic DMI administration produces a decreased cerebellar Purkinje neuron discharge rate. This change was observed as early as three days of treatment and continued for up to 21 days of treatment. The effect of DMI on discharge rate was reversed by propranolol (5 mg/kg ip). DMI induced slowing could not be observed in animals pretreated with 6 hydroxydopamine. In contrast to young rats, at 18-20 or 24-26 months the decrease in cerebellar Purkinje neuron firing rates after treatment with DMI for 21 days was not observed (see figure below). There was a significant age-drug response interaction at p<0.05 using an analysis of variance. There was a slight elevation of DMI levels in cerebellar tissue of the older rats.



Taken together these results suggest a decreased efficacy of tricyclic antidepressants in aged rats. (Supported by USPHS grant AG04418 and the VA Medical Research Service.)

214.7 **AGE-RELATED ALTERATIONS IN MONOAMINE RELEASE FROM RAT STRIATUM: AN IN VIVO ELECTROCHEMICAL STUDY.** G. Rose[1,2], G. Gerhardt[1], G. Conboy[1*] and B. Hoffer[1]. [1]Dept. of Pharmacology, University of Colorado Health Sciences Center and [2]Medical Research, VAMC, Denver, CO 80262

Deficits in psychomotor performance are a consequence of normal aging. Current evidence suggests that one CNS substrate for this behavioral abnormality involves a decline in striatal dopaminergic transmission. While postsynaptic modifications of striatal dopaminergic connections during aging (e.g., reductions in striatal dopamine receptor density) have been well documented, presynaptic changes have been less well defined. Therefore, in the present study, in vivo electrochemistry was used to examine presynaptic alterations in dopamine release in the striatum of aged rats.

Chronoamperometric determinations of monoamine release, induced by local micro-pressure applications of K^+, were made using Nafion-coated graphite epoxy electrodes; these electrodes minimize any signal derived from ascorbate or urate, or acidic monoamine metabolites. Recordings were made from the striatum of urethane-anesthetized Fisher 344 rats at 6, 24, and >29 months of age. Following the in vivo electrochemical experiments, the animals were sacrificed and the caudate nucleus removed for analysis of whole tissue levels of monoamines and their metabolites using standard HPLC techniques.

Overall, mean amplitudes of K^+-evoked releases from the striatum of 6 mo. and 24 mo. F344 rats did not differ significantly (6.8 uM $\pm$ 0.38 (S.E.M.) vs 6.0 uM $\pm$ 0.36; $p > 0.1$). However, this result was complicated by the observation that the mean values obtained from two separate groups of 24 mo. animals, recorded 6 months apart, were significantly different from each other (group 1--8.0 uM $\pm$ 0.64; group 2--4.8 uM $\pm$ 0.34; $p < 0.001$). Mean releases for the latter 24 mo. group were significantly less than for the 6 mo. group ($p < 0.01$). No difference was found in the release magnitudes of 6 mo. animals recorded contemporaneously with the two groups of 24 mo. rats. Release amplitudes for the >29 mo. group (4.0 um $\pm$ 0.37) were clearly less than from the 6 mo. animals ($p < 0.001$). Both groups of 24 mo. rats, as well as the >29 mo. animals, also showed significant prolongation in the timecourses of the K^+-evoked releases when compared to the 6 mo. group ($t_{1/2}$ values in sec: 6 mo.--87 $\pm$ 7.1; 24 mo.--130 $\pm$ 15; >29 mo.--130 $\pm$ 11; 6 vs 24, $p < 0.02$; 6 vs >29, $p < 0.01$). Whole tissue levels of the monoamines and their principal metabolites did not differ between ages (striatal dopamine levels $\pm$ 8500 ng/gm for all groups; DA/DOPAC ratios $\pm$ 5.5:1). Thus, dynamic alterations in presynaptic monoamine function, which are not reflected in whole tissue levels, occur in rat striatum with aging. (Supported by USPHS grant AG04418 and the Veterans Administration Medical Research Service.)

214.8 REDUCTION IN SOMATOSTATIN CONTENT IN THE SCIATIC NERVE OF THE AGING FISHER RAT. S.F. Lewis, A. Brodish and D.B. MacLean (SPON: P. Smith), Dept. of Med. and Physiol., Wake Forest Univ. Med. Ctr., Winston-Salem, NC 27103.

Somatostatin (SS) and substance P (SP), two widely distributed neuropeptides, are synthesized in primary sensory neurons within sensory ganglia and transported bidirectionally, towards the CNS and peripherally towards sits of innervation. Substance P may mediate nociception and chemoreception at synapses within the CNS, while peripherally it partly mediates neurogenic inflammation and perhaps other related (e.g. immune) responses. Although the functions of sensory neuron SS are undefined, it may inhibit both the release and actions of SP. The roles of these two peptides in altered autonomic and somatosensory responses that have been described with aging are unknown.

Male Fisher rats, either young (4 months), middle aged (12 months) or old (25 months) were decapitated and the sciatic and vagus nerves rapidly removed. Nerves were cut into equal 3mm segments and subsequently extracted. Neuropeptide content was determined using specific RIA's. The content of neuropeptide (pg, mean $\pm$ SEM) and protein (ug), expressed per 3 mm nerve segment, are shown below:

		SP	SS	Protein	SP	SS	Protein
	(n)	Vagus			Sciatic		
Young	(22)	56±4	0.8±0.1[a]	23±2	78±4	16±1[b]	151±6[b]
Middle	(26)	60±5	1.3±0.2[a]	29±3[b,c]	74±4	11±1[b,c]	194±5[b]
Old	(24)	60±6	0.9±0.1	31±3[b,c]	77±4	9±1[b,c]	227±8[b]

[a] $P<.05$ vs other groups [b] $P<.01$ vs younger group(s)
[c] NS vs middle aged group

These findings demonstrate: 1) that there is an increase in vagal and sciatic protein content with age that is not matched by an equal increase in neuropeptide content; 2) that SS content in the sciatic nerve declines as a function of age, unlike the vagus nerve in which peptide content is maintained or increased; and 3) most of the age related changes occur relatively early with a declining rate of change thereafter. The differential effect of aging on SS vs SP content in the sciatic nerve is similar to our observations in the chronic diabetic rat (Diabetes, suppl. 1985, #406). Further studies are in progress to determine whether the decline in content reflects increased neuropeptide turnover within the nerve, decreased synthesis/transport, or both.

Studies were supported by grant AG-04207 from the NIA.

214.9 CHOLINE ACETYLTRANSFERASE IN HUMAN CEREBROSPINAL FLUID: FACT OR ARTIFACT? L.I. Szweda*, D.D. Bloom*, J.L. Dahl, M.E. Salinsky*†, and C.D. Johnson*‡ (SPON: T.A. Rudy). Departments of Pharmacology, Neurology†, and Zoology‡, University of Wisconsin-Madison, Madison, WI 53706.

The activity of the enzyme choline acetyltransferase (ChAT), a specific marker for cholinergic neurons, is much reduced in cortical biopsy material from patients with Alzheimer's disease. There have been numerous suggestions that the activity of this enzyme in the cerebrospinal fluid (CSF) might be a useful indicator of the degree of central cholinergic deficiency and of the effectiveness of potential treatment strategies. However, reports of ChAT levels in the CSF have provided conflicting results. We therefore examined assay procedures for specific measurement of the activity of the enzyme in the CSF.

CSF was obtained by lumbar puncture from ten patients, eight with no evidence of neurologic disease, two with a diagnosis of Alzheimer's dementia. We used the radiometric assay of Fonnum [J. Neurochem. 24, 407 (1975)] to measure ChAT activity. In this assay [^{3}H]acetylcholine (ACh) synthesized from [^{3}H]acetyl CoA is extracted into an organic phase as a complex with tetraphenylborate (TPB). We obtained evidence for the CSF-dependent formation of an extractable radiolabeled product. However, the formation of this product was not affected by the presence of acetylcholinesterase in the assay or by boiling the CSF prior to assay. It was further observed that its extraction into the organic phase did not require TPB. These results indicated that the radiolabeled product was not ACh, and indeed in subsequent experiments we observed that the reaction product did not comigrate with authentic ACh when subjected to high-voltage paper electrophoresis at pH 1.9 [J. Neurochem. 2, 231 (1971)]. Similar results were obtained when CSF samples were dialyzed, frozen, or concentrated prior to assay.

Thus we could find no evidence for either the enzymatic or non-enzymatic production of ACh by CSF. Neither did we find any evidence for ChAT inhibitory activity in the CSF as it did not inhibit the enzyme isolated from chick brain. Our results call attention to the dangers inherent in the use of radiometric assays for assessing ChAT activity unless careful attention is paid to possible artifactual production of extractable radiolabeled material. They further suggest that if there is ChAT in lumbar CSF, the levels are too low to be detected by a conventional radiometric assay. On the other hand, they do not rule out the presence of ChAT in CSF from intracranial sites. Supported by funds provided by the Research Committee of the Graduate School of the University of Wisconsin to JLD.

214.10 PROPERTIES OF SINGLE MOTOR UNITS IN THE MEDIAL GASTROCNEMIUS MUSCLE OF MIDDLE-AGED AND OLD RATS. K.Kanda*, K.Hashizume*, E.Nomoto*, and S.Asaki* (SPON: H.Washio). Dept. of Physiol., Tokyo Metropolitan Institute of Gerontology, Tokyo 173, Japan.

Age-related changes in the mammalian neuromuscular system have been studied using physiological, histological, and histochemical techniques. Interpretation of the data from whole-muscle studies is difficult, however, because most muscles consist of motor units with widely varying properties. Our study therefore was directed at individual motor units; we examined various properties of such units in middle-aged (11-14 months) and old (26-30 months) male Fischer rats anesthetized with urethane and chloralose. The distal portion of the medial gastrocnemius (MG) muscle was carefully dissected from the surrounding tissues. The tendon of the muscle was attached to a strain gauge through a small steel hook. The mechanical properties of the motor units were studied by stimulating single MG axons in the dissected ventral root filaments. After the physiological experiments, the MG muscle was excised for histochemical studies. The wet weight of the MG muscle in the old rats decreased by about 24% compared with that in the middle-aged rats (0.63 g vs. 0.83 g). Data of 132 motor units taken from 8 middle-aged rats and 93 units from 6 old rats were analyzed. It was possible to classify all the motor units into 4 types (FF, FI, FR and S) using similar criteria ("sag" property in unfused tetani and fatigability) to those previously applied to cat hindlimb muscles. The composition by type for middle-aged rats was FF (fatigue index < 0.5) and FI (0.5-0.75) units, 37.2%; FR (> 0.75), 48.5%; and S, 14.4%. The MG muscle in old rats exhibited a relatively high proportion of S units (28.4%) and type I muscle fibers, but fewer FR units (35.8%). The twitch contraction time after maximum post-tetanic potentiation was shortened in FF and FI units (15.1 $\pm$ 2.1 ms for old rats vs. 17.0 $\pm$ 2.0 ms for middle-aged rats, t test, $P < 0.001$), but not in FR (15.4 $\pm$ 2.7 ms vs. 15.3 $\pm$ 2.1 ms) and S (27.9 $\pm$ 4.5 ms vs. 28.2 $\pm$ 5.5 ms) units. Post-tetanic potentiation of FF, FI and FR units was significantly less in the old rats. There was a large increase in the tetanic tension for S units (3.0 $\pm$ 1.7 gwt vs. 1.6 $\pm$ 0.6 gwt, $P < 0.001$), but a slight decrease for FF and FI (14.7 $\pm$ 6.9 gwt vs. 17.9 $\pm$ 8.1 gwt, $P > 0.05$) and FR (6.7 $\pm$ 4.0 gwt vs. 9.5 $\pm$ 4.3 gwt, $p < 0.002$) units. Conduction velocity of axons slowed down in all unit types (50.9 $\pm$ 11.2 m/s vs. 62.9 $\pm$ 4.2 m/s, $P < 0.001$ for FF and FI; 44.1 $\pm$ 10.3 m/s vs. 66.3 $\pm$ 3.5 m/s, $P < 0.001$ for FR; 46.1 $\pm$ 8.2 m/s vs. 57.6 $\pm$ 5.4 m/s, $P < 0.001$ for S). These results suggest that motor unit reorganization takes place in the late stage of the animal's life. However, the underlying mechanisms of the changes found in this experiment must await further studies.

214.11 SPECIFICITY OF INTRAHIPPOCAMPAL GRAFT-INDUCED IMPROVEMENTS ON COGNITIVE PERFORMANCE IN AGED RATS. F.H. Gage and Anders Björklund. Department of Neurosciences, University of California, San Diego, and Department of Histology, University of Lund, Sweden.

We have recently demonstrated that, a subgroup of aged rats are severely impaired in spatial reference memory, using the Morris swim maze, and that this impairment is selectively and specifically correlated with decreases in 2-deoxyglucose utilization in the hippocampal formation and the prefrontal cortex (Gage et al., J. Neurosci. 1984). We have subsequently reported that grafting of cholinergic neurons to the hippocampus of these age-impaired rats will result in a significant post-graft amelioration of this cognitive deficit (Gage et al., Science 1984). In order to better understand the nature of the functional recovery observed, both in terms of the extent of the behavioral recovery, and the mechanism by which the grafts induce restoration of function, we have replicated and extended our previous results. First, we have modified the behavioral test situation, so that both spatial and non-spatial memory can be measured. Secondly, we have administered pharmacological probes with physostigmine and atropine to determine the specificity of the cholinergic involvement, both with the initial deficit and with the recovery induced by the graft. Our results reveal that aged rats that are impaired are impaired on both spatial and non-spatial reference memory. However, the majority of aged rats are not impaired in either spatial or non-spatial reference memory. Those aged animals that are impaired show a transient and small improvement in non-spatial reference memory with a single injection of physostigmine. Only a small performance decrement was observed following atropine injections in the young control animals, while those aged animals that normally showed no reference memory impairments, were significantly impaired only in spatial reference memory following injections of atropine. Suspension grafts of basal forebrain rich in cholinergic neurons once again resulted in amelioration of the deficits in the age-impaired animals. The improvement was observed in both spatial and non-spatial reference memory. Injections of atropine reversed the positive effects of the grafts on both the spatial and non-spatial components of the task. These results suggest that the grafts are having their functional effects at least partly through the cholinergic system.

214.12 FORMATION OF CHOLINERGIC SYNAPSES IN THE DENTATE GYRUS OF BEHAVIORALLY-IMPAIRED YOUNG AND AGED RATS BY GRAFTED BASAL FOREBRAIN NEURONS. D.J. Clarke, F.H. Gage, and A. Björklund. University Department of Pharmacology, Oxford, UK (DJC), Department of Neurosciences, UCSD, La Jolla, California (FHG) and Department of Histology, Lund, Sweden (AB).

Previous studies have indicated that fetal cholinergic basal forebrain neurons grafted into the hippocampal formation can ameliorate learning and memory impairments in either young rats with lesions of the septo-hippocampal pathway or aged rats with age-related behavioral deficits. This study investigated the ultrastructural characteristics of the graft/host cholinergic connections in young and aged rats with graft-induced recovery in spatial learning (as assessed in the Morris' water-maze), using choline acetyltransferase immunocytochemistry.

The results showed that grafted ChAT-immunoreactive neurons were capable of forming extensive synaptic contacts with neuronal targets in the dentate gyrus. In young control rats, the majority of cholinergic boutons are known to be synaptic onto dendritic shafts. In the young grafted animals with lesions of the septo-hippocampal pathway, the quantitative relationship between somatic and dendritic synapses of the graft-derived fibers was abnormal such that somatic contacts predominated. In the aged rats, after completion of the behavioral testing (3 months after grafting), the intrinsic cholinergic afferents were removed 1 week prior to tissue processing by a fimbria-fornix lesion. Choline acetyltransferase immunostaining revealed grafts rich in cholinergic perikarya and fiber outgrowth into the host hippocampal formation. Ultrastructurally, the graft-derived boutons were seen to have formed predominantly symmetrical synaptic contacts with neuronal elements in the dentate gyrus.

The results suggest, therefore, that the ability of cholinergic grafts to compensate for behavioral impairments in both young and aged rats may be mediated via direct synaptic actions on host hippocampal target neurons.

214.13 AN ANIMAL MODEL OF HUMAN-TYPE MEMORY LOSS IN AGING BASED ON AGING, DRUG, AND LESION STUDIES WITH THE RAT. J.M. Ordy, R.C. Griffith, Pennwalt Corporation, Rochester, NY 14623, G. Thomas, University of Rochester, Rochester, NY 14624, and W. Dunlap, Tulane University, New Orleans, LA 70118.

Loss of recent memory represents an inevitable manifestation of aging, particularly of Alzheimer's disease. These age and disease related memory impairments have been related to degeneration and/or loss of neurotransmitter-specific neurons in the basal forebrain, and the septo-hippocampal entorhinal circuit. Research strategies for the development of effective drug treatments for human type memory loss in animal models have focused on the essential criteria for measuring trial-specific working memory for correlation with neural changes in neurotransmitter-specific memory circuits produced by aging, drugs, and lesions. The goals of this research program were to develop a valid test of trial-specific working memory with concomitent measures of motivation and neuromuscular performance for the rat in a T-Maze. Specific aims were to examine the effects of: 1) age, 2) basal forebrain, septal, amygdala lesions, and 3) physostigmine, scopolamine, and piracetam on memory, motivation, and motor performance of young, middle aged, and old rats. Aging significantly impaired working memory, motivation, and motor performance. Memory of septally lesioned rats was significantly more impaired than that of basal forebrain, or amygdala lesioned rats. Physostigmine improved, whereas scopolamine impaired memory. Physostigmine also blocked scopolamine impairment of memory. The "nootropic" drug piracetam did not improve memory, nor block scopolamine impairment of memory. The experimental findings of this geriatric research program provide direct support for the emerging view that clarification of age, lesion, and drug effects on transmitter-specific neural memory circuits in animal models represents a very important step in the research strategies for the development of effective drug treatments for recent memory loss in senile dementia and Alzheimer's disease.

214.14 NEUROENDOCRINE AND GENETIC ASPECTS OF AGING IN PLATYFISH. M. P. Schreibman, L. Halpern-Sebold* and H. Margolis-Nunno*. Biology Department, Brooklyn College, Brooklyn, NY 11210.

Age-related changes in the distribution of immunoreactive (ir-) luteinizing hormone releasing hormone (LHRH), serotonin (5HT) and tyrosine hydroxylase (TH) and in the size of cells where LHRH is localized were studied in brains of 8 to 30 (average life span) mo. old platyfish (Xiphophorus maculatus) genetically "constructed" to become sexually mature at two ages (early maturing males (E), 18 wks.; late maturing males (L), 59 wks.). The intensity of ir-LHRH in nucleus olfactoretinalis (NOR) perikarya and fibers in E is similar at 8, 24, and 30 mos.; it increases somewhat at 12, and more at 18 mos. The area of NOR cells in E decreases between 8 and 24 mos. and then shows a minor increase at 30 mos. In L, ir-LHRH in the NOR is most intense at 8 mos. when L are beginning puberty (stage 2). At all other ages there is considerably less NOR ir-LHRH with no elevation at 18 mos. as in E. NOR cell body area in L is smaller than E at 8 and 18, but larger at 12 mos. Ir-LHRH in nucleus preopticus periventricularis (NPP) perikarya of E increases at 18, decreases at 24, and is lowest at 30 mos. Ir-LHRH in L remains constant between 12 and 30, and is lowest at 8 mos. In E, NPP cell body area is maximum at 12 and lowest at 18 and 30 mos. NPP cell areas in L are not elevated at 12 mos. Ir-LHRH in the nucleus lateralis tuberis (NLT) is greatest at 18 mos. in E. In L, although ir-NLT perikarya are never seen, pale ir-fibers are present in the NLT at 12, 24 and 30 mos. In E, NLT perikaryal area decreases between 8 and 30 mos.; in L, it increases between 8 and 12 mos. and then decreases.

In E, ir-5HT in the forebrain is found in the wall of the third ventricle and its paraventricular organ (PVO) and in the pineal gland. Ir-5HT first appears in cell bodies of the nucleus preopticus (NPO) at 18 mos. in E. In L at all ages, ir-5HT is paler than, but similar in distribution to, E; however, ir-5HT is never seen in NPO perikarya of L.

In 12 mo. old E, ir-TH is localized in the NOR, NPO, and PVO. In NOR perikarya, ir-TH is greatest at 12 mos. and absent at 8 and 30 mos.; in the NPO it is highest between 12 and 24 mos. and in the PVO it is at low levels between 8 and 24, and absent at 30 mos. In L, low levels of ir-TH are found in the PVO only between 8 and 24 mos. and no ir-TH perikarya are localized in the NOR or NPO at any age.

Our report suggests that distinct changes occur throughout life in the distribution and relative quantities of the neuropeptides and neurotransmitters regulating the reproductive system and that the profiles of these changes are not similar in fish which differ in genotype for the time of sexual maturation. (Supported by NIA (AGO-1938) and PSC-CUNY).

214.15 AN AGE-RELATED DECLINE IN HIND-PAW SHOCK INDUCED ANALGESIA. R. J. Hamm and J. S. Knisely*. Department of Psychology Virginia Commonwealth University, Richmond, VA 23284.

In order to examine the function of an endogenous, nonopioid system of pain inhibition during aging, rats (3,14, and 24 mo old) were shocked on their hind paws. Rats were exposed to 90 sec of scrambled electric shock delivered to their hind paws. Because the 14 mo old and the 24 mo old rats had approximately a 0.1 mA higher shock threshold than the 3 mo old rats, the 3 mo old rats were exposed to a 1.6 mA shock intensity to induce analgesia while the 14 and 24 mo old rats were exposed to a 1.7 mA shock. After shock termination, tail-flick latencies were measured at 0,1,2,4,6,8,10,12 and 14 min.

To investigate the pharmacology and anatomy involved in the production of hind-paw shock induced analgesia, the effects of naltrexone (7 mg/kg), scopolamine (5 mg/kg), saline (1 ml/kg), and adrenalectomy were examined across age groups. A 3(Age) x 9(Time) analysis of variance of the tail-flick latencies following saline treatment revealed that there was an age-related reduction in the degree of analgesia produced by hind-paw shock ($p < 0.0001$). An analysis comparing naltrexone-treated animals and saline-treated animals demonstrated that naltrexone did not attenuate the analgesia observed following hind-paw shock in any age group. A similar analysis of the analgesia observed following adrenalectomy also found that adrenalectomy did not reduce hind-paw shock induced analgesia in any age group. An analysis of analgesia following saline and scopolamine treatment revealed that scopolamine significantly attenuated the analgesia produced by hind-paw shock ($p < 0.001$). In addition a measure of the effectiveness of scopolamine's blockade of analgesia revealed that there was an age-related decline in the degree to which scopolamine reduced hind-paw analgesia.

These results confirm that hind-paw shock activates a neurally mediated, nonopioid analgesic system that involves cholinergic sites. The results also demonstrate that as age increased there was a progressive reduction in the degree of analgesia induced by hind-paw shock. The age-associated changes in the effectiveness of scopolamine suggest that the decline in hind-paw shock induced analgesia is the result of an alteration in the function of the cholinergic system during aging.

214.16 OXOTREMORINE-INDUCED ANALGESIA IN MATURE AND SENESCENT RATS. J. S. Knisely* and R. J. Hamm (Spon: D. J. Mokler). Dept. of Psychology, Virginia Commonwealth University, Richmond, Virginia 23284.

In order to investigate the role of the central muscarinic cholinergic system in the production of analgesia during aging, rats (3-mo, 10-mo and 23-mo-old) were subcutaneously injected with oxotremorine (0.025, 0.05 or 0.10 mg/kg) in conjunction with 0.19 mg/kg scopolamine methylbromide (dose equimolar to 0.10 mg/kg oxotremorine). Before drug administration, baseline pain thresholds were determined using 3 tail-flick trials (1 minute ITI). Following the injections, tail-flick latencies were measured at 5 minute intervals through 30 minutes and at 45, 60, 75 and 90 minutes.

Post-drug TF latencies were converted to percent maximum possible effect (% MPE) and were analyzed by a 3(Age) x 3(Dose) x 11(Time) analysis of variance. The analysis revealed no age-related differences in oxotremorine-induced analgesia. However, there were main effects of Drug ($p < 0.001$) and Time ($p < 0.0001$) and a significant Drug x Time interaction ($p < 0.0001$). Thus increasing the dose of oxotremorine enhanced analgesia and the analgesia displayed, varied across time.

To more closely examine the dose-related analgesic effects of oxotremorine, a least-squares linear regression line was calculated for each age-group independently using % MPE scores at the peak-effect time (usually 15 to 25 minutes) for each dose of the muscarinic agonist. From this analysis, the correlation coefficient, slope and y-intercept of each regression line were determined and age groups compared. For the regression analysis, doses were converted to μg/kg. It was found that oxotremorine (plus methylscopolamine) produced analgesia in a dose-related fashion: correlation coefficients were 0.65, 0.77 and 0.59 for 3-mo-old, 10-mo-old and 23-mo-old rats, respectively. This analysis also revealed that 10-mo-old animals were slightly more sensitive to oxotremorine-induced analgesia than the other 2 age groups tested.

These results demonstrate a lack of age-related differences in analgesia produced by the stimulation of central muscarinic cholinergic receptors. This finding is supported by other research which has revealed that aged animals show an equivalent or increased pharmacological responsivity to exogenous cholinomimetics.

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214.17 AGE-RELATED DECREASE IN THE CATECHOLAMINERGIC INNERVATION OF THE MOUSE SPLEEN. A. Capocelli*, D.L. Bellinger, D.L. Felten and P.D. Coleman. Department of Anatomy, University of Rochester, Rochester, NY 14642.

Age-related alterations in immune function have been described in a number of mammals, including human and rodent. We are examining whether these alterations in immune function in mice may be related to possible changes in the catecholaminergic innervation of the organs of the immune system as a function of age. Male C57Bl/6 mice were obtained from the NIA colony at Charles River Breeding Laboratories and sacrificed at 6 ages from 7 to 38 months. Usually there were three mice at each age, with the exception of the oldest ages. The spleen, thymus, lymph nodes and brain were removed quickly, weighed, frozen on dry ice and placed in liquid nitrogen. Frozen sections of spleen were subsequently cut at 10 um and processed for induced fluorescence by the sucrose-phosphate-glyoxylic acid (SPG) method of de la Torre (1983). The sections were examined in a Nikon Biophot fluorescence microscope using epi-illumination. All microscope fields containing observable fluorescent fibers were photographed at 160x. Blind, qualitative examination of complete sets of micrographs permitted us to distinguish abundant innervation, moderate innervation, or sparse innervation of the spleen. These judgments in C57Bl/6 mice correlated with age. Younger animals showed abundant plexuses of varicosities surrounding both large and small vessels of the central arterial system in the white pulp, linear and punctate strands of varicosities in the parenchyma of the white pulp, and arrays of fibers associated with the splenic capsule and trabeculae. Age-related changes in older mice included progressive diminution of the size and fluorescent intensity of plexuses associated with blood vessels, diminished fluorescent parenchymal profiles in white pulp, and reduced presence of arrays of varicosities associated with the splenic capsule and trabeculae. In addition, the number of intensely autofluorescent cells in the splenic white pulp increased with age. Quantitative evaluation of fluorescent varicosities is under way. We tentatively conclude that catecholaminergic innervation of the spleen decreases with increasing age in the C57Bl/6 mouse.

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214.18 Mechanism of Nerve Resistance to Ischemic Conduction Failure with Increasing Age

J. D. Schmelzer*, K. K. Ward* and P. A. Low

Human peripheral nerve is more resistant to ischemia-anoxia with increasing age but the mechanism of this resistance to ischemic conduction block (RICB) is not known. We found that peripheral nerve of the Sprague Dawley rat also had increasing RICB with age and related RICB to oxygen consumption (VO_2), nerve ATP, creatine phosphate (CP) and lactate at rest and after anoxic stress (table; n>6).

Parameters	100 g	300 g	500 g
Time to 50% conduction block	8.1±0.5[a]	11.9±1.1[b]	25.9±1.8[c]
O_2 consumption (VO_2)(ml/kg/hr)	800.6±29.9	609.1±17.7[c]	386.2±17.1[c]
nerve ATP(nmol/mg)-at rest	1.07±0.07	0.69±0.05[c]	0.45±0.02[c]
- 15 min. anoxia (% of resting)	24.5±2.1	51.0±2.6[c]	70.5±2.8[c]
nerve CP(nmol/mg)-at rest	2.27±0.09	1.74±0.04[c]	1.32±0.05[c]
- 15 min. anoxia (% of resting)	4.7±1.0	13.9±1.4[c]	32.5±1.2[c]
nerve lactate(nmol/mg)-at rest	1.85±0.14	1.49±0.11[c]	0.93±0.06[c]
- 15 min anoxia (% of resting)	447.5±12.5	562.8±25.1[c]	588.6±20.5[c]

a = SEM
b = $0.001 < P < 0.01$
c = $P < 0.001$

The ages for 100 g., 300 g. and 500 g. rats were 30.3±0.6, 66.5±0.9 and 232.5±6.2 days respectively. Rat peripheral nerve underwent progressive RICB with age. Time to 50% block of conduction was increased by 47% and 220% for 300 g. and 500 g. rats respectively, as compared to the 100 g. rats. The major mechanism of RICB is a progressive reduction in energy requirements as indicated by a progressive reduction in O_2 consumption and in ATP and CP utilization of sciatic nerve with increasing age. An additional mechanism appears to be a slight increase in anaerobic metabolism as indicated by a greater percent increase in endoneurial lactate under anoxic stress.

214.19 EXPERIMENTAL ISCHEMIA IN AGED RATS: EFFECT ON BRAIN MICROVESSEL ß-ADRENERGIC RECEPTORS (ß-R). M.S.Magnoni*, S.Govoni and M.Trabucchi. Inst. of Pharmacol. and Pharmacognosy, Univ. of Milan and Chair of Toxicology, 2nd Univ. of Rome, Italy.

Right carotid occlusion (RCO) induces in the rat a more pronounced and persistent decrease in the density of ß-R located on cerebral capillaries of the controlateral hemisphere. On the other hand, the occlusion of the left carotid induces a greater ipsilateral decrease in ß-R number, suggesting that the left side of the brain is more sensitive to the ischemic insult, independently of the side of carotid occlusion. Corpus callosum section partially prevents the controlateral neurochemical changes induced by RCO, suggesting that the effects of ischemia in brain areas distant from the insult are mediated by transneuronal mechanisms. Thus, the different response of microvasculature of the two hemispheres to ischemia may be due to asymmetries in neuronal pathways controlling cerebral microcirculation. Along this line, the effect of ischemia on microvessel ß-R function was studied during aging, a condition associated with altered patterns of both neuronal activity and vascular regulation. Aged S.D. rats (24 months) underwent 48 hrs RCO and ß-R were measured in brain microvessels using the specific radioligand ^{125}I-iodocyanopindolol. The results indicate that aged rats show a reduced density of capillary ß-R. In addition the RCO does not induce a decrease of ß-R density in the controlateral hemisphere of old rats. The altered pattern of neuronal regulation of brain capillaries which occur during aging may lead to the reduced basal level of ß-R and to the loss of the asymmetric response of cerebral microvasculature to the ischemic insult. The qualitative difference in the brain microvascular reactivity to ischemia during aging may be of potential interest for the study of the acute cerebrovascular disease in the elderly.

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214.20 THE INTERACTION OF AGING AND ENDURANCE EXERCISE UPON SKELETAL MUSCLE OF PREDOMINANTLY DIFFERENT FIBER TYPE. R.P. Farrar[1,2], T.J. Walters[1], G. Cartee[1], and H.L. Sweeney[1]. [1]Dept. of Physical Education and [2]Division of Pharmacology, Univ. Texas at Austin.

Does aging induce an intrinsic decline in physiological function or are the declines observed as a result of decreased activity? This experiment was designed to control for the amount of exercise young and old rats were engaged in, and measure changes in aerobic capacity and myosin isozyme patterns in a predominancly slow twitch muscle, soleus (SOL), and a predominantly fast twitch muscle, extensor digitorum longus (EDL). Male, F344 rats were divided into 4 groups; young sedentary (YS), young trained (YT), old sedentary (OS), old trained (OT). YT and OT initiated training at 4 and 18 mo of age, respectively. Both groups trained at the same absolute workload throughout the study running 5 d/wk, up to 60 min/d, and up to 20 m/min. At the end of 6 mo of training both trained and age-matched sedentary groups were sacrificed. Training produced significant increases in oxidative enzyme activity over their age matched controls, and this increased activity was not significantly different between YT and OT. The EDL on the other hand showed no significant changes in any oxidative enzyme marker due to age or training. Electrophoresis of the myosin light chain patterns demonstrated no changes in the SOL, but a shift in the light chain patterns due to aging in the EDL. The young groups contained higher LC_3 content than the old groups. The OT showed higher LC_3 content than OS and this would infer a higher percentage of IIB fibers being maintained by training in the OT group, but still less than the young groups, trained or sedentary.

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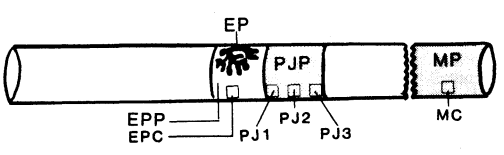
214.21 METABOLISM OF ACETYLCHOLINE AT THE NEUROMUSCULAR JUNCTION OF MATURE ADULT AND AGED RATS. M. H. Weiler and D. O. Smith. School of Pharmacy and Department of Physiology, Univ. of Wisconsin, Madison, WI 53706.

In rat diaphragm neuromuscular junctions, acetylcholine (ACh) levels and quantal size decline but evoked release remains constant during aging. This is associated with increased leakage of ACh from the cytoplasmic pool in aged animals. The causes of declining ACh levels are further examined in this study of ACh synthesis and release. Hemidiaphragms from rats aged 10 (mature adult controls) and 28 (aged) mos were dissected and preincubated in circulating oxygenated saline (37°C) containing physostigmine (50 uM). Endogenous and labeled ACh and choline (Ch) were then monitored using gas chromatography-mass spectrometry at various times following addition of $^2H_4$-Ch (10 uM) to the medium. In the older rats, endogenous Ch levels and efflux were consistently higher; moreover, incorporation of $^2H_4$-Ch was also greater. This incorporation must involve diffusion pathways, for uptake (V_{max}) by the high-affinity pathway has been shown to decrease with age. During stimulation, the synthesis of $^2H_4$-ACh equilibrated more rapidly in the 28-month animals, resulting in a 2-fold higher specific activity of labeled ACh within 8 min; in separate experiments, we found choline acetyltransferase activities unchanged with age. After 20 min, however, similar steady-state specific activities were reached. (Synthesis in noninnervated tissue was negligible). Thus, synthesis appears to occur at a more rapid rate in the aged rats. At rest and during stimulation, though, release of total ACh (endogenous plus tracer) was greater in the older animals. This was due to greater leakage of endogenous ACh during aging, because the specific activity of the released ACh was less in the aged group. Thus, ACh is synthesized more rapidly in the older preparations, but the newly synthesized ACh is not released as readily. We conclude that reduced ACh levels during aging are due primarily to increased leakage of the transmitter. Since this cannot be due to a larger concentration gradient in the older animals, it may be attributed to an age-related increase in membrane permeability to ACh. Supported by PHS grants AG01572 and MH17691 and a starter grant from the Pharmaceutical Manufacturers Association Foundation.

214.22 MICROLOCALIZATION OF ACH RECEPTORS IN YOUNG AND OLD C57 MOUSE MUSCLE. N.A.Anis* and N. Robbins, Dept. of Dev. Genetics & Anatomy, Case Western Res. Schl. of Med., Cleveland, OH 44106.

In limb muscles of aged C57 mice, min. end-plate potential (mepp) amplitude increases but there is no change in input resistance (Anis & Robbins, Neurosci. Abst. 10, ,1984). An accurate measure of AChR per endplate is necessary to elucidate this result, but the usual method of quantitating junctional AChR involves error due to the subtraction of the perijunctional component. Also, perijunctional AChR itself is of interest because it changes with disuse and denervation, and may relate to increased sprouting with age. Therefore, we carried out microdissection of individual muscle fibres combined with I-125 alpha-bungarotoxin (*BTX) labelling to assess junctional and perijunctional AChR in young (8-12 mo) and old (24-25 mo) C57 mice.

After incubation in and washout of *BTX, soleus muscles were teased into individual fibers, which were each cut in 3 pieces (see Fig.). Gamma counting, measurement of surface area, cholinesterase staina, and autoradiography were used to compute results. In both young and old mice, EPC and PJ1 grain counts were similar but PJ2 and PJ3 were less, so that a modified method of subtraction of gamma counts (EPP minus PJP) could be used to find true endplate AChR densities (sites per sq. micra). These were 7,975 +/-1235 S.E. and 7,301 +/-992 at old and young endplates respectively (total counts per endplate were also similar). The corresponding perijunctional values were 201 +/- 51 and 313 +/-97.



Thus, endplate AChR density does not change with age, and increased mepp amplitude must be due to other factors. Perijunctional AChR in old mice shows no changes characteristic of disuse or denervation.

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214.23 TRANSMITTER RELEASE AND ULTRASTRUCTURE OF YOUNG AND OLD MOUSE NEUROMUSCULAR JUNCTIONS IN LOW CALCIUM SOLUTIONS. M.A.Fahim and N.Robbins, Gerontology Research Center, University of So. California, Los Angeles, CA 90089 and Department of Developmental Genetics & Anatomy, Case Western Reserve School of Medicine, Cleveland, OH 44106.

Transmitter release increases at hindlimb neuromuscular junctions (nmj's) of aged CBF-1 mice, (Banker, Kelly & Robbins, J. Physiol., 339: 355, 1983) but the mechanism is unknown. Therefore, we studied the relation between external Ca and quantal content (mean EPP/mean mepp) in soleus muscles of young mature (7 mo.) and old (29 mo.) mice. In addition, we examined synaptic vesicle density in junctions fixed in zero Calcium solutions to minimize offects of fixation-induced release.

At Ca concentrations of 0.3 to 1.0 mM (with 2.75 mM Mg), quantal content was greater at nmj's of old mice, and a plot of quantal content vs. log Ca was similar but shifted to the left in old mice. This finding was consistent with a quantitative rather than a qualitative difference in transmitter release with age. In order to determine if this difference was related to the number of vesicles ready for release (i.e. within 200 nm of the nerve terminal membrane), morphometry was carried out on electron micrographs of 15-18 nmj's per age group, in muscles fixed either in 0 or 2.5 mM Ca. In 2.5 mM Ca, vesicle density was reduced in old muscles, as previously reported for the whole terminal (Fahim & Robbins, J. Neurocytol., 11: 641,1982). However, in muscles fixed in 0 Ca., there was no age difference in synaptic vesicle density (135 +/- 10 vs. 129 +/- 13 vesicles per unit area in young and old nmj, respectively).

We conclude that greater vesicle availability is not the source of increased transmitter release at the old soleus nmj. Rather, the previously reported age-related decrease in vesicle density in conventionally fixed muscles may reflect increased transmitter release of old nmj's during fixation. Since soleus muscles normally fire continuously in vivo, it is moot whether the conventionally fixed or the low-Ca-fixed nmj approximates the true in vivo situation.

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214.24 CALCIUM ENTRY, UTILIZATION AND CLEARANCE AT THE NEUROMUSCULAR JUNCTION OF AGED RATS. D. Blumberg, J.L. Rosenheimer, and D.O. Smith. Department of Physiology, University of Wisconsin, Madison, WI. 53706.

Confluent evidence from diverse preparations indicates that Ca^{2+} regulation in nerve cells may be less effective in aged animals. These possibilities were tested in the phrenic nerve of rats aged 10 and 28 mos.

Release requirements for calcium were indirectly assessed by measuring the magnitude of the postsynaptic response while varying extracellular [Ca^{2+}]. Double logarithmic plots of these data had slopes of 2.7 and 2.9 in the 10- and the 28-mos animals, respectively, indicating similar cooperativity in the action of Ca^{2+} in the release process of both age groups.

The frequency histogram of synaptic delays was used to determine the time course of transmitter secretion and, therefore, the kinetics of Ca^{2+} entry near the active zone. Synaptic delays were recorded at single end plates using focal extracellular recording techniques. The histogram of delays in the aged rats was shifted to significantly longer values; the average values were 0.28 and 0.40 ms in the 10- and the 28-mos animals, respectively.

The extent of synaptic facilitation provides an indirect measure of intracellular Ca^{2+} levels in the immediate vicinity of the transmitter release sites. To determine whether clearance of this residual Ca^{2+} is affected during aging, paired pulses were delivered to the phrenic nerve in Mg^{2+}-blocked preparations, and the magnitude of the synaptic facilitation was measured. The decay in facilitation as the inter-pulse interval became longer was well described by an exponential expression, and the time constants in 10- and 28-mos rats were 36 and 39 ms, respectively. Thus, Ca^{2+} clearance at the release site is apparently not affected during aging.

Indirect evidence obtained in a separate study further argued against major changes in intracellular free Ca^{2+} levels in this preparation. Neurofilament triplet proteins are readily degraded by a Ca^{2+}-dependent protease. The presence of these individual neurofilament subunits in the phrenic nerve was compared using SDS gel electrophoresis and immunoblot techniques. No quantitative age-related changes were seen.

Collectively, these results suggest that there are no significant age-related changes in intracellular free Ca^{2+} levels in peripheral axons. Longer synaptic delays, though, may indicate altered Ca^{2+} channel properties in the older animals. Supported by NIA grant AG01572.

SENSORY SYSTEMS: AUDITORY PATHWAYS III

215.1 FUNCTIONAL ORGANIZATION OF THE MUSTACHED BAT INFERIOR COLLICULUS: REPRESENTATION OF FM_2 FREQUENCIES CARRYING TARGET RANGE INFORMATION. W.E. O'Neill and R.D. Frisina, Center for Brain Research, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

The mustached bat, Pteronotus parnellii, emits multiharmonic, ultrasonic signals consisting of a long constant frequency (CF) terminated by a short frequency modulation (FM). Although it follows the basic mammalian plan, parts of the auditory system are specialized to process these two components of the biosonar signal. Target range information, coded by the time interval between FM components of the emitted pulse and echo, is processed in two cortical fields (FM-FM and DF). Neurons in these fields respond only to pairs of FM signals with specific time delays, and are thereby range-tuned. Target distance is represented by the locus of activity in these regions of cortex (O'Neill W.E. & Suga N., J. Neurosci. 2, 1982). To define subcortical pathways providing inputs to these cortical regions, we have studied the representation of FM frequencies of the second harmonic of the biosonar signal (FM_2:48-60 kHz) in the central nucleus of inferior colliculus (ICC). Unlike cortical units, units in ICC are not facilitated by pairs of FM signals (O'Neill W.E., J. Comp. Physiol., in press).

In the present study, we recorded from 884 neurons in 11 mustached bats. The locations of these units were reconstructed from stereotactic coordinates in each ICC studied. In the last four bats after detailed mapping of the ICC, we made focal iontophoretic injections of HRP to localize the physiologic maps and to trace the afferent and efferent connections of the FM_2 projection (companion abstract). Our results show that FM_2 frequencies are at the caudal border of the anterolateral division confined to a large slab which stretches across almost the entire width of the ICC. The slab measures about 1.4 mm dorsoventrally, 1.3 mm mediolaterally, and 0.6 mm rostrocaudally. The large region representing the reference frequency (61-62 kHz) lies just dorsocaudal to the FM_2 representation, while frequencies above 62 kHz are ventromedial. These findings are consistent with a previous study (Zook et al., J. Comp. Neurol. 231, 1985).

The injections of HRP made in the middle of the FM_2 region, in DAB sections, ranged from 200 to 750 μm in the a-p direction, and 300-950 μm in the other planes. The deposits did not exceed the physiologically-defined representation of FM_2 frequencies and clearly spared the cytoarchitectonic boundaries of the doroposterior and ventromedial divisions of ICC. Our reconstructions indicate that the cytoarchitectonic borders between these divisions correspond to the physiological borders between the FM_2, reference frequency, and high frequency representations. These borders thereby divide the ICC into functionally distinct pathways processing different aspects of sonar information reaching the bat's ears. Supported by NSF grant BNS 8311627 to WEO and NIH-NRSA NS07343 to RDF.

215.2 FUNCTIONAL ORGANIZATION OF MUSTACHED BAT INFERIOR COLLICULUS: CONNECTIONS OF THE FM_2 REGION. R.D. Frisina and W.E. O'Neill. Center for Brain Research, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

The FM components of the multi-harmonic, CF-FM biosonar signal used by the mustached bat for echolocation are important for encoding target-range information. We have mapped in detail the representation of the second harmonic component (FM_2) in the central nucleus of the inferior colliculus (ICC) (companion abstract). Here we report the afferent and efferent connections of this region as visualized by anterograde and retrograde transport of focally-injected HRP.

In TMB sections, regions contained darkly-stained Golgi-like cell bodies clearly organized into a laminar arrangement: Ipsilateral to the injection site - dorsal nucleus of lateral lemniscus (DNLL), intermediate nucleus of lateral lemniscus (INLL), dorsal and ventral divisions of ventral nucleus of lateral lemniscus (VNLL), lateral superior olive (LSO) and medial superior olive (MSO); Contralateral - DNLL, LSO, fusiform-cell layer of dorsal cochlear nucleus (DCN) and anterior and marginal divisions of anteroventral cochlear nucleus (AVCN). This laminar arrangement of cell bodies suggests that these nuclei are tonotopically organized. Other regions contained darkly-labeled cell bodies that were less clearly organized: Ipsilateral - all periolivary nuclei and ventral and lateral nuclei of trapezoid body; Contralateral - ventromedial periolivary nucleus (VMPO) and medial division of posteroventral cochlear nucleus (PVCN). Lightly-labeled cell bodies were found contralaterally in MSO, deep layers of DCN, posterior division of AVCN, and lateral division of PVCN.

In contrast to the inputs to the FM_2 region, its outputs are relatively uncomplicated. Heavily-labeled terminal ramifications were found ipsilaterally in an anteromedial region of the medial geniculate body of thalamus (MGB), dorsolateral MGB, posteromedial MGB, lateral pontine nuclei and deep layers of superior colliculus. Some darkly-labeled cell bodies and terminal ramifications were observed in contralateral ICC in a region roughly corresponding to the effective area of the injection site, and outside the effective area of the injection site in ipsilateral ICC. These findings suggest that some intracollicular processing takes place.

No labeling was observed in auditory cortex, cerebellum, nucleus of the brachium of inferior colliculus; contralaterally in MGB, INLL, VNLL, periolivary nuclei (except VMPO), nuclei of trapezoid body, and octopus cell region of PVCN; and ipsilaterally in medial nucleus of trapezoid body and cochlear nucleus. In sum, by confining our HRP injections within physiologically-defined borders of the FM_2 representation in ICC, we have identified pathways from cochlear nucleus to MGB which are critical for range information processing in this species. Supported by NSF grant BNS8311627 to WEO and NIH-NRSA NS07343 to RDF.

215.3 ANATOMICAL PROJECTIONS TO A SINGLE ISOFREQUENCY REGION: BASIS FOR AN ORGANIZATION OF BINAURAL RESPONSE PROPERTIES IN THE MUSTACHE BAT. L.S. Ross*, J.J. Wenstrup, and G.D. Pollak (SPON: L. Edds). Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Like other mammals, the mustache bat's inferior colliculus displays a tonotopic organization of frequencies, but there is an enlarged isofrequency region in which the 60 kHz component of its echolocation call is processed. This enlarged isofrequency region corresponds to the anatomically defined dorsoposterior division (DPD) of the central nucleus of the inferior colliculus (Zook et al., J. Comp. Neurol., 231:530,1985). Within the DPD, neurons having similar binaural response properties are found clustered together, and each binaural class occupies a specific region of the DPD. The central nucleus receives ascending projections from at least seven brainstem auditory nuclei, and the present study was undertaken to determine which brainstem nuclei project to the isofrequency region.

We began by placing large deposits of HRP (500 micron diameter) within the boundaries of the DPD as defined physiologically. These experiments have shown that the DPD receives a complete set of afferents from the lower brainstem auditory nuclei; that is, all of the nuclei projecting to the central nucleus also send projections to the DPD. The major projections to the DPD include contralateral projections from the cochlear nucleus, ipsilateral projections from the medial superior olive and intermediate and ventral nuclei of the lateral lemniscus, and bilateral projections from the lateral superior olive and dorsal nucleus of the lateral lemniscus. In addition, these projections appear to originate in the appropriate 6o kHz isofrequency regions of the brainstem nuclei. Thus, an isofrequency region receives a full set of afferents from the corresponding isofrequency regions of the brainstem nuclei.

Previous experiments have shown that these projections are not uniformly distributed within the central nucleus, suggesting that the organization of binaural regions occurs as a result of segregation of afferents. To investigate this possibility, small deposits of HRP (200 micron diameter) were placed in physiologically defined binaural regions within the DPD. The arrangement of binaural regions within the DPD is generally consistent with the observation of anatomical projections to the DPD. For example, deposits in regions of monaural EO cells results in the labeling of cells in monaural nuclei such as the cochlear nucleus and intermediate and ventral nuclei of the lateral lemniscus while the binaural brainstem nuclei tend to project to more binaurally dominated portions of the DPD.

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215.4 PREDICTING AN AUDITORY NEURON'S SOUND LOCATION SELECTIVITY. Z.M. FUZESSERY and J.J. WENSTRUP. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

A sound originating at a particular point in space generates a characteristic set of monaural and binaural spatial cues. A neuron's selectivity for that sound location is dictated by its selectivity for these spatial cues. Many auditory neurons exhibit complex monaural and binaural response properties. They may be excited by monaural input to one or both ears, and their responses facilitated or suppressed when these inputs are combined. Such neurons may be facilitated at certain interaural timing and/or intensity difference values, and suppressed at others. These influences may also vary with absolute stimulus intensity. To understand the mechanisms underlying the representation of auditory space, it is necessary to dissect out the influences exerted by a neuron's monaural and binaural response properties.

The present study examines the determinants of the spatial selectivity of the various binaurally excited neurons (E-O/F, E-O/F,I, E-E/O, E-E,F and O-O/F types) in the inferior colliculus of the mustache bat, and delineates a method for predicting their sound location selectivity from their monaural and/or binaural response properties. First, the directional properties of the external ears were determined to construct a matrix of the intensity levels reaching the cochlea from various points in the sound field, as well as the interaural intensity disparities generated throughout the sound field. Second, using dichotic stimulation, the response magnitudes of inferior colliculus neurons were documented over a relevant range of interaural intensity disparities and absolute stimulus intensity levels. From these data, the neuron's response magnitude at any point on the spatial matrix could be determined, allowing a prediction of the neuron's spatial selectivity. Finally, using free-field stimulation, the actual spatial selectivity of these same neurons was tested to measure the accuracy of the predictions.

In general, the experimental and predicted results were in close agreement. A feature observed in many neurons is that the various monaural and binaural properties of a neuron can exert opposing influences on spatial selectivity. They tend to shift a neuron's spatial sensitivity toward different points in the sound field, and the neuron is often most sensitive at an intermediate point. Predictions of spatial selectivity therefore require that the monaural and binaural influences acting on a neuron be evaluated in concert.

215.5 THE DESCENDING AUDITORY PATHWAY AND THE COCHLEAR NUCLEUS IN THE MUSTACHE BAT. J.M. Zook. Dept. of Zoological and Biomedical Sciences and OUCOM, Ohio University, Athens, OH 45701.

To date, most attention given to the centrifugal or descending auditory system has centered on the prominent olivocochlear projection from the superior olivary complex to the cochlea. However most, if not all of the principle nuclei of the auditory pathway receive centrifugal input in the form of cholinergic fibers descending from a more central auditory center. The cochlear nucleus is intriguing: although it contains cells and fibers which stain positive for Acetylcholinesterase (AChase) and is the target of descending auditory projections, there is no direct projection descending from cochlear nucleus to cochlea. This study focuses upon the AChase-positive regions of the cochlear nucleus of the mustache bat, Pteronotus parnellii, to suggest a way by which the cochlear nucleus may contribute indirectly to the descending auditory system.

Unlike other mammals, where AChase-positive cells and fibers are lightly scattered throughout the ventral cochlear nucleus, the cochlear nucleus of the mustache bat contains a circumscribed, densely AChase-positive region along the medial and posterior borders of the anteroventral cochlear nucleus. Named the marginal zone, this region contains an almost completely homogeneous population of large multipolar neurons which, along with the surronding fibers, are uniformly AChase-positive.

Single unit recordings were made to characterize the best-frequency response both in and around the marginal zone. Small deposits of horseradish peroxidase (HRP) or a peroxidase conjugate with wheat germ agglutinin (WGA-HRP) were placed at physiologically characterized loci from the recording micropipettes. In related experiments the retrograde transport of HRP was also traced from the cochlea to the cells of origin of the olivocochlear bundle. Retrograde transport of HRP from the cochlear nucleus confirmed that the marginal zone is the target of descending projections from more cells of the superior olive, lateral lemniscus and inferior colliculus than any other part of the ventral cochlear nucleus. Units with best frequencies between 50 and 80 kHz were recorded within the boundaries of the marginal zone. This range of best frequencies includes, but is not limited to the frequency band near the predominant frequency of the echolocation cry (which is disproportionately represented in most auditory nuclei of this species). These data suggest that the descending auditory input to the marginal zone might be specifically targeted to influence information carried by this 50-80 kHz band. Anterograde tracing of WGA-HRP and HRP suggest that some of the same cells of the periolivary nuclei which project to the cochlea may also receive input from the marginal zone. These data raise the possibility that the cochlear nucleus may have an indirectly descending contribution to the cochlea via the projecting cells of the superior olivary complex.

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215.6 FUNCTIONAL LAMINAR AND COLUMNAR ORGANIZATION OF THE AUDITORY CENTERS IN ECHOLOCATING JAPANESE HORSESHOE BATS. I. TANIGUCHI, N. SAITO* and O. ARAI*. Dept. Auditory Disorders, Med. Res. Inst., Tokyo Med. and Dent. Univ., Tokyo 113, Japan.

Functional macroscopic structures of the brain of echolocating bats were visualized by the autoradiographic 2-[^{14}C]deoxyglucose (DG) method. We used the Japanese greater horseshoe bat, Rhinolophus ferrumequinum nippon (Taniguchi, I., J. Comp. Physiol., 156, 185, 1985). This species emits an orientation sound (pulse) from the nostril, which consists of a long constant frequency (CF) component followed by a short downward frequency-modulated (FM) component. Duration of pulses is about 30 ms and the CF of resting pulses is about 65.5 kHz. Their hearing is most sensitive to around 66 kHz, slightly higher than the CF. When the bats are stimulated by the frequency near the optimal frequency as a mimetic echo, they vocalize frequently orientation sounds even in an experimental room.

Bats were injected peritoneally with 2 µCi DG in 0.2 ml normal saline, while they were unanesthetized. After injection, each animal hung from a toe-hold in a small bird-cage in a sound-proof room, with or without sound stimulation. To stimulate bats to vocalize, a train of tone bursts of 66 kHz was used. The duration and rise-fall time were 30 ms and 2 ms, respectively. The stimulus level was fixed at 60 dB SPL as measrured at the animal's head. Eighty minutes after the DG injection, the animal was killed by an overdose of sodium pentobarbital. The frozen brain was sectioned at 30 µ. The sections were exposed to a single emulsion X-ray film (Sakura, ^{3}H type) in X-ray film cassettes for 4 days. Autoradiographs made at the level of the inferior colliculus revealed an active laminar structure in the central nucleus of the inferior colliculus in echolocating bats, but such a structure was not readily detected in resting bats. One of the active laminae seems to consist of a cluster of neurons that respond to the optimal frequencies around 66 kHz. These laminae represent tonotopical organization of the inferior colliculus. A non-active lamina was also observed just above the active one. The neurons in the non-active lamina may optimally respond to frequencies slightly lower than 66 kHz. Probably the activity of these neurons was suppressed by lateral inhibition. The DG uptake to the medial geniculate body and the auditory cortex increased with echolocation. Furthermore, the columnar organization appeared in the auditory cortex during echolocation. They were arranged alternately active and less active. These results suggest that the auditory centers are influenced by vocalization and are organized in a laminar or columnar manner. Inputs to them may be sent from the sensory feedback via the auditory pathways or from the vocalization centers.

215.7 VISION GUIDES THE DEVELOPMENT OF AUDITORY LOCALIZATION IN BARN OWLS. Eric I. Knudsen and Phyllis F. Knudsen, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

To localize sounds, the auditory system associates sets of auditory cues with locations in space. However, during development, these auditory cues change as the head and ears grow. How, then, does the auditory system establish correct associations? We found that, in barn owls, visual experience guides the development of auditory localization and fine-tunes both its accuracy and its precision.

Sound localization was measured from head orienting responses to auditory stimuli. Noise bursts were presented from random locations in a darkened, sound attenuating chamber. The response of the owl was indicated by the position of an infrared beam that reflect from a mirror mounted on its head, and was quantified in degrees of error relative to the location of the target. The owls were tested beginning at 3 months of age.

Two owls that were raised with their eyes occluded oriented to sounds with significantly poorer precision (F - test; $p < .01$) than did birds raised with normal vision. In fact, the data from one bird indicated that it could not localize the vertical locations of sound sources at all. After the visual occluders were removed, the owls demonstrated normal precision in orienting to visual stimuli but not to auditory stimuli. The results imply that vision is required for owls to develop normal auditory localization precision.

Two additional owls were raised with Fresnel prisms in front of their eyes. The prisms deviated vision 10^{o} to the right. By 3 months of age both owls were orienting approximately 10^{o} to the right of acoustic stimuli. That is, they had adjusted their auditory localization to match the visual errors induced by the prisms. After the prisms were removed, the owls corrected their auditory localization errors over a period of about 3 weeks. Thus, during development the visual system supplies spatial information which can alter and fine-tune auditory localization.

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215.8 ORGANIZATION OF NUCLEUS LAMINARIS IN THE BARN OWL. C. Carr, N.Brecha and M. Konishi. Dept. of Biology, California Institute of Technology Pasadena, CA 91125 and CURE, VA Medical Center, Wadsworth, Los Angeles, CA 90073.

Sensitivity to interaural temporal disparities underlies aspects of sound localization. The first place where it appears is nucleus laminaris (NL), which is also the first recipient of binaural input. An analysis of the structure and connections of NL has been undertaken in an attempt to identify morphological correlates of the emergent sensitivity to interaural temporal disparities. The Golgi technique, immunocytochemical techniques and in vivo and in vitro intracellular dye injection were used.

In the barn owl, NL is a greatly hypertrophied brainstem nucleus, surrounded by afferent fiber tracts. NL contains numerous large (30-50 um) neurons in a central neuropil area, and these cells have many short stubby dendrites, from 10 to 50 um in length. NL neurons receive afferent input from both the ipsilateral and contralateral cochlear nucleus magnocellularis. Nucleus magnocellularis receives eighth nerve input via end bulbs of Held directly onto the unipolar somata of its neurons. Each neuron projects bilaterally to nucleus laminaris, and their terminals within NL are large, stubby and have few branches. These terminals end closely apposed to the large cells of NL.

Immunocytochemical methods have revealed that Calcium binding protein (CaBP) immunoreactivity is localized to both the neurons of nucleus magnocellularis and NL, although the target for NL efferents, nVLVa (anterior part of the ventral nucleus of the lateral lemniscus), which is also sensitive to interaural temporal disparities, displays no immunoreactive product. Glutamic acid decarboxylase (GAD) immunoreactivity has demonstrated the presence of a second afferent input to NL. GAD-positive terminals surround the somata of the large cells of NL. They are of unknown origin, and suggest that inhibitory processes may act in NL.

Sensitivity to a particular temporal disparity is conferred by the simultaneous arrival of afferent magnocellular inputs onto the cells of NL. The time difference in the incoming signals is compensated for within NL (Sullivan and Konishi, 1985). Physiological and ultrastructural techniques will be used to analyse how the required temporal advances and delays are produced.

215.9 CONSTRUCTION OF A NEURAL MAP OF INTERAURAL PHASE DIFFERENCE IN NUC. LAMINARIS OF THE BARN OWL. W. E. Sullivan and M. Konishi, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We studied the mechanisms underlying neuronal sensitivity to interaural phase difference in the barn owl's nuc. laminaris, the first-order binaural nucleus in the avian brainstem. Using microelectrodes, a large sinusoidal response to tonal stimuli, or "neurophonic" response, is recorded in this nucleus. The neurophonic has a 2 to 3 msec latency and is sharply tuned to frequency. These and other properties show its physiological origin. The neurophonic is probably due to the synchronized, phase-locked activity of a population of neurons. In the owl, neurophonic potentials can be recorded at frequencies of up to 8.5 kHz. and can be driven by monaural excitation of either ear or by binaural stimulation.

As the electrode moved ventrally within the nucleus, the neurophonic response to a contralateral stimulus showed a progressive phase advance whereas a phase delay was seen in successive ipsilateral ear responses. Binaural responses were sensitive to interaural time delay, a maximum response occurring when the monaural responses would be in phase and a minimum response occurring when they would be out of phase. Binaural tuning curves shifted towards a greater ipsilateral time lead with depth.

The results are consistent with the bilateral innervation pattern of nuc. laminaris by the cochlear nuclei and support a simple model for the genesis of binaural phase tuning and a neural map of interaural phase difference. Ipsilateral cochlear nucleus fibers enter nuc. laminaris from the dorsal side and contralateral fibers enter ventrally. The electrode moves towards the contralateral and away from the ipsilateral source as it advances ventrally, thus causing an earlier contralateral and a later ipsilateral response.

In our model, fibers from the two cochlear nuclei project through nuc. laminaris in opposite directions, representing the opposing "delay lines" proposed by Jeffress. This generates a dorsal-ventral gradient of neural delay difference. For coincidence of monaural signals to occur, increases in neural delay must be balanced by decreases in acoustic delay. Thus, a ventral movement causes binaural neurophonic tuning curves to shift towards a smaller acoustic delay in the ear for which a longer neural delay is seen: i.e., the ipsilateral ear. Laminaris neurons also respond maximally when the two monaural inputs coincide. This "coincidence detection" transforms the neural delay difference gradient into a map of interaural phase (or time) difference. (Supported by a Del Webb Fellowship to W.E.S. and PHS Grant NS-14617 to M.K.)

215.10 INTRACELLULAR RECORDINGS FROM OWL INFERIOR COLLICULUS. Andrew Moiseff, Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268.

The activity of space selective and space specific neurons were recorded from the inferior colliculus of adult barn owls (Tyto alba). Owls were tranquilized (Diazepam) and anesthetized (Ketamine), and placed in a stereotaxic frame with the animal's body suspended horizontally. A small craniotomy was performed to access the brain, a slit was made in the dura mater, and glass microelectrodes filled with 2M K-Acetate were advanced into the inferior colliculus under stereotaxic guidance.

Dichotically presented auditory stimuli were delivered through magnetic earphones mounted in each auditory meatus. It was often possible to record extracellular activity before impaling a cell. At such times the binaural parameters (interaural time difference and interaural intensity difference) were adjusted to maximize the unit's spike rate. Since receptive fields of closely located units are very similar this insured that the stimuli would be properly adjusted for exciting units when impaled intracellularly (even if the impaled cells were not the same as those recorded from extracellularly).

A standardized stimulus paradigm subjected the unit to various interaural time and intensity differences, monaural stimulation, and frequency tuning. Units showed strong, tonic, hyperpolarization (inhibition) in response to certain time and intensity differences. Inhibition lasted well beyond the duration of the 'offending' stimuli. 'Acceptable' stimuli elicited tonic depolarization above threshold for spiking. When the stimulus ended excitation ceased and the unit was inhibited for approximately 20 msecs.

The occurence of inhibitory and excitatory binaural parameters correlate with the center-surround organization of auditory receptive fields reported by Knudsen and Konishi (Science 202 :778) and demonstrate that this organization is, at least in part, the result of interactions at the level of space-selective and space-specific neurons. The temporal extent of inhibitory interactions, which far outlast the actual stimulus, is being further investigated. They may result in sharper receptive fields in response to moving auditory stimuli.

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215.11 PROJECTIONS FROM THE ACOUSTIC TUBERCLE TO OTHER AUDITORY BRAIN STEM NUCLEI IN THE RED-EARED TURTLE, CHRYSEMYS SCRIPTA ELEGANS. R.H. Browner, DM. Pierz, and D. Marbey. Department of Anatomy, New York Medical College, Valhalla, NY 10595.

It is important to determine the acoustic tubercle (AT) connections relative to the ability of the turtle's 8th nerve to regenerate to the cochlear nuclei (Marbey and Browner, Hearing Res. 15: '84). The interaction of these structures can effect the process of regeneration.

Ten red-eared turtles were anesthetized with Brevital Sodium (0.5 to 0.8%). Following this the animals were placed in a stereotaxic apparatus (Kopf) and anesthetized with Halothane, Nitrous Oxide, and Oxygen. The dorsolateral portions of the crania were drilled open, the dura mater opened and the cerebellum moved medially, exposing the surface AT. Glass micropipettes (tips: 15-30 µm) were placed in the AT. Injections were made rostal to caudal in the AT. 25% HRP or 20% HRP-WGA was iontophoretically (2.5 µamps) injected for seven minutes. The turtles survived for 5 days and then were perfused with Reptilian Ringers and the fixative for HRP (TMB Method). The brains were removed, embedded in gelatin-albumin, and sectioned at 40 µm in the standard planes. Three tracts were traced from AT. A dorsal tract coursed ventrally and medially under the 4th ventricle through both MLF. The fibers turned dorsally and entered the contralateral AT. It formed a band of terminals and fibers between the ependymal cells and the nuclei magnocellularis and laminaris. An intermediate smaller tract left the AT and crossed the midline, obliquely, ventrally to the contralateral ventrolateral superior olive (SO). The ventral pathway coursed ventrally and laterally and followed the outer contour of the brain stem to the ipsilateral SO. The fibers crossed the midline and joined the intermediate pathway in the area of the contralateral SO. These AT fibers entered the lateral lemniscus and coursed rostrally and dorsally, to enter the central nucleus (CN) of the torus semicircularis (TS). In the caudal portion of the CN the axons terminated in the large cell region. Some axons entered the medial smaller cell area. Ipsilaterally a small number of AT fibers coursed rostrally in the lateral lemniscus to enter the CN. There were no axon terminals in either laminar nucleus.

In the ipsilateral SO there were multipolar neurons retrogradely filled with HRP reaction product. In the contralateral nucleus magnocellularis there were HRP filled cells; in the contralateral SO there were axons and axon terminal fields. The cells in the nucleus of the lateral lemniscus had few axonal terminals; most fibers continued to enter CN of the TS.

Injections limited to the nucleus magnocellularis had fewer fibers entering the 3 tracts and terminating in the contralateral CN. Research supported by the Culpeper Foundation.

215.12 **REPRESENTATION OF COOPERATIVE FIRING ACTIVITY AMONG SIMULTANEOUSLY RECORDED NEURONS, G. Gerstein, A. Aertsen*, Dept. of Physiology, Univ. of Pennsylvania, Philadelphia PA 19104.**

Technical development of electrode structures and of spike shape sorters have made increasingly practical the simultaneous and separable recording of activity from some 20 to 30 neurons, thus allowing direct study of static and dynamic properties of neuronal assemblies. Analysis of such data is usually based on variants of cross-correlation of PAIRS of spike trains, and for combinatorial reasons requires examination of thousands of correlograms in order to draw conclusions about the neuron assembly. An analysis that treats the entire observed neuronal assembly as an entity rather than as a combination of pairs is clearly needed.

We examine here a new transformation of multiple simultaneously recorded spike trains (1) which represents each of the N observed neurons as a particle in an N space. Initially all particles are equidistant in the N space. Particle movements are governed by the timing of the neuronal spikes by the following rules. Each spike train is converted into a time varying "charge" for the associated particle, usually using a low pass filter. The force between any two particles is a vector proportional to the product of their charges, and is directed along the line between their positions. At each time step the movement of each particle in turn is determined by the vector sum of all pair forces acting upon it. As the calculation proceeds, particles representing neurons with correlated firing will tend to aggregate. Thus the developing spatial arrangement of the particles reflects the logical interactions of the neurons.

This simple representation can be modified in several ways for other emphasis (2). Change of sign in the charge product during the calculation produces spatial aggregation of those particles representing neurons which interact through inhibitory processes. Extension to a two-charge definition based on both forward and backward exponentials allows inference of causality (synaptic direction) rather than just correlated firing, as in the original version. Sensitivity of the "gravity" calculation is extremely high; typical data of 30 sec. duration produce interpretable aggregations.

Performance of this representation is best seen by projection from the original N space to a plane. Both "time exposures" of the particle trajectories and "snapshots" of the projected positions can identify the interactions within a neuronal assembly. Movies are useful to determine the dynamics of the aggregation process; this may reflect time or stimulus varying interactions within the observed assembly.

(1) Gerstein, Perkel, Dayhoff; J. Neuroscience 5, 881, 1985.
(2) Gerstein, Aertsen; J. Neurophysiology, in press, 1985.
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HORMONAL CONTROL OF BEHAVIOR

216.1 THE ROLE OF THE ALPHA ADRENERGIC SYSTEM IN THE MALE RING DOVE'S COURTSHIP BEHAVIOR. S.R.Barclay*and M.-F.Cheng.(Spon: M. Dalsass) Institute of Animal Behavior,Rutgers University,Newark,N.J.07102

We previously reported (Neuroscience Abstracts,1984) that an androgen dependent courtship behavior,the bow-coo, was depressed when male ring doves were treated with the alpha-adrenergic agonist, phenylephrine, and elevated when the males were treated with prazosin, an alpha-adrenergic antagonist. The purpose of the present study is to determine whether the modulation of the bow-coo behavior is mediated solely by the alpha-adrenergic system or whether the beta-adrenergic system is also involved.

In the present studies we followed the same experimental paradigms previously used to determine the effects of alpha-adrenergic agonists and antagonists on courtship behavior in the ring dove. In the first group of experiments we determined a dose response curve for each drug. Intact males were administered the drugs intramuscularly one to two hours prior to testing. Males were then paired with a receptive female in a breeding cage and observed for fifteen minutes. The second group of experiments determined whether a specific drug effect was hormone-dependent. Castrated males were primed with various steroids or vehicle administered in Silastic capsules for a week prior to behavioral observations. The primed animals were then treated with the lowest effective dose of the drugs and tested for courtship behavior with a receptive female. The third set of experiments attempted to show whether the observed drug effects were centrally mediated. Chronic cannulae were implanted in the third ventricle of intact males and drugs were infused into the ventricular fluid. A female was introduced into the male's cage thirty minutes after the infusion and courtship behavior was measured for fifteen minutes.

Consistent with our previous findings,methoxamine,an alpha-adrenergic agonist,also inhibited the bow-coo without affecting other androgen-dependent courtship behavior such as nest-coo. Norepinephrine reuptake blocker, desipramine,blocked all expression of male sexual behavior,including the bow-coo.

These results suggest that inhibition of the alpha-adrenergic system is part of the normal induction of courtship behavior in the male ring dove.

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216.2 PROGESTERONE ANTAGONIST DOES NOT PREVENT CHOLINERGIC FACILITATION OF LORDOSIS. G. Richmond* and L. G. Clemens. Dept. of Zoology, Michigan State University, E. Lansing, MI 48824

The sexual behavior of many female rodents is dependent upon sequential release of the ovarian steroids estrogen and progesterone (P). We have demonstrated that receptivity (lordosis) can be induced by intracerebral administration of muscarinic cholinergic agonists in estrogen-primed female rats. This effect is not enhanced by prior P administration, suggesting that while the facilitation is estrogen-dependent, it is independent of P. In Experiment 1 we investigated this possibility directly by blocking lordosis with a P antagonist (RU 38486) and examining whether the muscarinic agonist oxotremorine (OXO) can reverse this inhibition. We also examined whether the drugs affect behavioral desensitization to additional P. In a second study we investigated whether RU itself interacts with progestin receptors to cause desensitization to P given 25 hr later.

In Experiment 1 ovariectomized rats were implanted with stainless-steel cannulae in the lateral ventricles. They were treated for 3 days with .5ug estradiol benzoate (EB) and on Day 4 injected with RU (.5mg) or oil. One hr later they received P or oil. Three, 4, and 5 hr after this injection they were tested for lordosis; infused with OXO (.5ug/cannula) or vehicle; and tested again 5 and 20 min after infusion. On Day 5, 24 hr after the first P/oil injection, all females received .5mg P and were tested for lordosis 4, 5, and 6 hr later. RU blocked receptivity in animals receiving P. This inhibition was reversed 5 min after OXO infusion and disappeared by 20 min. Females that received EB + P on Day 4 showed a diminished response to a second P injection on Day 5. However, an even greater decrease in response to P was observed in animals that received RU on Day 4.

In Experiment 2 ovariectomized animals were treated for 3 days with EB. On Day 4, RU or oil was injected, followed an hr later by one of two concentrations of P (.5 or 1.5mg) or oil and 3 tests for lordosis (3, 4, and 5 hr after the last injection). A second P injection (.5mg) was given 24 hr after the first injection (on Day 5), followed 4, 5, and 6 hr later by lordosis tests. Animals that had been given RU with P again displayed little receptivity. After the second P injection, little or no receptivity was shown by females that had received RU alone as well as in combination with P on Day 4.

These experiments offer direct evidence for the hypothesis that the mechanism by which the cholinergic system facilitates lordosis is P-independent. The results also suggest that RU acts like a competitive antagonist to P, thus preventing P administered 1 hr and/or 25 hr after RU from inducing sexual receptivity.

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216.3 **DIFFUSION PATTERN OF CHOLINERGIC COMPOUNDS INFUSED INTO THE VENTRAL MEDIAL HYPOTHALAMUS OR MIDBRAIN CENTRAL GRAY.** T.C. Meyers*, G. Richmond* and L.G. Clemens (SPON: J. Zacks). Zoology Department and Neuroscience Program. Michigan State University, East Lansing, MI 48824.

Infusion of cholinergic agonists into the midbrain central gray (MCG) or the ventral medial hypothalamus (VMH) facilitates feminine sexual behavior (lordosis) in ovariectomized, estrogen-primed facilitate lordosis is uncertain due to the possibility of diffusion away from the MCG or VMH. In order to more thoroughly evaluate this possibility we have examined the diffusion pattern of a cholinergic compound infused into either the MCG or VMH.

Ovariectomized Sherman female rats were implanted with stainless steel cannulae terminating in the lateral ventricle and either the MCG or VMH. Animals were then injected with a dose of estradiol benzoate (EB) shown to be effective as a priming dose for facilitation of lordosis by infusion of a cholinergic agonist. EB was administered daily for three days. On the fourth day, tritiated scopolamine, a muscarinic cholinergic antagonist, was infused at a volume of .5ul into either the MCG or VMH. Cerebral spinal fluid (CSF) was extracted from the lateral ventricles at 5 and 15 minute and again at 3, 5, and 6 hours. CSF was then analyzed for presence of the isotope. The label was not found in the CSF at any of the above times. We therefore believe that cholinergic compounds infused into the MCG or VMH do not diffuse into the lateral ventricle.

In a subsequent experiment, we infused tritiated scopolamine into the MCG or VMH and determined the spread of the label through non-ventricular tissue. Animals were sacrificed by decapitation 5 or 15 minutes after infusion. The brains were removed and the MCG, VMH, cortex and various brainstem areas were dissected. When infused into the VMH, the label was found in VMH and MCG but no in cortex or brainstem. When the volume of scopolamine infused into the VMH was reduced to half the original, diffusion still occurred, but with a latency of 15 minutes.

These experiments indicate that at volumes and concentrations used in behavioral research, diffusion occurs from the VMH to the MCG, and perhaps to other periventricular areas. This may be an important consideration in attempting to localize the brain sites involved in the cholinergic regulation of lordosis.

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216.4 DORSAL MIDBRAIN LESIONS WHICH IMPAIR HAMSTER LORDOSIS ALTER THE RESPONSIVENESS OF REMAINING MIDBRAIN NEURONS. M.D. Havens and J.D. Rose, Dept. of Psychol. Univ. of Wyoming, Laramie, WY 82071

The midbrain provides a critical degree of control over the expression of lordosis by golden hamsters. Bilateral deep superior colliculus lesions in the hamster abolish lordosis and unilateral collicular lesions selectively impair elicitation of the response by stimulation of the contralateral flank. While central gray and ventral tegmental lesions sometimes impair lordosis, the effects of these lesions are not as consistent or as pronounced as those seen with tectal lesions. Neurons which respond to lordosis-triggering forms of somatic stimulation are widespread in the hamster's midbrain and the responsiveness of these neurons is enhanced by the synergistic effects of estrogen and progesterone. These findings imply that both tectal and nontectal neurons play an important role in the sensorimotor control of hamster lordosis and raise the possibility that tectal lesions impair lordosis by altering the sensory responsiveness of subtectal midbrain neurons. To evaluate this hypothesis, the responsiveness of single midbrain neurons to lordosis-controlling forms of somatic stimulation was assessed in three groups of ovariectomized, estrogen-progesterone treated hamsters. One group had received lordosis-blocking bilateral tectal lesions. The second group had received unilateral tectal damage and showed impaired lordosis responsiveness to contralateral flank stimulation. The third group consisted of surgical control animals. A total of 319 cells was recorded in the three groups of animals under urethane anesthesia. The percentage of cells which responded to some form of somatosensory stimulation was significantly higher in the bilaterally-lesioned group than in the unilaterally-lesioned or surgical control groups. In addition, more of the responsive cells were located in the ventral tegmentum of bilaterally-lesioned animals than in either of the other two groups. Also, the spontaneous firing rate was decreased by the bilateral lesions, relative to the unilateral lesion and surgical control groups. The tactile receptive fields of the cells were also altered by the lesions. A greater percentage of units in the unilateral lesion group responded to facial stimulation, whereas, a greater percentage of units in the bilateral lesion group responded to flank stimulation. Thus, dorsal midbrain lesions which impair the elicitation of lordosis by somatosensory stimulation also alter the responsiveness of remianing midbrain neurons. Functionally, the elevated responsiveness of ventral tegmental neurons in bilaterally-lesioned animals may result in the elicitation of locomotion rather than lordosis by flank stimulation, since ventral midbrain neurons in hamsters tend to show locomotion-related firing. (Supported by NIH Grant NS 13748)

216.5 EFFECTS OF PROGESTERONE ON MONOAMINE TURNOVER IN DISCRETE BRAIN NUCLEI: RELATIONSHIP TO SEXUAL BEHAVIOR. K.J. Renner, L.C. Krey and V.N. Luine. Lab. of Neuroendocrinology, Rockefeller University, New York, N.Y. 10021.

Progesterone facilitation of sexual behavior in the female rat is believed to be regulated, in part, by central monoaminergic systems. However, central sites for progesterone-monoamine interactions in regulation of behavior remain largely unknown. In the present study the effect of progesterone on monoamine levels and turnover were evaluated in brain nuclei in estrogen-primed rats.

One week after ovariectomy rats were injected s.c. with 0.5 mg progesterone (P) or oil vehicle 21 h. after s.c. treatment with 5 ug of estradiol benzoate. The initial levels of norepinephrine (NE), dopamine (DA), 5-hydroxyindole acetic acid (5-HIAA) and serotonin (5HT) were measured in animals injected i.p. with saline 3 hr after the administration of P. To estimate NE and DA turnover, oil or P treated animals (3h.) were injected with α-methyl-p-tyrosine (400 mg/kg) and sacrificed 2 h. later (5 h. after oil or P). Serotonin turnover was estimated in animals injected with pargyline (75 mg/kg) 20 minutes prior to sacrifice. Amine content was measured by high performance liquid chromatography with electrochemical detection in the following brain nuclei: lateral septum, vertical nucleus of diagonal bands (vDB), medial preoptic nucleus, anterior hypothalamic, periventricular region (PVE, anterior hypothalamic level), ventromedial hypothalamuis (VMN), midbrain central grey (MCG), arcuate-median eminence, and dorsal raphe. In addition, sexual behavior (lordosis response) and plasma LH were measured under these steroid-priming conditions.

Estrogen-primed rats treated with P showed high levels of lordosis responding and elevated plasma LH when compared with estrogen only primed rats indicating that the lag-time following E priming was sufficient for P to exert neuroendocrine effects.

Progesterone did not significantly affect initial levels of NE, DA, 5-HIAA or 5HT in any of the brain nuclei studied. In the PVE, P resulted in decreased NE turnover. DA turnover was elevated in the vDB of P treated rats. Progesterone treatment resulted in decreased 5HT turnover in the VMN and MCG. No other changes in monoamine turnover following P were noted.

These results suggest that progesterone influences monoaminergic activity in a few discrete brain nuclei of E-primed rats. The relationship of the changes in PVE NE and vDB DA turnover to sexual receptivity are not known. However, both regions have been implicated in the regulation of gonadotropin secretion. The decreased serotonin accumulation in the VMN and MCG following P treatment is consistent with the hypothesis that 5HT exerts tonic inhibitory modulation of lordosis behavior.

216.6 SEROTONIN IN THE MEDIAL BASAL HYPOTHALAMUS MEDIATES SEX DIFFERENCES IN HORMONE-FACILITATED LORDOSIS OF MALE RATS. J.Moreines, D.Pfaff, and B.McEwen, Rockefeller Univ., NY, NY 10021.

Developmental effects of perinatal androgens render adult male rats refractory to the activation of feminine sexual behavior (lordosis) by estradiol (E2) and progesterone (P). Recent evidence suggests that serotonin (5HT) may inhibit hormone mediated lordosis responding of female rats at the level of the ventromedial hypothalamus (VMH) (Luine,et al.,1984. Science 226: 1436). The hypothesis that this inhibitory input contributes to sex differences in behavioral responsiveness to hormones was assessed in gonadectomized adult male rats deprived of 5HT following bilateral stereotaxic injections of the neurotoxin, 5,7 dihydroxytryptamine (5,7 DHT) into the region of the VMH. Lordosis quotients (LQs) were determined at mating tests conducted 48 hr following sc insertion of Silastic capsules producing physiological E2 levels both before and 4 hr after P injection (0.5mg).

The results show that 5,7 DHT treatment enhances the effects of E2 in the absence of P. Increased sexual responsiveness was displayed at times when this treatment is known to reduce 5HT levels in the VMH of females (Luine,et al.,1984) (i.e., days 18 to 52 post surgery). The mean LQ achieved by these animals was 71 (N=4), which is comparable to that typically obtained by E2-primed females; in contrast, control animals attained a mean LQ of 25 (N=4) ($p=.01$). In addition, the 5,7 DHT treatment appeared to reverse the insensitivity of males to synergistic P effects. LQs of hyper-responsive males were elevated to 81 when P was injected 48 hr following E2 ($p<.05$). In contrast, LQs of controls (LQ=23) remained unaffected by P administration. Evidence that P potentiates E2-induced lordosis following 5,7 DHT injections was further demonstrated when E2-priming was reduced to 6 hr. This E2 stimulation was sufficient to induce lordosis 24 hr later only after exogenous P in 2 of 4 hyper-responsive males (LQ=40).

While 5,7 DHT injections appear to reverse sexually dimorphic responses to hormone-facilitated lordosis, this treatment fails to alter sex differences in gonadotrophin effects of E2 and P. Neither control, nor lesioned males, displayed a surge of luteinizing hormone following E2 and P treatment sufficient to mediate behavior of hyper-responsive males.

These results suggest that 5HT input into the VMH interferes with hormone-responsive lordosis mechanisms in males as in females. Interestingly, some lesioned males remain hyper-responsive more than 50 days post 5,7 DHT, i.e., when behavioral function and 5HT levels in the VMH of females have recovered (Luine,et al.,1984). Whether basal levels of 5HT are reinstated in these latter males, and what particular cellular mechanisms mediate the reversal in sensitivity to E2 and synergistic P are under investigation. Supported by grant NS07030.

216.7 INTRACEREBRAL DOPAMINERGIC CONTROL OF MASCULINE SEXUAL BEHAVIOR IN RATS. E.M. Hull, D. Bitran, E.A. Pehek, R.K. Warner, L.C. Band, and G.M. Holmes. Dept. of Psychology, State Univ. of New York at Buffalo, 4230 Ridge Lea Rd., Amherst, NY 14226.

Systemically administered dopaminergic drugs have been reported to facilitate sexual behavior of men and male rats. The locations within the brain that contribute to this facilitation have not previously been determined, though the caudate-putamen (CP), nucleus accumbens (NA), and lateral septum (LS) have been suggested as candidates. In addition, the medial preoptic area (MPOA) is important for masculine sexual behavior and receives dopaminergic terminals of incertohypothalamic (A14) neurons. In order to localize dopaminergic effects on copulation, we microinfused the dopamine agonist apomorphine (APO) into each of the sites noted above, as well as into the lateral ventricle (LV) as a control placement.

The lowest dose of APO (0.2 µg) infused into the LV decreased the number of ejaculations, the rate of intromitting and the percentage of mounts on which the male gained vaginal intromission. The higher two doses (0.5 and 2.0 µg) infused into the MPOA and, in some cases the LV, increased the number of ejaculations and the percentage of mounts with vaginal intromission, and decreased the latency to begin copulating, the latency to ejaculate, and the postejaculatory interval before resuming copulation.

Infusions into the CP and the LS did not affect any aspect of behavior. Infusions into the NA produced only a slight, dose-related decrease in latency to begin copulating.

The copulatory impairments associated with infusions of the lowest dose into the LV may have resulted from stimulation of dopamine autoreceptors; the facilitative effects of the two higher doses into the MPOA and LV may have been due to stimulation of postsynaptic receptors.

216.8 GENETIC INFLUENCES ON COPULATORY AND AGGRESSIVE BEHAVIORS IN MICE. B. E. F. Wee, D. R. Weaver, K. L. Sinchak*, and L. G. Clemens. Neuroscience Program and Department of Zoology, Michigan State University, East Lansing, MI 48824.

While castration results in reduction of a variety of hormone-dependent behaviors, McGill and Manning (Anim. Beh. 24:507-518, 1976) have shown that one hybrid strain of mice, the B6D2F1, continues to copulate up to one year after castration. The purpose of this study was to examine the effects of castration on copulatory and aggressive behaviors in this hybrid and the parental strains : C57Bl/6J and DBA/2J.

In experiment 1, sexual behavior was measured in adult male mice of the three strains : B6D2F1 (F1) (resulting from a cross between a C57 female and a DBA male), DBA, and C57. Sexually experienced males of each strain were castrated or sham operated. Following surgery, males were tested for an additional 25 weeks.

Castrated F1 males showed high levels of copulatory behavior, and some F1s continued to show the ejaculatory pattern 25 weeks after castration, thus confirming the observations by McGill and Manning. Neither parental strain retained the ability to ejaculate in the absence of testicular androgens.

To determine whether other "hormone-dependent" behaviors also persist after castration in B6D2F1 males, the effects of castration on aggression were examined. In experiment 2, DBA, C57, and B6D2F1 males were tested for aggressive behavior. After four biweekly aggression tests with an olfactory bulbectomized, stimulus male, the mice were either sham operated or castrated. Four additional biweekly aggression tests followed the surgery. Aggression was measured as the number of attacks by the experimental male within a 5 minute test session.

Levels of presurgical aggressive behavior were as follows : DBA > C57 > B6D2F1. Aggression was maintained at precastration levels after sham operation, but castration reduced aggressive behavior in all strains. However, when tested 7 weeks after castration, 60% of the DBA males continued to show aggressive behavior, while less than 20% of the C57 and F1 males were aggressive.

The results indicate that retention of copulatory behaviors by B6D2F1 castrates may be mediated by a nonhormonal mechanism. However, the effect of castration on aggressive behaviors does not follow this same pattern.

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216.9 ESTROUS CYCLE DEPENDENT VARIATION IN BALANCE BEAM PERFORMANCE IN FEMALE RATS. Melissa M. Miller*, Sara A. Westgate*, Peter J. Snyder*, Mary A. Kalafut* and Jill B. Becker (SPON: D.G. Green). Psychology Dept. and Neuroscience Laboratory Bldg., The University of Michigan, Ann Arbor, MI 48104-1687

During the estrous cycle in female rats, the amphetamine-stimulated release of striatal dopamine (DA) is greater on estrus than on proestrus or diestrus 1. Amphetamine-stimulated or electrical stimulation-induced rotational behavior show a very similar pattern of change across the estrous cycle. In the experiment reported here, we examined the influence of estrous cycle on a natural behavior requiring the active integration of sensori-motor information. Given what is known about the normal function of the nigro-striatal DA system, we hypothesized that fluctuations in striatal DA activity would be reflected in balance beam performance.

Female Holtzman rats were maintained on a 14:10 light:dark cycle (N=9). Estrous cycle phase was monitored by daily vaginal smear examinations. The ability of each rat to walk lengthwise across a balance beam 0.5" X 4.0 ft was tested daily, on two separate trials. Prior to each trial, the front and back feet were painted with different colors of tempera paint. The animal was placed on the beam and the time it took to walk the length of the beam for cookie mash reward was recorded. Footfaults were scored by an independent observer, blind to the estrous condition of the animal, at the end of each day by examining the footprints on each beam. A footfault was scored for each footprint that extended off the top of the beam onto the side of the beam. Animals were tested over 2 complete estrous cycles, 3-4 h after the lights went on in the animal room. Then animals were given 6 weeks to adapt to a reversed light:dark cycle and the procedure was repeated 3-4 h after lights off (under red light conditions).

There was a significant effect of estrous cycle on balance beam performance. When testing occurred 3-4 h after lights on, female rats made significantly fewer footfaults on estrus than on proestrus ($p<0.001$) or diestrus ($p<0.007$). When testing occurred during lights off, female rats made fewer total footfaults on estrus than on diestrus ($p<0.035$). Under both lighting conditions, fewer footfaults were consistently made with the front feet than with the back feet. In addition, there was no effect of estrous cycle on the time it took to walk the length of the beam.

It is intriguing to note that estrous cycle dependent variation in balance beam performance corresponds to the pattern of striatal DA release across the estrous cycle. However, further research is necessary to determine the underlying neural basis of the estrous cycle dependent variation in footfaults on the balance beam.

216.10 EFFECTS OF ESTRADIOL (E_2) ON PROTEIN SYNTHESIS IN VITRO, IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (VMN) AND PREOPTIC AREA (POA) OF THE FEMALE RAT. K.J. Jones, B.S. McEwen, and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

It is generally thought that the functional consequences of steroid hormone action on target tissues are effected via mechanisms which include protein synthesis. Early changes in cell nuclear morphology in VMN neurons during behaviorally-relevant paradigms of E_2 exposure are consistent with this view(Jones et al.,1985). In this study, we examined, after similar paradigms, effects of E_2 on protein synthesis in 2 E_2-concentrating regions of female rat brain, VMN and POA.

Ovariectomized rats (OVX) were either implanted with E_2 for 2h or for a discontinuous schedule of 2h-on-E_2/7h off/2h on-E_2, or sham-implanted for controls. Punches of VMN and POA were collected and in vitro labelled with ^{35}S-methionine and -cysteine for 4h. Proteins were separated by 2-dimensional gel electrophoresis, fluorographed, and exposed to autoradiographic film. The fluorograms were quantified by computerized scanning densitometric and normalization procedures.

In the VMN, 2h of E_2 produced increases in intensity of labelling in some proteins and decreases in intensity of labelling in other proteins, normalized and relative to the controls. After 2h-on/7h-off/2h-on of E_2, intensity of labelling changed in a different set of proteins, normalized and relative to the controls. The populations of proteins increased by E_2 did not overlap in the 2 paradigms, whereas, approximately 1/2 of the populations of proteins decreased by E_2 were the same in both treatment groups. In the POA, 2h of E_2 also produced increases in intensity of labelling of some proteins and decreases in intensity of labelling of others, normalized and relative to the controls. After 2h-on/7h-off/2h-on of E_2, intensity of labelling changed in a different set of proteins. Approximately 1/2 of the populations of proteins increased and decreased by E_2 were the same in both treatment groups. Most of the proteins in VMN affected by E_2 differed from those proteins in POA affected by E_2.

Differential effects of E_2 on protein synthesis in the VMN are in accord with ultrastructural data suggesting a cascade of events from the first 2h of E_2, and later through the second 2h of E_2. Taken together, these data support the hypothesis that estrogen has more than one phase of action on the VMN. The differences in the population of proteins affected by E_2 action in the VMN vs. POA imply regional specificity of steroid hormone action on protein synthesis in the brain. For example, these differences could contribute to VMN/POA differences in the control of female sex behavior, male behavior, parental behavior, and ovulation. Supported by MH-15125 and HD-05751.

216.11 THE EFFECTS OF IC ADMINISTRATION OF OXYTOCIN ON PUP-DIRECTED BEHAVIOR AND SELF-GROOMING IN PREWEANLING AND WEANLING RATS.
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Intracerebroventricular (ICV) administration of oxytocin (OXY) has been shown to increase maternal behavior in estrogen-primed adult female and also to increase self-grooming in intact adult male and female rats. Weaning rats (18-24 days of age, female and male) have been reported to display rapid onset maternal-like behavior towards young rat pups. The latency of onset of maternal-like behavior towards pups increases markedly after age 24 days concimitant with an increase in general neophobia. We have tested the effects of central administration of OXY on behavior directed towards young pups in rats at various ages before and during weaning.

Preweanling or weanling Sprague-Dawley rats (18, 22 or 26 days of age) were each allowed to habituate to a mouse cage for four hrs the day prior to behavioral testing. On the day of testing each animal received either 2 ug of OXY in normal saline (NS) or NS vehicle alone intracisternally (IC) while under light ether anesthesia. Fifteen minutes after IC injection, three 1-4 day old rat pups were placed in each cage. Each animal was observed for a one-minute period every ten minutes over the next two hrs. Behavior occurring during each five-second interval of observation was recorded. Among a large number of behaviors that were scored were forepaw holding with and without licking (FHL) of pups and self-grooming. Rats 22 days of age displayed far more FHL of pups after OXY than did those after NS injection ($F = 27.64$, $p < .0001$, Student's t test). In rats 18 days of age there was a trend towards more FHL of pups after OXY ($F = 3.41$, $p < .08$), but in rats 26 days of age there was no difference between OXY and NS recipients ($F = 1.39$, $p < .26$). Self-grooming behavior, however, was significantly increased in OXY recipients in each age group ($p < .0001$). Thus age, stage of weaning or the onset of neophobia appears to influence sensitivity to a pup-directed behavioral effect of OXY but does not alter sensitivity to a self-directed behavioral effect of OXY.

216.12 COMBINED CENTRAL PLUS PERIPHERAL IMPLANTS OF DIHYDROTESTOSTERONE INCREASE SEXUAL BEHAVIOR OF MALE RATS
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The inability of dihydrotestosterone (DHT), a nonaromatizable androgen, to stimulate sexual behavior in castrated rats is usually taken as support for the hypothesis that male sexual motivation is primarily influenced by estrogenic metabolites of testosterone (T). However, reports that DHT can facilitate mating in castrated monkeys, guinea pigs, and some strains of rats and mice have raised questions about the generality and validity of this idea. Although the mechanism for this species difference is unknown, it is possible that reduced bioavailability of DHT to the rat brain may contribute to its ineffectiveness in this species, since exogenous DHT is metabolized rapidly by male rats. The present experiment was therefore designed to bypass potential problems of biotransport by directly stimulating the medial preoptic area (MPOA) with DHT.

Fifty-two adult, male rats (Long-Evans), prescreened for sexual behavior, were castrated (Day 0) and implanted subdermally with either a blank implant or a silastic capsule of DHT. On day 21, subjects received bilateral guide cannulae (22 ga.) stereotaxically aimed at the MPOA. Males were given mating tests on days 28 and 31 to assess the effects of peripheral DHT alone on sexual behavior. On day 32, inner cannulae (28 ga.) containing 1 mm pellets of crystalline cholesterol, DHT, or T were lowered into the brain of males treated with peripheral DHT. Mating tests were conducted 12 and 15 days after central hormone application.

The number of rats mounting or ejaculating in response to peripheral DHT alone did not differ significantly from untreated controls. In contrast, more of the males treated with intracranial DHT displayed mounting and ejaculatory behaviors than those receiving central implants of cholesterol ($\chi^2(1) = 14.672$, $p<.001$, and 5.392, $p<.05$, respectively). The behavior of the males given central DHT was not significantly different than the behavior of males treated centrally with T. Analyses performed on the implant site coordinates revealed no significant differences in cannula placement between the groups, which clustered around the medial portion of the POA and anterior hypothalamus.

The fact that combined treatment with central and peripheral DHT is sufficient in itself to significantly increase male sexual behavior is inconsistent with the hypothesis that androgens must be aromatized to estrogens for the activation of male reproductive behavior. These results are more consistent with the idea that the relative ineffectiveness of peripheral DHT in male rats is due to decreased bioavailability of this hormone to the brain regions necessary for the expression of mating behavior.

216.13 COPULATION-ILLNESS ASSOCIATIONS FACILITATED BY AN ODOR CUE: NORMAL BLOOD LEVELS OF LUTEINIZING HORMONE.
G. J. Lawrence, S. W. Kiefer, T. L. Steele, and S. K. Quadri*. Depts. of Psychology/Anatomy-Physiology, Kansas State Univ., Manhattan, KS 66506.

Normal copulatory behavior in male rodents consists of a relatively stereotyped sequence of behaviors that are accompanied by specific endocrinological responses. For instance, there is a surge of luteinizing hormone (LH) in male mice when they are exposed to an estrous female (Coquelin et al., J. Neuro., 1984, 4). Copulatory behavior of male rats can be modified by using illness as punishment, and avoidance of copulation can be enhanced if a novel almond odor is present on the female (Lawrence & Kiefer, Neurosci. Abst., 1984, 8). The present study was conducted to determine whether alterations in LH response were present in male rats trained to avoid copulatory behavior.

Male rats were trained to avoid copulatory behavior by pairing copulation with lithium chloride (LiCl) induced illness; males were made ill after each encounter with an estrous female, noncontingent on mating responses. Males in the AO group (n=13) were trained with estrous females sprayed with an almond odor and rats in the NO group (n=16) were trained with estrous females without the almond odor. Controls (n=8) for odor, illness, and test environment were included. Eight training trials were conducted; on the ninth trial males were tested as before but without illness. Immediately following the ninth trial males were sacrificed and trunk blood was collected. The blood was assayed for LH levels as described by Fayez et al. (Proc. Soc. Exp. Bio. Med., 1985, 178).

On the ninth trial, males in the AO group showed a significant avoidance of copulatory behavior (85% failed to initiate copulatory behavior on the last trial compared with 38% in the NO group and 0% of controls). Males in both experimental groups showed significantly longer latencies for each mating response (mount, intromission, and ejaculation) compared with controls. No differences were found in the LH blood levels between males in the AO, NO, and control groups. Correlations between LH levels and avoidance or response latencies were nonsignificant. These data tentatively suggest a dissociation between normal endocrinological response of male rats to the presence of estrous females and the normal behavioral response of copulation.

216.14 THE ROLE OF ANDROGENS IN BEHAVIORAL MASCULINIZATION OF RATS.
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A critical question is the identity of the active differentiating hormone or combination of hormones that mediate behavioral masculinization. This is important for our eventual understanding of the neural mechanisms underlying differentiation of sexual behaviors. Problems associated with exposing rats to either testosterone or estradiol can be overcome by giving the synthetic steroid, methyltrienolone (R1881 = 17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one). R1881 binds with high affinity to putative neural androgen receptors and doesn't compete for neural estrogen binding. Moreover, it is not presumably metabolized into either estrogens or other behaviorally less potent androgens.

Experiments were carried out to determine whether perinatal exposure to androgens, independent of estrogen stimulation, could mimic the differentiating action of the testes. In these studies, neonatal exposure to R1881 was combined with prenatal aromatase inhibitor (ATD) and neonatal castration. The rationale is that the combination of an aromatase inhibitor and neonatal castration should eliminate estrogen stimulation during the differentiating period.

Pregnant Spraque-Dawley rats were injected with either the ATD (5 mg/.1 ml/day) or vehicle from Day 10 of gestation until parturition. Males were castrated at birth and implanted with Silicone capsules containing R1881 or cholesterol; the implants were removed 10 days later. Another group of neonatally castrated males was given daily injections of 100 ug of hormone for the first five postnatal days. Some males, exposed prenatally to ATD or vehicle, were castrated at 60 days of age. At 90 days of age, rats received daily injections of 200 ug of testosterone propionate; testing began 10 days later.

Rats exposed prenatally to ATD or vehicle and castrated after the developmental period exhibited ejaculatory behavior following androgen treatment in adulthood. Males castrated at birth, irrespective of the prenatal treatment, did not show high levels of mating behavior as adults. As compared with neonatally castrated males, postnatal exposure to R1881 enhanced the ability to display masculine behavior (increased mounts and intromissions) but the rats did not ejaculate.

These results suggest that estrogen is necessary for the masculinzation of the ejaculatory system but not the mounting system. Based on this outcome, we are now determining whether exposure to estrogen in combination with R1881 mimics the masculinizing action of the testes.

216.15 AUTORADIOGRAPHIC LOCALIZATION OF ANDROGEN AND ESTROGEN CONCENTRATING CELLS IN THE BRAIN OF THE JAPANESE QUAIL. J.T. Watson and E. Adkins-Regan. Field of Neurobiology, and Department of Psychology, Cornell Univ., Ithaca, NY, 14853

The autoradiographic method was used to identify cells in the quail brain which accumulate testosterone(T) and two of its meatabolites: estradiol(E) and dihydrotestosterone(DHT). Adult (6wks.-8mons.) male and female Japanese quail (Coturnix coturnix japonica) were photically castrated and implanted with T or E implants to induce normal sexual behavior. The implants were removed, and 48 hrs. later the birds were injected with 250uCi per bird tritiated E, T, or DHT. 90 minutes after injection, the birds were decapitated, and their brains were processed for autoradiography. Following exposure for 3 to 19 months, sections were developed, stained with cresyl violet, and examined with light microscopy. One half of each of 20 coronal sections from each bird was examined in entirety for labelled cells. The number of silver grains over a cell was compared to the expected number of grains for an area of the same size assuming a Poisson distribution for the background grain level estimated in each field of view.

Labelled cells were found following all three steroid treatments, but their distribution differed between treatments. Labelled cells were found in preoptic area(POA), nucleus taeniae (TN) of the archistriatum, the lateral posterior nucleus of the hypothalamus(PHL), the periventricular magnocellular nucleus (PVM), the lateral septal region(SL), the infundibulum(INF), and nucleus intercollicularis(ICo) of the midbrain. The distribution of labelled cells by treatment was: (++=many labelled cells, +=few labelled cells, 0=no labelled cells; * indicates a group significantly different from other steroid groups)

NUCLEUS	E	T	DHT
POA	++	++	0 *
TN	++	++	0 *
PHL	++	++	+ *
PVM	++	++	+ *
SL	+	+	+
INF	++	++	+ *
ICo	+ *	++	++

These results, taken with previous studies on the specificity of activation of sexual behavior with androgenic and estrogenic metabolites of testosterone, suggest that the metabolites act at different sites in the quail brain to activate the various components of sexual behavior.

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216.16 RESTORATION OF MASCULINE COPULATORY BEHAVIOR AND BINDING OF TESTOSTERONE, ESTRADIOL, AND DIHYDROTESTOSTERONE TO ANDROGEN AND ESTROGEN RECEPTORS IN MALE RATS. M.Y. McGinnis* and R.M. Dreifuss* (SPON: J.E. Shriver) Dept. of Anatomy, Mt. Sinai School of Medicine, CUNY, New York,N.Y. 10029.

The purpose of this study was to examine the effects of physiological doses of testosterone (T), its two major metabolites dihydrotestosterone (DHT) and estradiol (E_2), and DHT in combination with E_2, on masculine copulatory behavior, and on cell nuclear androgen and estrogen receptor binding in brain and pituitary of male rats.

For behavioral studies, all animals were required to complete two ejaculatory series on the pretest for inclusion in the study. Copulators were then castrated and three weeks later tested for the absence of copulatory behavior. Non-copulators, randomly divided into 5 treatment groups, received Silastic capsules inserted under the abdominal skin as follows: 1) 2 10mm T, 2) 1 5mm 10% E_2 (diluted with cholesterol), 3) 1 5mm DHT, 4) 1 5mm 10% E_2 plus 1 5mm DHT, 5) 1 10mm Blank (Bk) capsule. Tests for restoration of copulatory behavior were given 2-4, 5-7, and 14-16 days post-treatment. Most of the rats receiving T treatment completed 2 ejaculatory series by 5-7 days. Treatment with 10% E_2 + DHT also restored copulatory behavior by 5-7 days, but not as effectively as T. In rats receiving 10% E_2 copulatory behavior was restored only after 14-16 days of exposure. DHT and Bk capsules had no effect on restoration of copulatory behavior.

For cell nuclear androgen receptor and estrogen receptor binding assays, tissues from 6 animals in each treatment group were pooled. Pituitaries were removed, and brain samples were taken from hypothalamus, preoptic area, amygdala and septum. Half the pituitaries and samples from one half of each brain area were assayed for androgen receptors, the other half for estrogen receptors. For all tissues, high levels of androgen receptor binding, were found only in the T treatment group, and high levels of estrogen receptor binding were found only in 10% E_2, and 10% E_2 plus DHT treatment groups. Our results suggest that in physiological doses, T is the major gonadal steroid that binds to androgen receptors and E_2 is the major gonadal steroid that binds to estrogen receptors. Thus, restoration of copulatory behavior in castrated rats appears to be correlated primarily with testosterone binding to androgen receptors, and it is not correlated with DHT or E_2 binding to androgen receptors or with T binding to estrogen receptors either as estradiol or as testosterone.

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216.17 CYTOSOL ANDROGEN RECEPTORS IN GUINEA PIG BRAIN AND PITUITARY. M. Bonneau*, H.B. Ahdieh*, J. Thornton and H.H. Feder. Institute of Animal Behavior, Rutgers University, Newark, New Jersey 07102.

In contrast to the rat, male sexual behavior of castrated male guinea pigs is fully restored after treatment with 5α-dihydrotestosterone in the absence of exogenous estrogen (Alsum & Goy, Horm. Behav., 5:207, 1974). This suggest that guinea pig brain may be highly sensitive to non-aromatizable androgens, yet there are no reports of the characteristics of androgen receptors in brain of this species. In addition, the possibility that noradrenergic transmission may influence the concentration and/or distribution of brain androgen receptors has not been investigated, although such an influence of adrenergic transmission on progestin and estrogen receptors has been demonstrated (Nock & Feder, Neurosci. Biobehav. Rev., 5:437, 1981; Clark et al., Brain Res., 1985, in press).

Brain and pituitary "cytosol" receptor had an apparent K_D of 0.04nM and was androgen specific (methyltrienolone > 5α-dihydrotestosterone > testosterone = estradiol > progesterone). The concentrations of "cytosol" androgen receptors in castrated adult male guinea pigs (N=8) were 12.2±1.5, 11.6±0.9, 6.9±0.6, 2.6±0.3, 1.3±0.3 femtomoles/mg protein (mean±s.e.m.) in anterior pituitary, hypothalamus, medial preoptic area, amygdala and cerebral cortex respectively. No significant difference in the concentration of receptors was observed between castrated adult male and female guinea pigs. The systemic injection of 5mg/kg of Prazosin (an α_1-adrenergic antagonist) had no significant effect on the concentration of receptors in castrated adult males in any brain area.

The very high affinity of guinea pig brain "cytosol" androgen receptors and their localization to brain areas involved in reproductive processes is consistent with the previous report that non-aromatizable androgens stimulate masculine sexual behavior in this species. These results suggest that, at least in guinea pigs, brain androgen receptors might be crucially involved in the regulation of male sexual behavior.

216.18 ESTROGEN INCREASES CYTOSOLIC ANDROGEN RECEPTOR IN PITUITARY AND SPECIFIC BRAIN NUCLEI OF THE MALE RAT. R.J. Handa*, C.E. Roselli and J.A. Resko*. Dept. of Physiology, Oregon Health Sciences Univ., Portland, OR 97201 and Dept. of Reproductive Biology and Behavior, Oregon Regional Primate Res. Center, Beaverton, OR 97006

In the rat, estrogen synergizes with androgen to facilitate male reproductive behavior. To determine the biochemical basis of this synergism, we examined the in vitro binding of the synthetic androgen, R1881, to brain and pituitary (PIT) cytosol of male rats exposed to estrogen for 7 days. Animals were castrated 7 days before implanting 2.5 cm Silastic capsule containing crystalline 17β-estradiol (E). Controls were sham implanted. Purified cytosols were prepared from PIT or hypothalamus-preoptic area (HPOA). Incubation with increasing concentrations of [^{3}H]-R1881 revealed the presence of a single, saturable, high affinity receptor with a K_d of 0.9 X 10^{-10}M in both PIT and HPOA. Triamcinolone acetonide (10 μm) was added to the incubation tube to prevent binding to the progesterone receptor. Competition studies using excess radioinert steroids showed that the binding of [^{3}H]-R1881 was specific for androgen receptor (AR). Single point determination of AR in PIT cytosol showed significantly greater amounts in E-treated males (42.2 ± 3.0 fmol/mg protein [mg], n=9) compared with controls (26.4 ± 1.65 fmol/mg n=11; $p<0.01$). Values were comparable when [^{3}H]-DHT was substituted as the radioligand (43.0 ± 2.4 vs 24.0 ± 1.0 fmol/mg n=5, $p<0.01$). In addition, we measured AR in discrete brain nuclei and subregions from animals perfused with 10% cold DMSO. Brains were frozen, sliced at 300 μm and 16 brain nuclei were punched according to the method of Palkovits. Nuclei from 5-6 animals were pooled for each determination. Highest levels of AR were found in the ventromedial n. (17.2 ± 0.6 fmol/mg), medial preoptic n. (MPOA, 12.8 ± 1.6 fmol/mg) bed n. of the stria terminalis (BNST 11.6 ± 1.5 fmol/mg), lateral septum (12.4 ± 1.8 fmol/mg) and medial amygdala (MA, 10.6 ± 0.7 fmol/mg). E treatment increased ($p<0.05$) AR in the MPOA (15.5 ± 0.5 fmol/mg), BNST (15.6 ± 0.8 fmol/mg) and MA (14.9 ± 1.5 fmol/mg). No differences were detected in nuclei with intermediate levels of binding (5-10 fmol/mg; suprachiasmatic n., periventricular preoptic n., lateral preoptic n., periventricular ant. hypothalamic n., ant. hypoth. n., arcuate/median eminence, or hippocampal CA_1) or in 4 nuclei with low levels of binding (1-5 fmol/mg). These data demonstrate that E can modify AR numbers and that these increases are restricted to the pituitary and specific brain regions which appear to mediate male reproductive behavior. Supported by HD 06731, HD 18196. HD 07133.

216.19 TESTOSTERONE BLOCKS THE AUTORADIOGRAPHIC LABELING OF NEURONS BY [^{3}H]ESTRADIOL IN THE BRAINS OF FEMALE RHESUS MONKEYS. H.D. Rees and R.P. Michael. Dept. of Psychiatry, Emory Univ. Sch. of Med., and Georgia Mental Health Inst., Atlanta, GA 30306.

The administration of testosterone (T), either systemically or intracerebrally, increases female sexual receptivity and motivation in ovariectomized rhesus monkeys. The aromatization of T to estrogenic metabolites may play a role in its behavioral actions. The activity of aromatizing enzymes varies widely in different regions of the brain. Autoradiography was used to localize precisely the brain regions in which aromatized metabolites of T may influence estrogen target neurons in female rhesus monkeys. Five females, weighing 5.9-7.6 kg, were ovariectomized and injected sc with either vehicle (controls) (N=2), 2 mg testosterone propionate (TP) (N=2), or 2 mg dihydrotestosterone propionate (DHTP) (N=1) daily for 4 days beginning the day of ovariectomy. Four hours after the last injection, each female received 1 mCi [2,4,6,7-^{3}H]17β-estradiol iv (Amersham; 93 Ci/mmol). One hour later, the animals were killed and the left halves of brains and samples of peripheral tissues were frozen and stored in liquid nitrogen. Frozen sections were cut at 4 μm in a cryostat and thaw-mounted on emulsion coated (NTB3) slides. After exposure for 20 weeks at -19^{o}C, the slides were developed and stained with methyl green-pyronin. A cell in the brain was considered labeled if its nucleus had a silver grain density at least twice that of adjacent neuropil background.

Labeled neurons in the vehicle-treated controls were located in the medial preoptic nucleus (n.), anterior hypothalamic area, arcuate n., ventromedial n., paraventricular n., premammillary n., supramammillary n., lateral septal n., bed n. of stria terminalis, medial, cortical and accessory basal amygdaloid n., and claustrum. In the brains of the TP-pretreated females, the labeling of neurons was virtually abolished except in the arcuate n. and lateral septal n., where the percentage of neurons labeled remained high and similar to that of the controls. In contrast to TP, pretreatment with the non-aromatizable androgen DHTP did not affect labeling in any brain region. Neither TP nor DHTP pretreatment had any effect on the labeling of cells in the pituitary gland, uterus, vagina, or clitoris. These data suggest that metabolites of T occupy nuclear estrogen binding sites in estrogen-concentrating brain regions, except the arcuate n. and lateral septal n. Thus, T appears to act as a regionally specific estrogen in the female primate brain.

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216.20 SOCIAL STIMULI INCREASE PROGESTIN RECEPTOR LEVELS IN HYPOTHALAMUS BUT NOT IN PREOPTIC AREA OF THE FEMALE PRAIRIE VOLE, AN INDUCED OVULATOR. M.Cohen-Parsons, C.S.Carter* and E.J.Roy. Depts. of Psychology and Ecol., Ethol., and Evol., University of Illinois, 603 E. Daniel, Champaign, IL 61820.

Cross-species comparisons reveal a general pattern of steroid target cell distribution in the vertebrate brain. In female animals, high concentrations of estrogen (E) concentrating cells are found in the medialbasal hypothalamus (MBH), preoptic area (POA) and the limbic forebrain. In intact laboratory rats, the binding of E to brain cell nuclei fluctuates with the secretion of ovarian E over the estrous cycle. In these females, E induces the synthesis of receptors for progesterone (P), thereby priming the female for maximal responsivity to P in areas of the brain which are known to regulate sexual behavior and ovulation.

In contrast to the spontaneous ovulators, in prairie voles E and P do not synergize to facilitate behavioral estrus. Female sexual behavior in prairie voles is E-dependent, and pheromonal and tactile stimulation from males cause an increase in cell nuclear E receptor binding in the brain. Although the level of cytosol progestin receptors increases in the MBH of sexually activated females, no difference in progestin receptor levels occurs in the POA. These results indicate that although the presence of E-accumulating cells is a general characteristic of the vertebrate brain, the ability of cells in different brain regions (MBH vs POA) to respond to E may differ among species. These differences may be related to the differential control of sexual behavior and/or ovulation among species.

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216.21 ACTION OF ESTROGEN IN MALE HIPPOCAMPUS. Y.L.T. Ting* and T.J. Teyler (SPON: D. Riccio). Neurobiology Program, N.E. Ohio Univerisities College of Medicine, Rootstown, OH 44272.

A crossed hormone effect on tissue excitability has been demonstrated in hippocampal slice preparations. Active forms of estradiol increases the population spike amplitude of hippocampal slices taken from male rats by as much as 250%. A less prominent effect of testosterone on slices from female rats has also been observed (increase of 150%) (Teyler et al., Science, 209:1017, 1980). Such changes appeared rapidly, occuring within 5 minutes after drug administration. To demonstrate that such electrophysiological changes are not limited to slice preparations, the experiment has been replicated in intacts.

Varying doses of beta-estradiol-3-benzoate dissolved in safflower oil was injected I.P. into male hamsters weighing 95-130 grams. I.V. injections of 17-beta-estradiol suspended in PVP were also used. Stimulation of the Schaffer collaterals and recoridng from CA1 pyramidal cells of the hippocampus was achieved with tungsten electrodes. A constant level of anesthesia was maintained with an infusion pump of nembutol-saline solution. After baseline determinations, the tissue response to the drug was tracked for 45 minutes.

Preliminary data shows that intact hippocampus excitability has a dose dependant property parallel to that of slice preparations. In addition, the changes occurred rapidly as in the slice, suggesting the operation of a mechanism of action other than that of the classical steroid hormone.

217.1 CHOLECYSTOKININ MODULATION OF DOPAMINE-MEDIATED BEHAVIOR: IMPLICATIONS FOR TOURETTE SYNDROME. H.K. Kulmala, J.W. Boja*, and J.A. Nielsen, Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Tourette Syndrome (TS) is a developmental disorder which appears to involve a hypersensitivity of striatal dopamine (DA) systems. Unilateral asymmetries in DA, itself, or in DA receptor concentrations in rodent striata are reflected by circling behavior. Thus, changes in rotational activity can be used to predict the efficacy of agents for treating TS. We have utilized this behavior to determine the possible efficacy of cholecystokinin-8 (CCK) in TS.

Rats received injections of 2ul of artificial CSF or 100 ng CCK in this vehicle through indwelling right lateral ventricular cannulae 1 hr. prior to testing. Two and four days later, animals received artificial CSF, followed by testing. Rotational activity was evaluated every other day in darkened, computerized circular chambers. Total left versus right, one-quarter and full turns were determined for 15 minutes before and 30 minutes after rats received either 2.5 mg/kg d-amphetamine (AMPH) or saline administered intraperitoneally. Once baseline behavior returned, a second dose of CCK or CSF was administered and animals were tested as above.

Animals acclimated to the chambers by the second or third trial. Some rats showed an inherent left or right turning bias, whereas others showed little bias. Saline injections did not significantly change the direction or quantification of circling. The effects of AMPH were consistent in any one animal, but varied across the group. Left-biased animals showed little change in total rotational activity or directional preference, whereas right turning was significantly amplified. Intraventricular CCK had differential effects on left- and right-biased rats. Rotation was decreased or changed to right-turning in previously left-biased animals, an effect still seen at 2 days after CCK. In right-biased animals, a decrease in right-turning was evident.

CCK had been reported to decrease DA receptor number. Thus, following unilateral intraventricular injection, we expected to create a hyposensitive striatum, resulting in spontaneous or amphetamine-revealed right-turning. The data in left-turning animals is consistent with this expectation. The data in right-biased animals indicates that CCK also may have effects not related to the action on DA receptors. Further experiments are in progress to expand upon these findings.

Supported by a research grant from the Tourette Syndrome Association to H.K. Kulmala.

217.2 **GABAERGIC AND CATECHOLAMINERGIC SYNAPTIC INTERACTIONS IN THE MACAQUE HYPOTHALAMUS: DOUBLE LABEL IMMUNOSTAINING WITH PAP AND COLLOIDAL GOLD.** K.K. Thind, T. Song* and P.C. Goldsmith. Dept. of OB/GYN and Repro. Sci., U.C.S.F., San Francisco, CA 94143.

Gamma-aminobutyric acid (GABA) has been implicated as an inhibitory neurotransmitter in control of gonadotropin secretion. In this study, we analyzed GABA-CA neuronal interactions in the anterior ventral periventricular area (AVPV), and the arcuate nucleus (ARC) and adjacent periventricular zone (ARC-PVZ) of the primate hypothalamus.

Adult male Rhesus monkeys were perfused with aldehydes. Coronal Vibratome sections (40 microns) were immunostained sequentially for tyrosine hydroxylase (TH) with the peroxidase anti-peroxidase (PAP) technique, and for glutamate decarboxylase (GAD) with immunogold (15 nm), so that the location of both enzymes could be visualized simultaneously at the light microscopic (LM) and electron microscopic (EM) levels. Rabbit anti-TH (#16) and sheep anti-GAD (#1440-4) were generously supplied by A.W. Tank and V. Weise respectively. At the **LM level**, TH-immunoreactive (-IR) perikarya (brown) were observed in the ARC, ARC-PVZ, and the AVPV, where positive fibers were plentiful. GAD-IR processes (red), present throughout the hypothalamus, appeared to contact some TH-IR neurons. GAD staining was especially dense in the AVPV, where a substantial overlap of TH and GAD fibers occurred. At the **EM level**, PAP was present in perikarya, dendrites, axons, and axon terminals of TH-IR neurons. Colloidal gold particles were found only in dendrites and axon terminals of GAD-IR neurons. Labeled GAD terminals typically contained small, clear synaptic vesicles, while TH terminals contained these and sometimes one or two dense core vesicles. No evidence for cross-labeling was observed, and appropriate controls confirmed the specificity and selectivity of the method. In the **ARC and ARC-PVZ**, asymmetrical (Gray I) axo-dendritic synapses occurred between GAD and TH-IR profiles, with TH/GAD directionality more prevalent. Symmetrical (Gray II) synapses were less common, with either TH or GAD pre-synaptic in axo-dendritic and dendro-dendritic contacts. GAD/GAD interactions were not seen, but TH/TH contacts appeared to be dendro-dendritic. In the **AVPV**, only symmetrical synapses were observed, and their directionality was difficult to determine. GAD- and TH-IR dendrites frequently formed dendro-dendritic synapses, but GAD/TH dendro-somatic synapses were rare. Again no GAD/GAD interactions were seen, but TH/TH contacts were often dendro-dendritic and rarely dendro-somatic.

These results illustrate the complex interactions of GAD and TH containing elements in the neuroendocrine hypothalamus. Since both GABA and CA neurons concentrate estrogens, they also suggest how their connections might affect gonadotropin secretion in primates.

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217.3 VIP STIMULATION OF SECRETION AND ADENYLATE CYCLASE IN RAT PAROTID ACINAR CELLS. M.K. McMillian*, M.M. Freiden* and B.R. Talamo. Neurosciences Labs, Tufts Medical School, Boston, MA 02111

Vasoactive intestinal peptide, VIP, and acetylcholine are co-localized to nerves in the rat parotid gland. As with acetylcholine and α-adrenergic agonists, VIP is a weak stimulator of exocytosis of amylase from parotid acinar cells, cell aggregates or tissue fragments. Unlike these agonists, however, it does not stimulate increases in calcium levels as measured by 22Na+ uptake.

It has been suggested that VIP stimulates exocytosis via cAMP. VIP also has been reported to elevate cAMP in rat parotid acinar cells, but at maximally active concentrations it does not detectably elevate cAMP accumulation in our system. However, under some conditions VIP can activate adenylate cyclase in parotid cells, albeit far less effectively than do β-adrenergic agonists, which are also more potent stimulators of exocytosis. Forskolin or a phosphodiesterase inhibitor, RO 20-1724, greatly potentiate VIP effects on cAMP level and amylase release. Forskolin enhances the secretory response without significantly shifting the EC50 for VIP, which is about 1nM for cell aggregates. Although cAMP accumulation can be boosted (4 fold) by using a combination of VIP and phosphodiesterase inhibitor, maximal amylase release comparable to that elicited with isoproterenol cannot be achieved. Paradoxically, VIP is as effective as isoproterenol in activating adenylate cyclase in a membrane preparation. The EC50 for enzyme activation is comparable to that for stimulating cAMP accumulation or amylase release from intact cells.

This association of very low cAMP accumulation with amylase release parallels the effects seen with isoproterenol, where the capacity to stimulate cAMP accumulation can be shown to be far in excess of the amount of cAMP which accumulates under conditions of maximal exocytosis. Unlike isoproterenol, with VIP there do not appear to be spare receptors for the secretory response; the amylase release curve parallels the cAMP accumulation dose-response curve (detected in the presence of forskolin).

Muscarinic and α-adrenergic agonists, which inhibit adenylate cyclase stimulation by isoproterenol in the heart, do not inhibit either the accumulation of cAMP or the release of amylase stimulated by VIP or isoproterenol in this system. This work is supported by NIH grants RO1-NS17331 and PHS P30AM39428.

217.4 ROLE OF CHOLINERGIC NEURONS IN THE CENTRAL CARDIOVASCULAR ACTIONS OF SUBSTANCE P. G.R. Trimarchi*, W.C. Glisson*, W.M. Thompson*, J.C. Van Lingen* and J.J. Buccafusco (SPON: R. Borison). Dept. Pharmacology and Toxicology, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

The role of cholinergic neurons in central cardiovascular regulation is not well understood, however, activation of brain cholinergic neurons in several species evokes a hypertensive response. It also has been reported that central cholinergic blockade in hypertensive animal models produces an antihypertensive response. It is possible that brain acetylcholine may, at least in part, play a role in the maintenance of elevated blood pressure. As with central cholinergic stimulation, intracerebroventricular (icv) injection of substance P (sP) elicits a pressor response in unanesthetized rats. The purpose of this study was to determine whether the cardiovascular effects following icv injection of sP are mediated by central cholinergic neurons. Male, Wistar rats were anesthetized, and chronic indwelling arterial and icv cannule were implanted. Mean arterial pressure (MAP) and heart rate (HR) were recorded from unanesthetized, unrestrained animals. Icv injection of sP (0.05, 0.5 and 5 ug) produced a dose-related increase in MAP of 21 ± 7, 27 ± 3 and 41 ± 2 mmHg, respectively and an increase in HR of 110 ± 44, 145 ± 8 and 178 ± 13 beats/ min, respectively. The maximal increase in MAP occurred between 5 and 25 min after injection, as the dose administered was increased. For the highest dose, MAP was significantly elevated even 45 min after injection. HR increased rapidly just after injection, the response peaked with 5 min, and then returned towards baseline by 30 min. Hemicholinium-3 (HC-3), when administered centrally, causes a marked depletion of brain acetylcholine. One hr following HC-3, icv injection of sP (0.5 - 5 ug) elicited a pressor response significantly reduced in magnitude and duration. For example, an increase of only 7 ± 6 mmHg was observed following 5 ug of sP, and this was maintained only briefly. HC-3 pretreatment did not significantly alter the entire course of the sP-induced tachycardic response, however, there was a significant inhibition in the initial rise in HR, and an increase in the time to peak of the response. These findings indicate that functioning cholinergic neurons are required for the full expression of the cardiovascular response to central injection sP. Since HC-3 is a presynaptic inhibitor of cholinergic neurons, it is possible that sP elicits its central pharmacological actions through the enhanced release of acetylcholine. Supported by HL30046 and the Veterans Administration.

217.5 CHOLECYSTOKININ BLOCKS EFFECTS OF GABA ON HIPPOCAMPAL POPULATION SPIKE. J.D. Stittsworth, Jr. and W.J. Giardina
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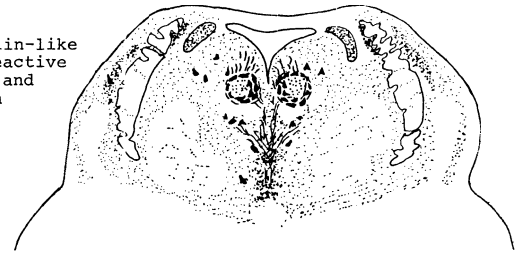
Cholecystokinin (CCK) and GABA immunoreactive material has been reported to coexist in hippocampal neurons (Somogyi et al., The Journal of Neuroscience, 1984). Bradwejn and deMontigny (Nature, 1984) reported that i.v. administration of benzodiazepines reversed CCK-induced activation of CA1 or CA3 hippocampal neurons. The benzodiazepine receptor is thought to be coupled with the GABA receptor. Because CCK and GABA immunoreactive material coexist in hippocampal neurons and because of the CCK-benzodiazepine interaction, a question of functional significance is raised. We have studied the effects of CCK_8, CCK_4, bicuculline (BIC) and GABA on the population spike (PS) recorded from CA1 hippocampal pyramidal neurons.

The PS was recorded from 400 µm thick hippocampal slices prepared from brains of guinea pigs. The slices were placed in a recording chamber at the gas-fluid interface, in a noncirculating bath. A stimulating electrode was placed in Schaffer Collaterals and a recording electrode was placed in the pyramidal cell layer of CA1. The stimulus was delivered at a rate of once every 10 seconds. The PS was averaged (n=2) once every min. When the amplitude of the PS was stable, ten control recordings were made and their average amplitude calculated (control = 100%). Compounds were added to the chamber by micropipetting (20 µL) near, but not on, the slice. The PS amplitude was recorded every min for 65 min following administration of the test compound. 100 nM CCK_8 had no effect on the PS amplitude. 1 µM CCK_8 caused a slow increase in the amplitude of the PS, beginning 25 min after application to the bath; the amplitude, after 65 min, was 150% over control. The effect of 10 µM CCK_4 could be seen earlier beginning 10 min after bath application. At the end of the 65 min, the CCK_4-induced enhancement was 220% over control. The effect of the GABA antagonist BIC (300 nM) was an immediate enhancement of the PS to 150% over control. Application of 100 µM or 30 µM GABA caused a suppression of the PS. At 100 µM the GABA-induced suppression was seen immediately, and 30 µM GABA caused an initial enhancement (70% over control) followed by nearly total suppression within 45 min. When CCK_8, CCK_4 or BIC were added to the bath 15 min prior to GABA application, the GABA-induced suppression was blocked. 100 nM CCK_8, a concentration which has no effect on the PS, also blocked the GABA-induced suppression. The interaction of CCK and GABA is similar to that of BIC and GABA. These data suggest that CCK may affect GABA neurotransmission in the hippocampus.

217.6 RELATIONSHIP BETWEEN CATECHOLAMINE, ENKEPHALIN, SUBSTANCE P AND NEUROTENSIN IMMUNOREACTIVE NEURONAL SYSTEMS IN THE INFANT HUMAN BRAIN. N. Sakamoto*, J-P. Michel*, K. Kitahama*, N. Kopp* and J. Pearson. Osaka Univ., Japan, Faculté de Médecine and Hôpital Neurologique, Lyon, France and NYU Medical Ctr., New York, NY 10016.

Three human infant brains were studied after perfusion fixation with formalin using an immunoperoxidase technique modified to greatly improve sensitivity and to reduce background to virtually zero. High dilution of primary antibody, very prolonged incubation, extended rinses, blocking of nonspecific absorption by normal serum at all 3 stages involving antibodies, and metallization using nickel ammonium sulfate in the final incubation mixture give superior results.

In the medulla substance P and enkephalin axons are present in a newly demonstrated aggregate of adrenergic (phenylethanolamine N-methyl transferase-reactive) neurons dorsal to the main part of the solitarius nucleus. In the upper pons an enkephalinergic nucleus lies dorsal to the medial longitudinal fasciculus and is particularly richly innervated by substance P. Substance P-immunoreactive cell bodies are present in large numbers in the upper pontine tegmentum and junction with the mesencephalon. Substance P, enkephalin and catecholamine fibers have complex, sometimes complimentary relationships in the basal ganglia. Neurotensin neurons are prominent in thalamic and limbic systems; in the latter they are related to substance P neurons. Multiple catecholamine and peptide neurons are present in the subcortical white matter of the cerebral hemispheres. Basal ganglia and cortical terminal pattern distributions are distinctive for each substance. Preliminary data indicate that developmental changes may result in different patterns of terminal density for some transmitters in the adult.



Enkephalin-like immunoreactive neurons and axons in human infant pons.

217.7 DENERVATION SUPERSENSITIVITY TO CHOLECYSTOKININ AND DOPAMINE IN THE RAT NUCLEUS ACCUMBENS: MICROIONTOPHORETIC STUDIES. X-T. Hu* and R.Y. Wang (SPON: H. Benzinger). St. Louis Univ. School of Medicine, Department of Pharmacology, St. Louis, MO 63104.

Hökfelt et al (Nature 285:476-478, 1980) reported that approximately 40% of the dopamine (DA) neurons in the ventral tegmental area (VTA or A10) also contain cholecystokinin (CCK). These DA/CCK neurons send their projections primarily to the medial and caudal parts of the nucleus accumbens (NAc). To study the interaction between DA and CCK on NAc cells, we have shown that iontophoresis of sulfated CCK octapeptide (ionto-CCK) activated primarily cells in the medial NAc. Concurrent ionto-DA reversed CCK-induced activations and suppressed the activity of NAc cells (White and Wang, Brain Res. 300:161-166, 1984). The purpose of the present study was to investigate the development of denervation supersensitivity to DA and CCK on NAc cells after lesions of DA fibers in the medial forebrain bundle (MFB).

Rats were pretreated with desipramine, a norepinephrine (NE) uptake blocker, 30 min prior to the direct injection of 6-hydroxydopamine (6-OHDA, a relatively selective neurotoxin for catecholamine systems) in the MFB to protect the NE systems. Two other groups of rats were injected with either the vehicle or 5,7-dihydroxytryptamine (5,7-DHT, a relatively selective neurotoxin for serotonin systems) and served as controls. Five to seven days after the injection, rats were anesthetized with chloral hydrate and prepared for single cell recording and microiontophoretic studies. The ejecting current of CCK needed for increasing the discharge rate of spontaneously active cells to 80% above their basal rate (ED_{80}) or the current needed to activate quiescent cells to 2 Hz was compared in control vs. experimental groups. ED_{80} was selected because not all cells can be driven by CCK to 100% above basal rates. Glutamate was also used to activate those quiescent cells to 2 Hz of firing rate. ID50s for DA were systematically compared on spontaneously active cells as well as on those quiescent cells activated by glutamate in both 6-OHDA pretreated and control rats.

The sensitivity of NAc cells to iontophoretically applied DA or CCK was markedly enhanced (approximately 3 fold) in 6-OHDA pretreated rats but not in vehicle or 5,7-DHT pretreated groups. In addition, the enhanced sensitivity to CCK was more obvious on cells in the medial NAc than in the lateral part. No such regional differences were detected for DA. Interestingly, the number of spontaneously active cells increased in the medial but not the lateral part of the NAc.

In conclusion, the results indicated that lesions of DA/CCK fibers by 6-OHDA were effective in inducing the development of denervation supersensitivity to both CCK and DA on NAc cells. (This research was supported by USPHS grants MH-34424, MH-38794 and a RSDA MH-00378.)

217.8 DOES CHOLECYSTOKININ POTENTIATE DOPAMINERGIC ACTION IN THE NUCLEUS ACCUMBENS? R.Y. Wang and X-T. Hu*. Department of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Recently Crawley et al. (Neurochem. Int., 6:755-766, 1984) reported that cholecystokinin (CCK) injected directly into the nucleus accumbens (NAc) of the rat potentiated apomorphine-induced stereotypy and dopamine (DA)-induced hyperlocomotion. Because this facilitation of DA receptor mediated behavior by CCK was not observed when CCK was injected in the neostriatum, Crawley et al. suggested the potentiation of DA by CCK may be specific to the mesolimbic neurons, where CCK and DA coexist. We have previously shown that iontophoresis of sulfated CCK octapeptide (ionto-CCK) activated the cells in the medial but not the lateral NAc. Furthermore, concurrent ionto-DA would reverse CCK-induced activation (White and Wang, Brain Research, 300:161-166, 1984). The purpose of this study was to determine whether CCK would potentiate the effect produced by DA on NAc cells by using single cell recording and microiontophoretic techniques.

The rat was anesthetized with chloral hydrate and then mounted on a stereotaxic apparatus. Standard electrophysiological techniques were used.

As previously described (White and Wang, 1984) many NAc cells had very low discharge rates or were quiescent. Therefore, the effect of CCK on DA was studied on spontaneous activity as well as glutamate (GLU) activated cellular activity in the NAc. DA suppressed both the spontaneous as well as GLU-activated activities. However, the GLU-activated quiescent NAc cells appeared to be more sensitive to DA inhibition. Consistent with our previous finding, CCK was much more effective in activating medial than lateral NAc cells. In the medial NAc when CCK concurrently iontophoresed with DA, the ejecting current was adjusted so that CCK by itself did not have direct effect on the cellular activity, CCK attenuated markedly DA-induced inhibition. CCK was also effective in reversing DA-induced inhibition on lateral NAc cells. Of 15 NAc cells studied, under no circumstance was there any sign of potentiation of DA by CCK. Furthermore, when CCK was applied concomitantly with GLU (n=7), CCK enhanced GLU-induced excitation.

In conclusion, CCK potentiated GLU-induced excitation and attenuated DA-induced inhibition on NAc cells, suggesting that CCK and DA have opposite roles in modulating the activity of NAc cells. Our results, therefore, do not support the view that CCK potentiates DA at cellular level. (This research was supported by USPHS grants MH-34424, MH-38794 and a RSDA MH-00378.)

217.9 EFFECTS OF NALTREXONE ON MONOAMINES IN ESTROGEN-PRIMED RATS. D. L. Allen and V.N. Luine. (SPON: I. Wajda)
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Opiates are known to be involved in the hormonal control of gonadotropin release and male sexual behavior, and may have a role in the control of female sexual behavior. We have recently shown (Allen, et. al., Hormones and Behavior 19:98, 1985) that the opiate antagonist, naltrexone (NTX, 3 mg/kg s.c.) facilitates lordosis responding in estradiol benzoate (EB)-treated female rats. The facilitation of behavior was blocked by pargyline, a monoamine-oxidase inhibitor, suggesting a possible interaction of monoamines and opioids in the regulation of female sexual behavior. The purpose of the present experiment was to further investigate possible opiate-monoamine interactions in the regulation of sexual behavior.

The effect of naltrexone on monoamine levels and serotonin turnover in discrete brain regions was investigated. Ovariectomized rats were injected with EB (5 ug), and 48 hours later with naltrexone (3 mg/kg) or saline. Three hours later, when naltrexone-treated rats would be sexually receptive, all rats were injected with pargyline (75 mg/kg) or saline, and sacrificed twenty minutes later in the dark. Discrete brain regions were analyzed by the punch technique. The brain regions sampled were the dorsomedial nucleus and the lateral portion of the ventromedial nucleus of the hypothalamus, anterior hypothalamic nucleus, arcuate-median emminence, medial preoptic area (POA), medial nucleus of the amygdala, and the dorsal and lateral mesencephalic central gray. Levels of monoamines were detected by HPLC with electrochemical detection.

Naltrexone in estradiol-primed female rats produced limited effects on monoamine levels and serotonin accumulation. There was no effect on dopamine, norepinephrine or serotonin levels in any of the brain regions. Naltrexone treatment decreased the rate of accumulation of serotonin after pargyline in EB-primed female rats only in the preoptic area (14.0 $\pm$ 3.4 pg/ug protein/hr in EB vs. -1.1 $\pm$ 3.7 in EB + NTX, $p < .01$), suggesting decreased turnover.

Thus, estradiol and naltrexone, in doses that facilitate sexual behavior, result in limited effects on monoamine levels and serotonin accumulation in the areas examined. The only change was a dramatic decrease in 5-HT accumulation after pargyline in the POA, a region involved in the control of female sexual behavior. Possible interactions between opiates and monoamines in the control of sexual behavior need to be further examined.

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217.10 THE SAME POTASSIUM CONDUCTANCE IS INCREASED BY ACTIVATION OF μ-OPIOID RECEPTORS AND α_2-ADRENOCEPTORS. J.T. Williams and R.A. North. Neuropharmacology Laboratory, 56-245, M.I.T., Cambridge, MA 02139.

Intracellular recordings were made from neurons in the locus coeruleus in slices cut from rat pons and maintained in vitro. Membrane currents were recorded with a single electrode voltage-clamp amplifier. Opioids and catecholamines produced outward currents at membrane potentials between -40 and -110 mV; these currents have been shown previously to result from opening of membrane potassium channels. The receptors activated by the opioids and catecholamines on locus coeruleus neurons have been shown by determinations of dissociation equilibrium constants for antagonists to be μ and α_2 types respectively. The purpose of the present experiments was to test the hypothesis that the different cell surface receptors were coupled to different populations of potassium channels. Addition of quinine (50 μM - 1 mM), substitution of barium for calcium, or substitution of rubidium for potassium, in the superfusion solution decreased the outward currents induced by both opioids and α_2-adrenoceptor agonists to a similar extent. The maximum outward currents produced by [Met]enkephalin (30 μM), normorphine (10 μM) and clonidine (1 μM) were the same (279 $\pm$ 28 pA, n = 13). The currents induced by a combination of any two agonists ([Met]enkephalin and normorphine; [Met]enkephalin and clonidine; noradrenaline and normorphine; [Met]enkephalin and noradrenaline) were not larger than the maximum current observed in the same neuron in response to any agonist applied alone. For example, the outward current observed during application of [Met]enkephalin (10 μM) was not further increased when the superfusion solution was changed to one which contained both [Met]enkephalin (10 μM) and clonidine (100 nM or 1 μM), or to one which contained both [Met]enkephalin and normorphine (1 or 10 μM). The results indicate that α_2-adrenoceptors and μ-opioid receptors activate the same potassium conductance in an individual neuron, presumably through a shared intracellular second messenger.

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217.11 CCK-8 PEPTIDES ALTER THE RELEASE OF ENDOGENOUS DA FROM THE RAT NUCLEUS ACCUMBENS IN VITRO. M.M. Voigt, R.Y. Wang and T.C. Westfall. Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

We have previously demonstrated that sulfated CCK-8 (CCK-8S) can alter the release of DA from the nucleus accumbens (NAc) in vivo. The purpose of the present study was to examine the effect of CCK-8 on DA release in vitro in an attempt to further explore the interaction between CCK and DA release.

Male Sprague-Dawley rats (270-320 g) were decapitated and the brain quickly removed and placed into an aluminum brain mold on ice. The NAc was dissected out in two consecutive 1 mm slices which were kept separated and designated the anterior (ANT) and posterior (POST) portions, respectively. The NAc was dissected from the frontal sections using the landmarks of Horn et al (1974). 300 μm slices were prepared and incubated in a Krebs-Ringer bicarbonate buffer (KRB). Using a previously published basket method, release of endogenous DA from slices was quantitated using HPLC coupled with electrochemical detection. Slices were subjected to two 5 min pulses of high-K^+-containing KRB separated by 20 min. The effects of the indicated drugs were tested during the 5 min preceeding the second stimulation period (A2) as well as during the second stimulation period (S2).

CCK-8S was found to alter basal release from the POST section of the NAc only, whereas the unsulfated octapeptide (CCK-8US) had no effect on basal release from either region. Marked differences between the ANT and POST NAc were observed with respect to their responses to CCK-8S section on K^+-evoked DA release. CCK-8S caused a dose-dependent decrease in evoked release from the ANT NAc (1 nM to 1 μM) whereas it attenuated release from the POST NAc at only very low concentrations (0.1 and 1.0 nM). CCK-8US also attenuated release from the ANT NAc at a concentration of 1 nM, whereas in the POST NAc this concentration enhanced release. At 100 nM, this peptide had no effect on release from either region. Additionally, this peptide (at 100 nM) could block the effects of CCK-8S in both regions. Proglumide (PRG) also decreased release by itself in the ANT NAc and could not block the effect of CCK-8S in this region of the NAc. However, PRG had no effect of its own on release from the POST NAc and could block the action of CCK-8S in this region. The DA antagonist sulpiride had no effect on CCK-8S action in either region of the NAc.

These results suggest that there may be two forms of CCK receptors in the NAc that differ with respect to PRG and CCK-8US. In addition, it appears that CCK-8S does not act via the DA autoreceptor. Finally, there also seem to be major differences between ANT and POST NAc in the regulation of DA release.
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217.12 *IMMUNOCYTOCHEMICAL VISUALIZATION OF DOPAMINE, NORADRENALINE, ACETYLCHOLINE, SEROTONIN, TRYPTAMINE AND 5-METHOXYTRYPTAMINE IN THE RODENT CENTRAL NERVOUS SYSTEM.* *A. McRae-Degueurce, J. Dulluc* and M. Geffard. IBCN-CNRS, 1 rue Camille Saint-Saëns, 33077 Bordeaux cedex France.*

Antisera were raised against dopamine (DA), noradrenaline (NA), acetylcholine (ACh), serotonin (5-HT), tryptamine (T) and 5-methoxytryptamine (MT) by conjugating each molecule to bovine serum albumin and to human serum albumin via glutaraldehyde. Antibody specificity was tested in vitro with the ELISA method. A perfusion mixture composed of glutaraldehyde, allyl alcohol in a cacodylate buffer at pH 11 allowed immunocytochemical visualization of DA, NA, ACh, 5-HT, T and MT in the same rodent brain. No cross-reactivity was found among the various antisera.

This immunocytochemical approach allowed the visualization of DA cell bodies and ACh fibers in the substantia nigra (SN). ACh cell bodies were found in the striatum, medial septum and the cortex. 5-HT fibers were equally noticed in the SN. NA-containing cell bodies were restricted to the locus coeruleus and other noradrenergic cell body regions (A1, A2, A5). Neuronal populations containing HT, T and MT were found in the raphe dorsalis (RD), raphe centralis superior (RCS) and the B9 group. Specific neurotoxins such as 6-hydroxydopamine (6-DHDA) and AF64A were used to further examine the specificity of the antisera. Ten months following a unilateral injection of 6-DHDA in the medial forebrain bundles no immunoreactive DA cells were found in the SN ipsilateral to the injection site. However ACh fibers were still present. One to 3 weeks following a unilateral injection of AF64A in the SN a substantial loss of ACh fibers was noted, but the DA cell bodies were still present.

Visualization of these neurotransmitters is also possible at the ultrastructural level. This immunocytochemical approach should further our knowledge concerning the distribution, localization and synaptic organization of these neurotransmitter systems in the CNS.

217.13 NICOTINIC- AND MUSCARINIC-INDUCED RELEASE OF DOPAMINE IN CAT AND RABBIT CAROTID BODY. B.G. Dinger, L. Almaraz* and S.J. Fidone. Dept. of Physiology, Univ. of Utah School of Medicine, Salt Lake City, UT 84108.

The arterial chemosensory tissue of the carotid body consists of morphologically distinct parenchymal cells in synaptic association with afferent terminals of the carotid sinus nerve (CSN). Type I parenchymal cells contain multiple neuroactive substances, including catecholamines and acetylcholine. Natural stimuli, such as hypoxia, induce a calcium dependent release of dopamine (DA), a major catecholamine of the carotid body. Because type I cells possess cholinergic receptors (which bind ^{3}H-QNB and ^{125}I-α-BGT), and cholinergic agents have a profound effect on CSN discharge, we have investigated the possibility that DA release from the carotid body is modulated by nicotinic and muscarinic agents.

In the present experiments the release of ^{3}H-DA (synthesized from ^{3}H-tyrosine) was monitored in the in vitro cat and rabbit carotid body preparations in response to nicotinic and muscarinic agonists and antagonists. Cat carotid bodies superfused with 100% O_2 equilibrated media responded to 100 μm ACh with a 5 fold increase in ^{3}H-DA release. Atropine (0.5 μM) partially inhibited ACh evoked release, and the addition of 50 μM mecamylamine further reduced release to near basal values. Conversely, release was elevated by both nicotinic and muscarinic agonists.

In the rabbit carotid body, on the other hand, nicotinic and muscarinic receptors appear to have divergent effects on ^{3}H-DA release. In 100% O_2-equilibrated superfusion media, basal release was unaffected by bethanechol (100 μM), and ACh stimulated release only in the presence of atropine (0.5 μM). Under hypoxic conditions, however, bethanechol resulted in a 30% decrease in evoked release, suggesting that muscarinic receptors are primarily inhibitory to DA release. In contrast, nicotine produced a 50% increase in release, and preliminary data suggest that this nicotinic component can be abolished by ACh (100 μM) or bethanechol (100 μM).

Our data imply fundamentally different actions for muscarinic receptors in cat vs. rabbit carotid bodies. Interestingly, the increase in ^{3}H-DA release produced by both nicotinic and muscarinic receptors in the cat, and their mutually antagonistic effects in the rabbit carotid body, correspond precisely to the actions of nicotinic and muscarinic agents on CSN impulse activity in these two species. We are currently studying the possibility that endogenous ACh may influence DA release evoked by natural stimuli.

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217.14 THE EFFECTS OF ALPHA-ADRENERGIC AGENTS ON CALCIUM ANTAGONIST BINDING SITES. H.Matsubayashi*, S.Kito, S.Katayama, and R.Miyoshi (SPON:M.Shimoyama). Third Dept. of Int. Med., Hiroshima Univ. School of Med., 1-2-3 Kasumi Minami-ku, Hiroshima, 734, Japan.

Ehlert and Itoga described the specific ^{3}H-nitrendipine(^{3}H-NTD) binding to neurons themselves. At the last meeting of Society for Neuroscience, the authors showed that there was an interaction between the alpha-adrenoceptor and Ca antagonist binding sites. In Ca antagonist binding experiments, ^{3}H-nitrendipine has been exclusively used as radioactive ligand. In this study, ^{3}H-verapamil (^{3}H-VER) and ^{3}H-diltiazem (^{3}H-DIL), which were structually different from nitrendipine, were used to characterize binding sites of these drugs in the brain. In addition, the authors tried to study how alpha-adrenergic agents modulated Ca antagonist binding. The effects of Ca antagonists on brain adenylate cyclase activity was also studied. The 10% synaptosomal fraction of the rat cerebral cortex was prepared. In the ^{3}H-VER and ^{3}H-DIL binding experiments, the buffer was 50mM Tris/HCl, pH 7.4 (0.1% bovine serum albumin) and the incubation condition was 60min at 25°C. Adenylate cyclase activity was measured following the method of Salomon et al. The standard assay mixture consisted of 50mM Tris/HCl, 11mM creatin-phosphate, 10mM $MgCl_2$, 8mM theophylline, 0.035units/ul creatin-phosphokinase, 0.1mM ATP, 0.19uM ^{3}H-ATP and synaptosomal membrane pretreated with 100uM Gpp(NH)p for 30min at 30°C. The incubation was performed at 30° C for 10min. Through these experiments, the following results were obtained. 1) Saturation analysis of ^{3}H-VER binding in the rat cerebral cortex revealed a single binding site whose Kd value was 1.26nM and Bmax 19.4fmol/mg protein. As for ^{3}H-DIL binding sites they were not detected in our experimental conditions. 2) The inhibition experiments of ^{3}H-VER by various Ca antagonists revealed inhibitory potency of the drugs in order of diltiazem, nicardipine and nifedipine with IC_{50} values $3.5X10^{-7}$, $1.9X10^{-6}$ and $2.6X10^{-4}$ (M) respectively. 3) Yohimbine and prazosin inhibited ^{3}H-VER binding with IC_{50} values $3.5X10^{-5}$ and $3.3X10^{-5}$ (M) while they did ^{3}H-NTD binding with IC_{50} values $2.0X10^{-5}$ and $7.9X10^{-4}$ (M). Clonidine was much less potent to inhibit ^{3}H-VER binding with 30% inhibition at 10^{-4}M and it had no effect on ^{3}H-NTD binding. 4) Gpp(NH)p had no effect on ^{3}H-VER binding whereas ^{3}H-NTD was considerably inhibited. 5) Of these four kind of Ca antagonists, nifedipine was only drug to inhibit adenylate cyclase activity. The inhibition rate was 20% at the concentration of 10^{-3}M. All these results showed that there was heterogenecity of Ca antagonist binding sites in the brain and these heterogenious characters were interacted each other. Futhermore, these binding sites are deeply associated with alpha-adrenoceptor. It was also assumed that nifedipine and nitrendipine binding sites were coupling with Gi protein.

217.15 CHANGES IN BETA-ADRENERGIC RECEPTOR NUMBER AND FUNCTION FOLLOWING SELECTIVE LESIONS OF SEROTONIN AXONS. C.A. Stockmeier, A.M. Martino* and K.J. Kellar, Department of Pharmacology, Georgetown University, Washington, DC 20007.

Functional relationships between central serotonin (5-HT) and norepinephrine (NE) systems have been proposed based on anatomical, electrophysiological and biochemical evidence. We have measured the effects of selective lesions of 5-HT axons with p-chloramphetamine (PCA) or 5,7-dihydroxytryptamine (5,7-DHT) on adrenergic receptor binding and function in several areas of rat forebrain.

Rats received PCA (5 mg/kg, i.p.) on days one and three, or 5,7-DHT (7 ug free base) was injected into both the dorsal and median raphe nuclei of anesthetized rats that had been pretreated with desipramine. Five to seven weeks later the extent and specificity of the lesions were determined by measuring the uptake of [^{3}H]5-HT and [^{3}H]NE into fresh homogenates from various forebrain areas. The uptake of [^{3}H]5-HT was reduced by PCA (60-70%) and by 5,7-DHT (> 90%) in both frontal cortex and hippocampus. In contrast, neither the uptake of [^{3}H]NE nor the NE content in either area was significantly reduced by the lesions.

Lesions with PCA resulted in a 33% increase in the binding of [^{3}H]dihydroalprenolol ([^{3}H]DHA) to beta-adrenergic receptors in the hippocampus and an equivalent increase in isoproterenol-stimulated production of cyclic AMP. The binding of [^{3}H]DHA was also increased in the frontal cortex (26%). In a similar manner, lesions with 5,7-DHT increased [^{3}H]DHA binding to beta-adrenergic receptors in the frontal cortex (40%), hippocampus (46%) and hypothalamus (21%). Scatchard analyses indicated that the number of beta-adrenergic receptors was increased by 30% and 80% in the frontal cortex and hippocampus, respectively, following the lesions of 5-HT axons with 5,7-DHT. There was no change in the affinity of [^{3}H]DHA for the receptors, nor was the Hill coefficient of binding altered by the lesion. Competition studies indicated that L-isoproterenol was 2 to 3 times less potent in competing for [^{3}H]DHA binding sites in cortex from rats lesioned with either PCA or 5,7-DHT than in cortex from control rats. A similar shift in affinity of isoproterenol for beta receptors following lesions of 5-HT axons has been reported previously by Manier et al., 1983. In contrast to the increase in beta-adrenergic receptors, neither the binding of [^{3}H]prazosin to $alpha_1$-adrenergic receptors nor [^{3}H]p-aminoclonidine to $alpha_2$-adrenergic receptors was altered by either type of 5-HT lesion. These results indicate that 5-HT neurons may regulate beta-adrenergic receptor number and function in brain. This regulation offers a possibly critical link between these two neurotransmission systems, both of which have been implicated in depression and other CNS disorders.

218.1 AUTORADIOGRAPHIC MAPPING OF PROTEIN KINASE C IN RAT BRAIN WITH A PHORBOL ESTER, ^{3}H-PDBU: EFFECTS OF NEUROTOXIN LESIONS. P.F. Worley*, J.M. Baraban and S.H. Snyder. (SPON: R. Schnaar). Johns Hopkins Univ., Dept. of Neuroscience, Baltimore, MD 21205.

Protein kinase C is a calcium and phospholipid stimulated, phosphorylating enzyme present in high concentrations in the brain. Its activity appears to be linked closely to the phosphoinositide (PI) cycle, since diacylglycerol which is generated by the PI cycle may be an endogenous activator of protein kinase C. Phorbol esters are potent tumor promoters which bind to specific receptor sites with high affinity. Several lines of evidence indicate that the phorbol ester receptor is identical to protein kinase C (Niedel et al., Proc. Natl. Acad. Sci. USA 80:36-40, 1983). Accordingly, one can assess the distribtuion of protein kinase C by monitoring the binding of the potent phorbol ester ligand [^{3}H]phorbol 12,13-dibutyrate [^{3}H]PDBU). Quantitative autoradiography of rat brain sections demonstrates marked heterogeneity with dense binding in the cortex, hippocampus, striatum, cerebellum and substantia gelatinosa. To ascertain the cellular localization of receptor binding sites, we examined the effects of neurotoxin lesions upon grain densities in various areas of the brain. Administration of ibotenic acid unilaterally near the substantia nigra produces no loss of [^{3}H]PDBu binding in either the nigra or the caudate despite extensive destruction of nigral neurons. However, unilateral injections of quinolinic acid into the caudate deplete binding of [^{3}H]PDBu markedly in the ipsilateral substantia nigra as well as in the caudate itself. Accordingly, most phorbol ester receptor binding in the substantia nigra appears to be associated with terminals of the descending striatonigral fibers rather than intrinsic nigral neurons. Unilateral injections of quinolinic acid into the hippocampus produce a marked loss of grains in both the stratum oriens and stratum radiatum ipsilateral to the injection. By contrast, receptors are unchanged in the ipsilateral molecular layer of the dentate gyrus following the lesion. Thus, the loss of [^{3}H]PDBu binding in the stratum oriens and stratum radiatum reflects phorbol ester receptors associated with intrinsic neurons while the preserved binding in the molecular layer may be associated with afferent terminals. Localized injections of kainic acid into the cerebellum produce regions where Purkinje cells are destroyed with no apparent loss of granule cells. In regions of Purkinje cell loss, lower grain density in the molecular layer coincides with Purkinje cell degeneration. Accordingly, a large portion of phorbol ester binding sites in the molecular layer of the cerebellum appear to be associated with dendrites of Purkinje cells. These experiments indicate that protein kinase C is largely associated with neurons and is concentrated in both pre- and post-synaptic elements.

218.2 Ca^{2+}-ACTIVATED, PHOSPHOLIPID-DEPENDENT PROTEIN KINASE (C-KINASE) ACTIVITY IN THE HERMISSENDA NERVOUS SYSTEM. J.T.Neary, S.Naito*, and D.L.Alkon. Lab. Biophys.,NINCDS-NIH,MBL, Woods Hole, MA 02543.

Long-lasting reductions in early and late K+ currents are correlated with associative learning in Hermissenda (Alkon, Science 226, 1037, 1984). These currents can be reduced by increasing the cytosolic Ca^{2+} concentration, $[Ca^{2+}]c$, and a series of experiments suggest that an increase in $[Ca^{2+}]c$ is an early event in conditioning. However, the rise in $[Ca^{2+}]c$ is likely to be brief, whereas the physiological response is sustained. We are interested in biochemical mechanisms that underlie the sustained response to the Ca^{2+} transient, and one possible mechanism involves the activation of C-kinase by Ca^{2+} and diacylglycerol (DG), a product of phosphoinositide metabolism. In mammalian systems, DG increases the sensitivity of C-kinase for Ca^{2+} and phospholipid, thus converting it to a form which is active at low $[Ca^{2+}]c$ in a membrane environment (Nishizuka, Science 225, 1365, 1984). Here we report on C-kinase activity in the Hermissenda nervous system.

Enzyme activity is measured by the phosphorylation of endogenous substrates (Mr=20K, 28K, 54K, 56K, 71K, 82K, 87K, and >200K) and lysine-rich histone, a substrate for mammalian C-kinase. After homogenization in Tris, pH 7.0, EGTA, DTT, and centrifugation at 23,000xg for 30 min, 80-85% of the C-kinase activity is in the supernatant and 15-20% in the particulate fraction. This centrifugation step isolates cytosolic C-kinase from calmodulin kinase because the latter is present almost entirely in the membrane fraction. Cytosolic C-kinase activity is dependent on phosphatidylserine (PS) and is increased by Ca^{2+} and DG; without PS neither Ca^{2+} nor DG is effective. PS + 5-20 μM Ca^{2+} and PS + <0.5 μM Ca^{2+} stimulate ^{32}P incorporation in lysine-rich histone 10- and 3-fold, respectively, over basal control (EGTA) or Ca^{2+}. Most endogenous substrates are phosphorylated by PS + <0.5 μM Ca^{2+}, but 56K and 71K require higher Ca^{2+}. As with the mammalian enzyme, addition of DG, in the presence of PS, increases C-kinase activity, particularly at free Ca^{2+} levels of 0.5-5.0 μM. DG or 1-oleoly-2-acetylglycerol (OAG) is more effective than triacylglycerol. The major endogenous substrate for cytosolic C-kinase is Mr 56K; a protein with a similar Mr is a major substrate of endogenous cAMP-dependent protein kinase, and in intact ganglia depolarization with high external K+ increases phosphorylation in this band (S. Naito et al., this volume).

Recently it has been shown that treatment of Hermissenda photoreceptors with both a C-kinase activator (OAG) and a Ca^{2+} load produces reductions in K+ currents which last longer than those obtained with either treatment alone. This effect, which is likely to be mediated by C-kinase and calmodulin kinase, may be important for long-term storage of associative memory. (We thank L. Kaczmarek and S. DeRiemer for their C-kinase assay method.)

218.PO ELEVATED EXTERNAL POTASSIUM CAUSES PERSISTENT CHANGE OF SPECIFIC PROTEIN PHOSPHORYLATION IN HERMISSENDA NERVOUS SYSTEM. S.Naito*, J.T.Neary, M.Sakakibara*, and D.L.Alkon. Lab. of Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543.

Electrophysiological studies have indicated that protein phosphorylation plays a crucial role in reducing two potassium currents (I_A and $I_{Ca^{2+}\text{-}K^+}$) in type B photoreceptors; this reduction is similar to that observed following associative learning in Hermissenda. Recent evidence (Acosta-Urquidi et al., Science 224:1254, 1984; Alkon et al., in press; Sakakibara et al., in press) suggested that Ca^{2+}/calmodulin-dependent protein kinases and C-kinase are involved in the regulation of these potassium channels. Since it was demonstrated that membrane depolarization in the type B cell accumulated during acquisition of associative learning and that depolarization alone in type B cell induced behavioral modification in animals (Alkon, Science 210:1375, 1980; Farley et al., Science 221:1201, 1983), the effect of high external K+ induced depolarization on protein phosphorylation was examined in this study. Protein phosphorylation in Hermissenda nervous system (NS) was studied by incubating NS in artificial sea water (ASW) containing ^{32}P for 3 hr and treating it under test conditions for appropriate duration, then phosphoproteins in NS were analyzed by SDS-PAGE and autoradiography (Neary et al., Nature 293:658, 1981). High K+ (100 or 300 mM) was found to increase the state of phosphorylation of Mr 56K protein and decrease that of 25K; the latter had previously been described to be affected by 4-aminopyridine or high K+ (Neary and Alkon, J.B.C. 258:8979, 1983). High K+ also increased to a lesser degree the state of phosphorylation of 24.5K and decreased that of 20K protein. Experiments using a broken cell preparation suggested that among these proteins the 56K was phosphorylated by endogenous cAMP-dependent protein kinase and C-kinase (Neary et al., Soc. Neurosci. Abstr. 10:805, 1984; ibid., 11: in press). The reversibility of high K+ effect on protein phosphorylation was studied by transferring NS to a normal ^{32}P-containing ASW after high K+ treatment. Interestingly, the state of phosphorylation of 25K did not increase to a control level (15 out of 19 cases) for up to 30 min after removal of high K+. In contrast to the effect on 25K, the high K+-induced changes in state of phosphorylation of 56K, 24.5K and 20K did not persist after removal of high K+. Thus it is plausible that depolarization of NS causes lasting change(s) in protein kinase and/or protein phosphatase activity which specifically affect 25K phosphorylation. The role of Ca^{2+} on the depolarization-induced reduction of 25K phosphorylation is now being investigated. The present findings may provide a useful model for examining biochemical learning mechanisms in the medial type B cell previously shown to be a primary locus for long-lasting storage of associative memory (Alkon, Science 226: 1037, 1984).

218.4 PERFORMANCE CHARACTERISTICS OF THE DFPase/F^- (ENZYME) ELECTRODE IN ACETYLCHOLINESTERASE INHIBITORS OF THE ORGANOPHOSPHATE TYPE. K. S. Rajan and S. Mainer*, IIT Research Institute, Chicago, IL 60616, F. C. G. Hoskin, Illinois Institute of Technology, Chicago, IL 60616, and L. Luskus, Brooks AFB, TX

The enzyme electrode composed of DFPase attached to a fluoride ion specific electrode reported earlier (Rajan, K. S., Mainer, S., Hoskin, F. C. G. and Luskus, L., Biophys. Jl. 47, 9a, M.AM.B12, 1985) has been investigated with regard to its response characteristics in aqueous solutions of organophosphonates in the presence of cations such as Ca^{2+} and Mg^{2+} and anions such as diisopropylphosphate, NO_3^-, SO_4^{2-}, F^- and metal chelating agents. In the presence of 10^{-4}M to 10^{-2}M concentrations of the divalent ions, the electrode responds linearly to solutions of diisopropylphosphorofluoridate (DFP) in the concentration range 10^{-6} to 10^{-2}M with an efficiency of 95-98%. In the presence of 10^{-3}M NO_3^- and SO_4^{2-}, the electrode responses to DFP solutions (10^{-5}M - 10^{-2}M) are linear with an efficiency of 91%. Diisopropylphosphate and F^- ions (10^{-5} - 10^{-4}M) do not interfere with the efficiency and linear response of the electrode in DFP solutions. The effect of metal chelating agents such as EDTA, DTPA and citric acid on the performance of the electrode are being investigated in connection with their use as masking agents for trivalent cations such as Al^{3+} and Fe^{3+}. The results are considered from the point of view of the effects of the different cations and anions studied on the activity of the electrode-bound enzyme, DFPase.

218.5 ANALYSIS OF THE β SUBUNIT OF TRANSDUCIN (TD) AND IDENTIFICATION OF THE β SUBUNIT OF GUANINE NUCLEOTIDE REGULATORY PROTEINS (GNRPs) FROM RAT, HUMAN AND BOVINE BRAIN ON TWO-DIMENSION POLYACRYLAMIDE GELS. Heydorn, W.E. , Creed, G.J.*, Gierschik, P.*, Spiegel, A.M.*, Milligan, G.* and Jacobowitz, D.M. Lab. Clinical Science, NIMH, Bethesda, MD 20205

GNRPs are a family of proteins involved in signal transduction across cell membranes. All four GNRPs identified to date (TD, G_s, G_i and G_o) are heterotrimers with α, β and γ subunits. Previous studies have shown peptide map identity and immunochemical crossreactivity between the β subunit of TD and the other GNRPs. We have studied the mobility of the β subunit of purified TD using two-dimensional gel electrophoresis (2DE). In addition, we have identified the location, MW and pI of the β subunit of the GNRPs in rat, human and bovine brain on 2D gels.

In the initial experiment, 1 µg of purified TD β/γ isolated from bovine rod outer segment membranes was subjected to 2DE, then stained with silver. The majority of the protein migrated as a doublet (in the MW dimension) with approximate MWs of 36kDa and 34kDa (values similar to those determined previously for the β subunit of the GNRPs), and a pI about 5.8. In addition, small amounts of slightly more acidic forms of the β subunit could also be detected. The major 36kDa and 34kDa protein of TD β/γ separated by 2DE reacted on immunoblots with antiserum raised against both TD β/γ and the β subunit of bovine brain GNRPs, demonstrating that the β subunit of TD consists of two major polypeptides of MW 36kDa and 34kDa, and pI of 5.8. Subsequently, proteins contained within rat hippocampus, freshly dissected (during surgery) human cortex and bovine cerebral cortical membranes were studied by 2DE and immunoblotting using antisera raised against both TD β/γ and the β subunit of bovine brain GNRPs. In all three cases, the primary proteins visualized were a doublet of MW 36kDa and 34kDa and pI 5.7-5.8. Comparision of these results with those obtained using TD β/γ revealed that this doublet co-migrates with the β subunit of TD. Restaining the brain blots to identify orientation markers (e.g., actin), showed that in the rat the β subunit of the GNRPs corresponds to protein #43 of the rat brain atlas of Heydorn et al (J. Neurosci. 3:2597, 1983). In human, this protein is #M85 according to the system of Narayan et al (Clin. Chem. 30: 1989, 1984).

In summary, these results demonstrate: 1) The β subunit of TD migrates on a 2D gel as 2 polypeptides of MW 36kDa and 34kDa, and pI 5.8. 2) Rat, human and bovine brain all contain 2 polypeptides that co-migrate with the β subunit of TD and cross-react with antiserum raised against both TD β/γ and the β subunit of bovine brain GNRPs. This suggests that these are the β subunits of the GNRPs in brain. 3) The β subunit of the GNRPs is one of the major proteins present in brain.

218.6 POTENTIATING EFFECT OF PHORBOL ESTERS ON CYCLIC AMP ACCUMULATION IN RAT BRAIN CORTICAL SLICES. E.W. Karbon*, R.S. Duman* and S.J.Enna. (SPON: G.A. Robison). Depts. Pharmacol. and of Neurobiol. and Anat. Univ. Texas Med. School, Houston, Texas 77025.

Experiments were undertaken to examine the ability of phorbol esters, compounds known to stimulate protein kinase C, to influence neurotransmitter- and forskolin-stimulated cAMP accumulation in rat brain. cAMP production was studied in slices of rat brain cerebral cortex using a prelabeling technique. 12-O-Tetradecanoyl phorbol--13-acetate (TPA) markedly potentiated cAMP production induced by isoproterenol (ISO) or 2-chloroadenosine, while having little effect on cyclic nucleotide formation alone. The EC_{50} for TPA was approximately 1 µM. TPA also potentiated the cAMP response to VIP, PGE_2, and forskolin, suggesting that it influences the adenylate cyclase system at some point beyond the neurotransmitter receptor recognition site. The potentiating effect of TPA on ISO was additive with other agents known to facilitate cAMP responses such as α-adrenergic and $GABA_B$ receptor agonists. Also, these latter substances had no effect on the potency of TPA to facilitate ISO-stimulated cAMP production. The potentiating effect of TPA was not influenced by EGTA or quinacrine, which have been shown to diminish the interaction between ISO and α-adrenergic/$GABA_B$ receptor agonists. It was found that a preincubation period with TPA was necessary to observe a maximal response to this agent, a finding that is consistent with the notion that TPA is not directly modifying the receptor system. Moreover, RO 20-1724, a phosphodiesterase (PDE) inhibitor, did not alter the potentiating response to TPA with regard to ISO-stimulated cAMP production, indicating that the phorbol ester is not inhibiting PDE. Other tumor promoting phorbols such as 4 β-phorbol 12, 13-dibutyrate were also found to possess potentiating properties, whereas phorbols known not to stimulate protein kinase C such as 4 α-phorbol and 4 α-phorbol 12, 13-didecanoate did not. The results suggest that activation of protein kinase C modifies some component of the brain receptor-coupled adenylate cyclase system, resulting in an increased production of cAMP in response to neurotransmitter stimulation. It is possible that activation of this enzyme enhances the coupling between proteins involved in cAMP production or modifes adenylate cyclase to prolong its catalytic action. (Supported in part by grants from the National Science Foundation, the National Institute of Mental Health and Bristol-Myers, Inc.).

218.7 PHORBOL ESTERS: OPPOSING EFFECTS ON CALCIUM-DEPENDENT ACTION POTENTIALS OF DORSAL ROOT GANGLION NEURONS. M. A. Werz and R. L. Macdonald, Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

Stimulation of membrane inositol phospholipids has been suggested as a mechanism by which neurotransmitters mediate cellular actions. One of the products of membrane phospholipid turnover is diacylglycerol, which stimulates protein kinase C. Protein kinase C has been implicated in receptor transducing mechanisms in multiple systems. We have investigated the effects of phorbol esters, compounds that can stimulate protein kinase C at the same site as diacylglycerol, on action potentials of dorsal root ganglion (DRG) neurons grown in primary dissociated cell culture using intracellular recording techniques. Action potentials evoked from DRG neurons bathed in a tris buffered solution and impaled with microelectrodes filled with 4 M KAc have a duration of about 2 msec and have a convex inflection on the repolarizing limb. The initial component of action potentials is sodium-dependent with the convex inflection calcium-dependent. The phorbol esters, TPA (12-O-tetradecanoylphorbol-13-acetate) and PDBu (phorbol 12,13-dibutyrate), did not have visible effects on the configuration of these brief action potentials. However, when the calcium-dependent component of action potentials was augmented to durations of 20-80 msec by reduction of repolarizing potassium conductances, by either intracellular injection of the potassium channel blocker cesium or by addition of 5 mM tetraethylammonium to the recording medium, phorbol-mediated effects were observed. At membrane potentials larger than -50mV, PDBu (10 nM - 1 µM) and TPA (1 nM - 100 nM) augmented calcium-dependent action potential duration dose-dependently. The increased action potential duration produced by phorbol esters was associated with a decrease of after hyperpolarization and was blocked when intracellular cesium injection produced action potentials with durations greater than 400 msec and without after hyperpolarizations, consistent with substantial blockade of potassium conductance. With either membrane depolarization to about -35 mV or substantial blockade of potassium conductance, PDBu and TPA decreased calcium-dependent action potential duration dosedependently. The ester 4 α-phorbol was inactive at all concentrations tested (up to 50 µM). We suggest that the phorbol esters affect calcium-dependent action potential duration in two ways: by decreasing a calcium conductance and by decreasing a potassium conductance. These effects of the phorbol esters on calcium influx may have relevance for the intracellular events occurring at presynaptic terminals following neurotransmitter binding to surface receptors.

218.8 CALCIUM, cAMP, AND pH DEPENDENT PHOSPHORYLATION OF PLEUROBRANCHAEA NEURAL PROTEINS. J.M.Connor*, R.Gillette, and M.U.Gillette. (SPON: A.E.Applebaum). Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

Endogenous calcium and cAMP dependent protein phosphorylation of neural tissue affects several specific ion conductances in molluscan neurons, thereby changing their excitability. Recently small changes in intracellular pH (0.05-0.2 units) have also been shown to have profound effects on ion conductances and cAMP metabolism of the opisthobranch Pleurobranchaea (Calhoon, R. D. and R. Gillette Brain Research 271:371 1983; see Green and Gillette, this volume). In order to characterize the phosphoproteins of the nervous system we studied endogenous phosphorylation in calcium, cAMP, and pH stimulated homogenates of CNS. Phosphoproteins were identified by separation on one-dimensional polyacrylamide gel electrophoresis after $^{32}PO_4$ labeling of proteins. Calcium and cAMP stimulated phosphorylation was notable in 45 and 30 kilodalton proteins respectively. An 80 kilodalton protein was phosphorylated in the presence of either cAMP or calcium but only at pH 7.0 and not at pH 7.5 or 8.0. Phosphorylation of the 45 and 30 kilodalton proteins was not markedly sensitive to pH. The unique substrate specific pH sensitivity of the phosphorylation of the 80 kilodalton protein may relate causally to to the pH sensitivity of the excitability of many neurons. These studies provide the basis for future attempts to link changes in the excitability of Pleurobranchaea neurons to intracellular protein modification. This work was supported by NSF BNS 8308551 to R. G.

218.9 **THE KINASE C ACTIVATOR 1,2-OLEOYL ACETYL GLYCEROL MIMICKS NOREPINEPHRINE'S EFFECT ON THE VOLTAGE DEPENDENT CALCIUM CURRENT OF EMBRYONIC CHICK DORSAL ROOT GANGLION NEURONS.**
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Diacylglycerol (DAG), a product of hormone-induced polyphosphoinositide turnover, is a second messenger which initiates protein kinase C-dependent phosphorylation. The membrane-permeable analogue of DAG, 1,2-oleoyl acetyl glycerol (OAG), has been shown to induce kinase C activity in whole cells when applied extracellularly. We have tested the effects of OAG on voltage-dependent macroscopic currents recorded from embryonic chick dorsal root ganglion (DRG) neurons grown in tissue culture.

Ca currents were recorded with the whole-cell variation of the patch clamp technique. The patch pipette solution contained (in mM): 150 CsCl, 10 HEPES, 5 MgATP, 5 BAPTA and the external solution contained (in mM): 133 NaCl, 1 $CaCl_2$, 10 TEA, 25 HEPES, 0.3 uM TTX.

OAG (25 ug/ml) reduced Ca current in all cells tested (mean decrease 49.8 + 4.5%, 22 cells), an effect similar, if not identical, to that produced by 10 uM NE which reduced Ca current by an average 41.9 + 6.7%, 14 cells. I-V plots showed that NE and OAG affected only the amplitude of the peak Ca current. Neither compound changed the voltage threshold for current activation, the potential at which current was maximal, or the null potential for the current.

To determine whether NE and OAG-induced decreases in Ca current share a common pathway, the additive effects of the two drugs applied together were tested. One drug was first applied to give a maximal decrease in Ca current, after which the second drug was instantaneously applied in the continued presence of the first drug. Application of the second drug caused no further decrease in Ca current, regardless of the order in which NE and OAG were applied. This result is consistent with the idea that both NE and OAG act through a common, saturable pathway.

It appears unlikely that the NE- and OAG-induced decreases in net inward current result from an increase in an outward current. When Ca was replaced with Ba in the external solution (thereby further decreasing the likelihood of activating Ca-dependent outward current), NE- and OAG- induced decreases in inward current were even greater (OAG mean 87.9 + 4.2%, NE mean 75.9 + 7.2%, 4 cells each). Furthermore, in an external solution containing 10 mM Co and TTX (to block all inward current), OAG did not affect the voltage-dependent outward current recorded with 150 mM KCl in the patch pipette (5 cells). Similar results have been previously described for NE (Dunlap and Fischbach, J.Physiol. 317, 519-535, 1981).

In some non-neuronal cells OAG has a secondary effect (besides kinase C activation) of raising intracellular Ca concentration. The minimal concentration of OAG required to elicit this effect (50 ug/ml) is double the concentration used for this study. Furthermore, for DRG neurons the actions of OAG and NE were unaffected when intracellular Ca was buffered to either very low (less than 10^{-8} M) or very high (greater than 10^{-6} M) levels with either BAPTA or EGTA in the patch pipette solution. It is doubtful that the decreaes in Ca current due to either OAG or NE can be attributed to an elevation of intracellular Ca.

The experiments described for this study provide evidence that the diacylglycerol/protein kinase C system may mediate the NE-induced decrease in voltage-dependent Ca current of embryonic chick DRG neurons. This second messenger/kinase system could have general applicability in the study of how neurotransmitters modulate voltage-dependent ion channels.

218.10 INCREASED PLASMA CALMODULIN LEVELS FOLLOWING STRESS. K. Fliegner*, C.C. Loullis, D.D. Davis*, S.R. Dunlop*, P.A. Shea, H.C. Hendrie* and B. Beer. (SPON: S.K. Fisher). Dept. of CNS Research, Medical Research Div. of American Cyanamid, Lederle Labs, Pearl River, NY 10965 and Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46222.

The prevalence and importance of calcium as a regulator of biochemical activity is by now well established. Little, however, is known about the presumed role played by this ion in mediating the body's mobilization of resources in response to stress. Preliminary data from our laboratory indicated that calmodulin (CaM), an ubiquitous calcium-binding protein, could be measured in human plasma using a radioimmunoassay procedure. Hypothesizing that any special stress-related function of calcium may be reflected by changes in CaM levels, we have measured plasma CaM levels in high and low trait anxious subjects under baseline and stressful conditions.

Blood samples were drawn from 18 low anxious and 19 high anxious students three days prior to and then just before a mid-term examination in their undergraduate psychology course. Subjects were assigned to low and high anxiety groups on the basis of their scores on the Trait Anxiety Inventory (Spielberger et al., 1970). Both baseline (three days pre-exam) and stress (morning of exam) plasma CaM levels were then determined for both groups by radioimmunoassay.

A 2 x 2, two-way (Anxiety x Stress) analysis of variance with repeated measures on stress revealed that plasma CaM levels increased with stress ($p < 0.05$) irrespective of anxiety group. Mean $\pm$ S.E.M. values for baseline and stress levels were 334 $\pm$ 45 and 498 $\pm$ 72 ng/mL, respectively.

These preliminary results indicate that appreciable circulating levels of CaM are present in the periphery and that plasma CaM levels are modulated by stress. Interestingly, these same subjects exhibited a similar pattern of change in whole blood serotonin (5-HT) levels (Davis et al., in press). In light of these findings and the known involvement of CaM in central neurotransmission, further examinations of these stress-related changes would be important. Such investigations might reveal that peripherally circulating CaM levels can serve as a marker for central neurochemical changes that occur in psychiatric disorders and following behavioral and/or pharmacological manipulations.

218.11 INCORPORATION OF CALMODULIN INTO CENTRAL NEURONS: IMPLICATIONS FOR NEUROTRANSMISSION. C.C. Loullis, K.H. Fliegner*, J.A. Joseph, C. Sus-Binderwald* and D.H. Hellhammer. Dept. of CNS Research, Medical Research Div. of American Cyanamid, Lederle Labs, Pearl River, NY 10965 and Psychology Inst., Munster Univ., 4400 Munster, Fed. Republic of Germany.

Calmodulin (CaM), a small, ubiquitous, acidic, thermostable calcium-binding protein, plays a crucial role in cell function by virtue of its calcium-dependent ability to regulate a wide variety of enzyme systems. In brain, CaM has been shown to be involved in neurotransmission and receptor responsiveness. The role of CaM in neuronal function has been presumed, thus far, to be mediated only through its intracellular actions.

However, initial observations from in vivo push-pull experiments in our laboratories indicated the presence of CaM in brain extracellular fluid and its modulation by peripheral administration of testosterone. Data from both push-pull and slice experiments revealed that perfusion of CaM into the extracellular fluid could modulate the release and metabolism of neurotransmitters. Moreover, the amount of continuously perfused CaM recovered through the cannula was considerably less than expected, suggesting that extracellular CaM may be incorporated into neurons. To investigate this possibility, we incubated whole brain crude synaptosomes with ^{125}I-CaM at 0° C and 37° C. Cytosolic and particulate fractions were separated by centrifugation and radioactivity quantified in a γ-counter. Results indicated a temperature- and time-dependent incorporation of CaM. This incorporation was Ca^{2+}-dependent and could be abolished by the addition of excess cold CaM.

These data provide preliminary evidence that CaM exists in extracellular fluid, that its extraneuronal levels can be manipulated pharmacologically, and that CaM can be incorporated into neural cells. This incorporation process may be partially responsible for CaM's ability to modulate neurotransmitter release and metabolism. Although the precise role of extracellular CaM is only beginning to be elucidated, these preliminary data suggest the possibility that this extracellular pool, in addition to intracellular stores of CaM, is important for proper neuronal function.

218.12 INCREASED BRAIN FATTY ACID CYCLOOXYGENASE ACTIVITY IS ASSOCIATED WITH DRUG INDUCED CLONIC CONVULSIONS. T.W. Lysz, M. Centra, and P. Keeting, Depts. of Pharmacology and Biochemistry, UMDNJ-New Jersey Medical School, Newark, N.J. 07103.

We recently reported that drug-induced convulsions produced a marked increase in brain fatty acid cyclooxygenase (CO) activity. (Federation Proceedings 1985, Abs. #6184). The resulting increased activity of the CO may play a role in the enhanced prostaglandin (PG) formation measured at the onset of the first clonic convulsion. This study was undertaken to determine whether the elevated CO activity induced by pentylenetetrazol (PTZ) could be reversed by anticonvulsants.

Adult Swiss Webster mice (25-30 Gm) were injected with either 500 mg/kg ethosuximide (ESM) (ip) or saline, followed 1 hour later with 85 mg/kg PTZ (ip). Two minutes after the onset of clonic convulsions (or 4 minutes following convulsant administration if no convulsions were observed), the mice were decapitated and brain microsomes were prepared as previously described (Lysz and Needleman, J. Neurochem. 38 (1982) 1111-1117). Microsomal CO from PTZ treated mice, determined by RIA after a 5 minute incubation at 37 °C with 17 µM arachidonic acid (AA), 1 mM epinephrine, and 1 mM reduced glutathione, consistantly generated 60 to 90% more PGE_2 then did the microsomes from saline injected controls (56.8 $\pm$ 6.1 pmoles/mg protein for PTZ treated mice ($\bar{x}$ $\pm$ S.E.M., N=4) versus 31 $\pm$ 5.6 pmoles/mg protein in controls ($P < .05$). ESM effectively eliminated the clonic convulsions induced by PTZ and prevented the increase in brain CO activity associated with the PTZ challenge (38 $\pm$ 7.8 pmoles/mg protein). ESM administered ip to control mice did not affect microsomal CO activity nor did addition of the anticonvulsant in vitro. Similar results were obtained for $PGF_{2\alpha}$ production, the other major AA metabolite produced by the brain microsomes.

CO activity was significantly ($P < .05$) elevated 2 minutes after the onset of clonic convulsions. An increased, though not significant, activity of CO was measured 30 seconds after the injection of PTZ but before observable clonic convulsive activity. The CO activity returned to control levels by two hours after the last observed clonic convulsion.

These results, together with our previous findings, suggest that mouse brain fatty acid CO undergoes modification with seizure activity. The enhanced activity of the CO can be effectively reversed by anticonvulsant pretreatment. Supported by NIH grant NS-19478.

218.13 MODULATORY EFFECTS OF ASCORBIC ACID ON NEURONAL ACTIVITY IN THE NEOSTRIATUM DURING IONTOPHORESIS OF DOPAMINE AND GLUTAMIC ACID. T.W. Gardiner* and G.V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Although little is known about the functions of ascorbic acid (AA) in the brain, recent data suggest that this compound plays an important -- perhaps neuromodulatory -- role in CNS regulation. To assess the potential for AA to influence neuronal activity directly, we have begun to examine the effects of administering AA iontophoretically to neurons in the neostriatum of chloral hydrate anesthetized rats. Iontophoresis was performed using a glass, multibarreled pipette that was glued to the tip of a tungsten recording electrode. Drug solutions (0.5 M) were administered from the outer barrels of the pipette, whereas the center barrel (containing 4M NaCl) was used routinely for current balancing. In more than 30 neostriatal units examined thus far, about one-third responded to AA (15-80 nA) with a marked, dose-dependent excitation of firing rate. Moreover, when AA was administered to neurons during simultaneous activation by glutamic acid (GLU), more than two-thirds showed excitatory responses to AA. Many of these GLU-activated cells did not respond to AA alone. Ejection of AA with higher currents (80-120nA) usually produced an even greater excitation of neuronal activity, although occasionally an inhibition of activity was observed. Ejections of anodal or cathodal current alone did not produce these effects, whereas administration of isomeric AA produced effects that usually were less pronounced than those of AA.

In 10 additional neurons, AA antagonized the inhibitory effects of iontophoretically-applied dopamine (DA) in an additive manner. Although other data suggest that AA may modulate DA-receptor binding, the additivity of these responses suggests that AA and DA exert their effects upon neostriatal unit activity via independent mechanisms. Further work will explore this issue, as well as attempt to characterize the mechanisms underlying AA's neuronal effects in the neostriatum.

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218.14 SIGNIFICANT BIOPTERIN CONCENTRATIONS IN NUCLEAR FRACTIONS OF REGIONAL RAT BRAIN PREPARATIONS. S. Knapp and M. Merwin.* Dept. of Psychiatry, UC San Diego Sch. of Med., La Jolla, CA 92093

Tetrahydrobiopterin (BH_4), the required cofactor for tryptophan and tyrosine hydroxylases (TPOH and TOH), regulates the activities of these key enzymes in the biosynthesis of three neurotransmitters serotonin (5-HT), dopamine (DA), and norepinephrine (NE). Its cofactor role of making molecular oxygen available to the mixed-function oxidases has provided BH_4 with a critical functional role in the brain. However, few relevant neurochemical measures have been correlated with changes in regional BH_4 levels, and none have been examined in regional subcellular fractions. We have recently demonstrated (Knapp, S. and Merwin, M., Fourth Winter Workshop on Biochemical and Clinical Aspects of Pteridines, 1985) that the fractional distribution of BH_4 in neurotransmitter-specific regions did not correlate with previously determined TPOH activity distributions (Knapp, S., et al., JPET 189: 676, 1974).

Significant portions of regional brain BH_4 are associated with the nuclear (P_1), synaptosomal (P_2), and cytosolic (S_2) fractions rather than occurring selectively in fractions associated with neurotransmitter systems, the synaptosomal and supernatant fractions. The localization of BH_4 in other than neurotransmitter-specific fractions is consistent with the existence of multiple pools of BH_4 that may be differentially sensitive to pharmacological manipulations.

Of 30 psychoactive drugs examined, only d-amphetamine, and neither cocaine nor methylphenidate, reduced striatal BH_4 levels (Mandell, A., et al., JPET 213: 569, 1980). Amphetamine also reduces protein synthesis in cell-free systems in vitro, probably via decreased tRNA acylation (Nowak, T., and Monroe, H., BBRC 77: 1280, 1977), and disaggregates brain polysomes (Moskowitz, M., et al., PNAS 72: 834, 1974). D-amphetamine disassociates polysomes in rat brain with a time course similar to ones describing loss and recovery of TPOH activity, certain behavioral responses, and striatal BH_4 levels. We now report that most of the BH_4 decrement observed in total striate preparations after treatment with d-amphetamine (1.0 mg/kg) occurs in the S_2 cytosolic fraction. These data are consistent with there being a pool of BH_4 particularly sensitive to amphetamine's interference with tRNA acylation and/or polysomal-dependent protein synthesis.

218.15 ISOLATION OF A PLASMA ACID PROTEIN MODULATOR FOR THE HUMAN PLATELET IMIPRAMINE BINDING SITE. K.I. Abraham*and L.R.Meyerson. CV-CNS Research Section, Medical Research Division of American Cyanamid Co., Lederle Labs, Pearl River, NY 10965. Recently a substantial effort has been implemented in search of mammalian circulating modulators. The serotonin (5HT) system is an excellent target for an endogeneous modulator due to the observed diurnal variations in serum 5HT levels, platelet 5HT uptake velocity and tricyclic binding. Fresh human platelet rich plasma was obtained from normal volunteers and the platelets and plasma separated for binding assays. Platelet free plasma (PFP) when added back to platelet membranes dose responsively inhibited [3H]-imipramine(IMI) binding. In purifying the plasma modulator, PFP was applied to a blue sepharose CL-6B column to remove albumin and other plasma proteins. The unbound effluent from this column contained >75% of original IMI binding inhibitory activity. The blue sepharose CL-6B effluent was then subject to chromatography on a concanavalin A sepharose 4B column in order to remove complex carbohydrates and select glycoproteins. The effluent from this procedure contained >66% of the original IMI binding inhibitory activity and was then subject to ion exchange chromatography on a FPLC Mono Q HR 10/10 column. One peak of IMI binding inhibitory activity was eluted across a linear NaCl gradient with peak inhibitory activity eluting at 0.22 M NaCl. This peak was then chromatographed on tandem gel permeation columns (Zorbax Bio-Series GF-250) using 200 mM sodium phosphate pH 7.4 as the eluant. The apparent molecular weight of the single active fraction was ca. 48 kdal. This peak of inhibitory activity was then applied to a Mono P HR 5/20 chromatofocusing column and found extremely acid in nature (pI<4.0). Purity of the fractions containing inhibitory factor was assessed by SDS-PAGE at each step. The GF-250 fraction was insensitive to heat and acid exposure. The purified GF-250 fraction was immunoreactive with rabbit antiserum to human alpha-1-acid glycoprotein, although this does not necessarily confirm positive identity as such. The relevence of a modulator of this type is noteworthy with respect to control of the 5HT transporter.

218.16 EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF FORSKOLIN AND 2'-5'-DIDEOXYADENOSINE ON SLEEP IN RATS. S.R. Glaum, G.M. Yanik, N.M. Porter, E.H. Chen*, and M. Radulovacki. Depts. of Pharmacology and Biometry Program, University of Illinois College of Medicine, Chicago, IL 60612.

Adenosine (Ado) and related compounds are thought to affect sleep through Ado A_1 and A_2 receptors linked to adenylate cyclase (AC). Ado and Ado analogs administered in nanomolar quantities activate A_1 receptors, reduce AC activity and decrease the formation of cAMP. Activation of A_2 receptors requires micromolar quantities of Ado and Ado analogs, which stimulate AC and increase formation of cAMP. Systemic administration of low doses of Ado analogs increases deep slow-wave sleep (S_2) in rats, presumably by acting at A_1 receptors. In contrast, higher doses decrease sleep, possibly through action at A_2 receptors (Radulovacki, M., et al. JPET 228: 268, 1984).

To test the hypothesis that sleep is mediated by changes in the levels of cAMP, forskolin and 2'-5'-dideoxyadenosine (2'-5'-DDA), two compounds that interact directly with the catalytic subunit of AC, were administered to rats. Forskolin is a potent activator of AC and increases cAMP in rat brain membranes (Seamon, K.B. and Daly, J.W., J. Cyc. Nuc. Res. 7(4): 201-204, 1981), whereas 2'-5'-DDA is an antagonist of the AC catalytic subunit and decreases formation of cAMP (Londos, C., Wolff, J., and Cooper, D.M.F., In: Purinergic Receptors p. 289-322, ED. G. Burnstock, Chapman and Hall, London 1981).

Male Sprague-Dawley rats (300 gm) implanted with EEG and EMG electrodes were polygraphically recorded for 6 h following intracerebroventricular administration of 2.4, 24.0, 120.0, 300.0 or 600.0 nmoles of forskolin or .098, 0.98 or 9.8 nmoles of 2'-5'-DDA over 2 minutes. Records were analyzed as waking, light slow-wave sleep, S_2, REM and total sleep (Radulovacki, M., op. cit.).

Forskolin at all doses produced no significant change from control in the amount or quality of sleep. Similar results were obtained with 2'-5'-DDA at all doses. The results indicate that generalized stimulation or inhibition of AC is insufficient to mediate sleep in the manner of specific A_1 and A_2 agonists. We conclude from these results that non-specific modulation of cAMP levels cannot account for the alteration in sleep as seen with administration of Ado analogs. These results suggest the importance of receptor-specific changes in cAMP on the regulation of the sleep-wake cycle. (Supported by ONR Contract N000 14-79-C-0420)

218.17 MODULATION OF ADRENOMEDULLARY SECRETION OF CATECHOLAMINES AND ENKEPHALIN-LIKE PEPTIDES BY OPIATES. T. D. HEXUM AND B. A. BARRON. Dept. of Pharmacol., University of Nebraska Medical Center, Omaha, NE 68105

[Met^5]-enkephalin immunoreactive material (ME-IRM) and catecholamines are co-stored and co-released from the adrenal medulla. Splanchnic nerve stimulation brings about the secretion of these agents by releasing acetylcholine onto chromaffin cell nicotinic receptors. The presence of ME-IRM in splanchnic nerve [Hexum et al, J. Neurochem. 43 1500 (1984)] and opiate receptors on chromaffin cell membranes [Saiani and Guidotti, J. Neurochem. 39 1669 (1982), Castanas et al, Mol. Pharmacol. 25 38 (1984)] suggests that opiates may influence adrenomedullary secretion [Costa et al, Fed. Proc. 40 160 (1981)]. The effect of the opioid agonist, etorphine, and the antagonist, diprenorphine, on the release of catecholamines and [Met^5]-enkephalin-immunoreactive material (ME-IRM) from bovine adrenal glands was investigated using retrograde perfusion. Etorphine (5×10^{-7} M) inhibited the spontaneous outflow of ME-IRM by approximately 10 percent but had no significant effect on spontaneous catecholamine release. Acetylcholine (ACh) and 1,1-dimethyl-4-phenylpiperazinium (DMPP) stimulated catecholamine and ME-IRM release at concentrations ranging from 1 to 100 µM. The acetylcholine (5×10^{-5} M) and DMPP (5×10^{-5} M) stimulated release of ME-IRM and catecholamines was significantly decreased by the addition of etorphine. Diprenorphine (5×10^{-7} M) had no significant effect on the spontaneous outflow of either ME-IRM or catecholamines. However, diprenorphine reversed the inhibition of the DMPP-stimulated release caused by etorphine. After submaximal stimulation of the gland with DMPP (1×10^{-5} M), a further stimulation of release of ME-IRM and catecholamines was observed after the addition of diprenorphine alone, i.e., in the absence of etorphine. These results provide further evidence supporting the contention that opiates modulate the secretion of catecholamines and ME-IRM from the adrenal gland.

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SOMATOSENSORY CORTEX

219.1 THE THALAMOCORTICAL AND THE TRANSCALLOSAL BARREL-FIELD SLICE PREPARATIONS: PRESERVING EXTRINSIC CONNECTIONS IN VITRO. A. Agmon*, and B.W. Connors (SPON: M.W. Siegel). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Layer IV in the rodent primary somatosensory cortex contains discrete clusters of neurons and neuropil (barrels), forming an array that is topologically equivalent to the pattern of sinus hairs in the mystacial pad. Indeed, each barrel receives its main sensory input from the corresponding contralateral vibrissa. This unique structure-function relationship makes the barrel field an attractive region for investigating sensory information processing in the neocortex, and the synaptic circuitry underlying it. We chose to address these questions using the in vitro slice technique. In addition to the well-established technical advantages of the slice preparation (more stable intracellular impalements, direct access to all cortical layers, control over the extracellular environment), we have found it to offer another significant benefit: direct visualization of the barrels in the living, unstained tissue (Agmon and Connors, Soc. Neurosci. Abst. 10:493, 1984). A major drawback of the slice technique has been, so far, loss of connections with extrinsic structures. We report here two new slice preparations that include intact afferent/efferent pathways with their structures of origin: the thalamocortical and the transcallosal barrel-field slice preparations.

Rat barrel-field slices (500 µm thick) were prepared and viewed as described, with the following modifications. To preserve the thalamocortical pathway, the block of tissue to be sectioned included both the parietal cortex and the underlying striatum and thalamus, and was vibratomed in a vertical plane running anterolateral to postero-medial, approximately 45° from the coronal plane. To preserve the callosal pathway, a two-hemisphere block of parietal cortex was sectioned coronally. The afferent/efferent connectivity was assessed by extracellular stimulation and recording. In the thalamocortical preparation, responses in the barrel field were elicited by stimulation of the ventrobasal nucleus of the thalamus (VB), and vice-versa; responses in both VB and the barrel field were elicited by stimulation in the internal capsule. In the transcallosal preparation, responses lateral to the barrel field were elicited by stimulating the corpus callosum in both the ipsi- and the contralateral hemislices.

We believe that the unique opportunity to record from the barrel field in vitro under direct visual control, while stimulating specific input/output pathways, promises to make the barrel-field slice a preparation of choice for probing the synaptic circuitry of the neocortex.

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219.2 THE ROLE OF A CORTICAL BARREL IN VIBRISSAL SENSATION. R. B. Masterton and K. A. Hutson*. Department of Psychology, Florida State University, Tallahassee, FL 32306.

Three graded tactile discriminations, each requiring transduction by a single vibrissa, were investigated in rats with or without prior ablation of the vibrissa's cortical barrel. Behaviorally-derived psychophysical functions show that a single vibrissa's cortical barrel makes no measurable contribution to its vibrissa's sensitivity to sinusoidal oscillations, nor to the vibrissa's acuity for detecting changes in the frequency of oscillation. Extending the cortical ablation to include almost the entire barrel-field either contralaterally or bilaterally also has no appreciable effect on these tests of a vibrissa's spatial and temporal acuity.

In contrast, a task requiring vibrissal palpation of an object to detect its presence is completely abolished after ablation of the cortical barrel. In this latter test, the loss of a cortical barrel is indistinguishable from the loss of the vibrissa itself. Therefore, the barrel deficit in rats seems to be more of a vibrissa-specific agnosia than a vibrissa-specific anesthesia.

The similarity of the barrel deficit both to clinical astereognosis and to experimentally-derived deficits in "active" discriminations following somatosensory cortex ablations in rats and other mammals suggests that a cortical barrel's contribution to its vibrissa may not be qualitatively different than the contribution of any other (non-barrel) patch of somatosensory cortex to its (non-vibrissal) receptors.

(Supported by NINCDS-07726.)

219.3 CELLULAR PATTERNING OF METABOLIC ACTIVATION IN MOUSE SI VIBRISSAL CORTEX FOLLOWING SINGLE-WHISKER STIMULATION IN BEHAVING ANIMALS. J.S. McCasland and T.A. Woolsey. James L. O'Leary Division of Experimental Neurology and Neurological Surgery and The McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, Saint Louis, MO 63110.

The one-to-one representation of whiskers in functional columns of mouse somatosensory cortex offers an excellent opportunity to analyze the detailed spatial organization of mammalian cerebral cortex. The purpose of these experiments is to map the detailed spatial activity of a single cortical column. An in situ fixation method for cellular resolution of stimulus-dependent 2-deoxyglucose labeling (Durham et al.,1981) has been modified to achieve a ten-fold improvement in label retention. The results are consistent with the notion that the fixed 2DG is in glycogen.

Subjects had all large whiskers clipped except C3, and behaved freely for 45 minutes after 2DG injection. They were then anesthetized with phenobarbitol for 4 to 5 hours, a treatment which results in a doubling of brain glycogen. All perfusion steps are carried out at 0-4^{0}C, at pH 4, and in the presence of the glycolytic inhibitor iodoacetate. Under these conditions virtually no breakdown of 3H-2DG in glycogen can occur after the perfusion fluid has penetrated and cooled the brain. The labeling patterns obtained using this fixation scheme were very similar to those obtained with the Durham et al., '81, protocol, despite the ten-fold improvement in label retention. In control animals (no whiskers clipped) the vast majority of layer IV cells as well as adjacent neuropil regions in SI vibrissal cortex are labeled. The stimulus-dependent localization of the fixed label is seen in an isolated column of heavy label in the appropriate cytoarchitectonic zone after C3 stimulation in the behaving animal. In C3-only experimental animals, a much larger percentage of neurons are labeled in the stimulated C3 barrel than in adjacent "unstimulated" barrels. Taken together, these results indicate that the vast majority of active cells are labeled in our material.

These materials lend themselves to a detailed regional and cellular analysis of labeling patterns with respect to cortical depth and the identified columnar boundary of the barrel. Zones of heavy metabolic activity are correlated with the incidence of heavily-labeled single cells within a barrel. A regional analysis of these materials indicates a columnar correlation between patches of heavy label within the C3 barrel in serial 50u sections. The analysis also shows that the adjacent C4 and C2 barrels are differentially activated by C3 stimulation in the behaving animal such that labeling in C2 is heavier than that in C4.

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219.4 DISTRIBUTION AND FORMS OF GABAergic NEURONS IN RAT BARREL CORTEX: A COMPARATIVE IMMUNO- AND HISTOCHEMICAL STUDY OF GAD, GABA AND GABA-TRANSAMINASE. S. Lyon* and B.W. Connors (Spon: K.L. Chow). Dept. of Neurol., Stanford Univ. Sch. of Med., Stanford, CA 94305.

γ-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in neocortex. Neurons utilizing GABA are presumed to contain uniquely high levels of glutamic acid decarboxylase (GAD, the GABA synthesizing enzyme), GABA-transaminase (GABA-T, the GABA degradative enzyme) and GABA itself. We have attempted to identify GABAergic neurons in the barrel area of rat SmI cortex by using immunocytochemical methods to localize GAD and GABA, and a histochemical technique to localize neuronal GABA-T (Nagai et al., J. Comp. Neurol. 218:220, 1983).

Each stain labelled a very similar population of neurons, as judged by the nearly identical distributions of soma size and position and primary dendritic orientation. GAD, GABA, and GABA-T positive somata had maximal diameters ranging from 6-30 μm, with a mean of 14.4-15.8 μm in coronal sections. The largest cells tended to be in or near layer IV, although soma size was not strongly correlated with laminar position. On average, stained cells had a vertical orientation. In coronal sections the mean angle by which primary dendrites deviated from the normal to the pia was 30°; further, within layer IV cell profiles displayed significantly more primary dendrites in the coronal plane than in the tangential plane. However, clearly multipolar cells were commonly observed with each staining technique. The densities of GAD, GABA and GABA-T positive cell bodies were highest in layer IV, and lowest in layer I and the subcortical white matter. The distribution of stained cell bodies within barrels was examined in tangential sections through layer IV. Cell density (i.e. # of somata per unit area) was 20 to 100% higher near the wall of the barrel than in the centermost area.

All three methods stained clearly delineated punctate structures, interpretable as GABAergic terminals, whose density was highest in layer IV and lowest in layer I and upper layer V. In tangential sections through layer IV, each barrel was visible as a dense patch of stained puncta. The interbarrel septa were relatively unstained. Within single barrels there was no obvious gradient of specific terminal staining that could distinguish walls from hollows.

These results suggest that a high concentration of either GAD, GABA or GABA-T is a useful marker for GABAergic neocortical neurons. It seems likely that each histochemical method labelled the same set of neurons. Within rat barrel cortex, GABAergic neurons have diverse sizes and forms but relatively specific spatial distributions of somata and terminals.

We thank Donald Schmechel for providing the GAD antiserum. This work was supported by NIH grants NS 12151 and NS 19510.

219.5 ANGULAR SENSITIVITIES OF SMI BARREL NEURONS AND THE EFFECTS OF FENTANYL ANESTHESIA. D.J. Simons and P.W. Land. Center for Neuroscience, and Dept. of Physiology and Dept. of Anatomy and Cell Biology, Univ. of Pittsburgh Sch. of Medicine, Pittsburgh, PA 15261.

More than 200 single units in the layer IV barrels of the rat SmI cortex have been examined to quantitatively assess their responses to single vibrissa movements in eight different angles (i.e. in 45° increments relative to the horizontal alignment of the whisker rows). The vast majority of cells in the barrels responded to deflections of only one whisker; cells located in the septa between barrels responded to two or three adjacent hairs. Most cells responded maximally to whisker movements at one or two adjacent angles. For more than half of the units, responses at the "best" angle were significantly greater than to movements in 4 or more other directions; less than 20% of the units displayed no angular preference, i.e. their polar plots were circular. Among moderate to highly tuned cells, more responded best to up or up-and-back whisker displacements (42%) than to down or down-and-forward deflections (17%); less than 5% of tuned cells responded best to down-and-forward displacements. These findings may reflect a non-random distribution of mechanoreceptive endings in the whisker hair follicles.

Units recorded successively within a vertical electrode penetration through lower layer III and layer IV display qualitatively similar angular preferences. Small horizontal displacements of the recording electrode, on the order of 50-75 um, often are accompanied by a slight shift in the cells' overall angular preference. The horizontal distance required to observe a substantial shift in angular preference (i.e. through more than 270°) is considerably less than the full diameter of a barrel. In addition a single barrel does not appear to contain a simple "clock-like" distribution of best angles. One possibility is that there are two, or three, representations of angular space within a barrel each of which may correspond to one of the several metabolically active regions in the barrel centers revealed by cytochrome oxidase histochemistry.

Moderate doses of Fentanyl, a morphine analogue, increase spontaneous activity and mean discharge to whisker displacments; for a given cell the degree of angular tuning is reduced but the overall angular preference is unaffected. At low doses (4 ug/kg/hr) unit responses more closely resemble the unanesthetized state. By contrast Nembutal (sodium pentobarbital, 15-30 mg/kg) diminishes unit responsiveness and greatly reduces the ability to isolate responsive cells using fine-tipped glass microelectrodes.

Supported by NIH grant NS-19950.

219.6 INTRACELLULARLY RECORDED RESPONSES OF RAT SmI CORTICAL NEURONS TO CONTROLLED STIMULATION OF THE MYSTACIAL VIBRISSAE George E. Carvell* and D. J. Simons. (SPON: T. Plant). Center for Neuroscience and Dept. of Physical Therapy and Dept. of Physiology, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Extracellular recording studies have shown that cells in the SmI barrel cortex respond selectively to temporally and spatially patterned stimulation of the vibrissae. Intracellular recording techniques were used to examine membrane potential changes that may underly the neuronal integration of multi-whisker inputs. To date, data have been obtained from more than 40 neurons. Results show: 1) Whisker displacements elicit short-latency (7-10 ms) EPSP's of 10-15 ms duration. 2) The columnar whisker elicits the shortest latency EPSP's and, typically, action potentials (AP's). Adjacent and more distant whiskers elicit longer latency EPSP's that are smaller in amplitude and have a slower rise time. Such subthreshold EPSP's are observed even in the middle cortical depths where only a single whisker elicits AP's. 3) EPSP's with or without AP's are followed by longer-latency hyperpolarizations of the membrane potential (IPSP's) whose amplitudes are reduced with hyperpolarizing current. 4) IPSP amplitudes may be as large as 10 mV and are maximal 20-30 ms after stimulus onset; the membrane potential repolarizes over a 50-75 ms period (i.e., up to 100 ms post-stimulus). 5) Termination of IPSP's is often associated with a rebound depolarization and occassionally AP's. 6) The appearance of IPSP's coincides with an absence of spontaneous AP's. 7) AP's from deflection of a second (or third) adjacent whisker are abolished when that whisker is displaced during the period of maximal IPSP amplitude. Such response suppression can be observed even when the first (i.e., conditioning) deflection fails to elicit an AP. 8) The magnitude, and in some cases the presence, of IPSP's is related to certain spatial aspects of the sensory stimulus; these include the angle in which a single whisker is deflected, which particular whisker is stimulated, and the combination of vibrissae comprising a patterned multi-whisker stimulus.

These membrane potential changes parallel the results of extracellular studies which have suggested that the responses of cortical neurons to patterned stimulation of the vibrissae can be understood in terms of excitatory and inhibitory components of their receptive fields. The present findings also have important implications for interpreting alterations in receptive field properties of cortical neurons following peripheral deafferentation or sensory deprivation.

Supported by NIH grant NS 19950 and by the Foundation for Physical Therapy.

219.7 RECEPTIVE FIELDS OF VIBRISSAE-DRIVEN UNITS IN THE AWAKE RAT SI CORTEX: EFFECTS OF ACTIVE MOVEMENT AND ANESTHETICS. J.L.U. Guise and J.K. Chapin. Dept. of Cell Biology and Anatomy, U. of Texas Health Sci. Cntr., Dallas, Tx. 75235.

We have evaluated sensory responses of single units in the whisker region of the SI cortex (the "barrelfield") of unanesthetized, unrestrained rats. Standardized mechanical whisker stimulation techniques were employed for this purpose. Major reasons for utilizing this awake preparation were to: 1) observe the effects of spontaneous whisker movement on responses to applied whisker stimulation, and 2) compare responses of the same single neurons to equivalent stimulation in the awake, and halothane anesthetized states. A hand-held rotary motor-driven device was used to mechanically stimulate the whiskers in a relatively natural fashion by moving a bar at a constant speed and constant displacement across the whisker field. This bar was brought across the whiskers once every second in alternating directions. Rats were habituated to this procedure so that they remained relatively still.

Perievent histograms of the response to such stimulation when the whiskers were not spontaneously moving generally showed a 20ms duration peak, followed by an inhibition lasting from 20-100ms. During low amplitude spontaneous whisking movements these peaks were often unchanged in amplitude, but the post-excitatory inhibitions were markedly reduced, or even replaced by excitatory peaks. Occasionally, especially during greater amplitude whisker movements, even the short latency response peaks were facilitated or suppressed. Some cells were directional, a property which appeared to interact in complex ways with movement.

Halothane was used to test anesthetic effects because it may be quickly administered and reversed. When a neuron was isolated in the awake rat, administration of halothane typically severely reduced responses, even in low doses. Nevertheless, spontaneous activity was even more sharply reduced, thus greatly increasing the signal/noise ratio. These effects persisted for at least 30 minutes, even in the absence of behavioral effects.

To conclude, the direct, short latency responses to whisker stimulation appear to be modifiable by behavioral and drug manipulation. However, these are much less modifiable than either the longer latency responses to the whisker stimulation, or the spontaneous discharge.

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219.8 ANESTHETIC EFFECTS UPON RESPONSE PROPERTIES OF NEURONS IN THE SUPERFICIAL SI CORTEX. J.D. Greenspan and D.A. Dreyer*. Dept. of Physiology and Dental Research Center, Univ. of North Carolina, Chapel Hill, NC 27514.

A previous report described a class of neurons located in the upper laminae (I-III) of cat SI cortex which had unusual response properties, including 1) a preference for slowly moving stimuli across the skin (<5.0cm/s), and 2) suppression of activity by fast, repetitive stimuli (McKenna, et al, J. Neurophysiol., 51, 1984). This report futher describes the response properties of these "slow brush" neurons, both before and after the administration of nitrous oxide or alpha-chloralose.

Many of these units exhibited a directional preference in their sensitive range of excitatory stimulus velocities. With repeated application of computer-controlled stimuli, units would show inconsistent responses to initially excitatory stimuli. In some cases responses grew in intensity, and in other cases responses decreased in intensity with repeated brushing. Even in cases where there was no apparent trend in response across trials, the variability in a unit's response was considerably greater than that observed in the more typically described SI neurons.

Preliminary data indicate that these superficial "slow brush" neurons are very sensitive to anesthetics. Two units have been tested before and after nitrous oxide administration. A 33% concentration of nitrous oxide eliminated directional selectivity but retained velocity selectivity in one unit. A 75% concentration of nitrous oxide almost entirely eliminated another unit's responsivity.

Three out of three slow brush units were entirely suppressed by I.V. administration of 40mg/kg chloralose. In contrast, one of one layer IV "fast brush" neuron was unchanged following the same dose of chloralose. More data will be presented to futher delineate the relationship between these units' physiological and pharmacological properties.

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219.9 ACTIVITY OF POSTCENTRAL SOMATOSENSORY CORTICAL NEURONS CHANGES PRIOR TO ACTIVE MOVEMENT. R.J. Nelson. Dept. Anatomy, Univ. Tennessee, Ctr. Hlth. Sci., 875 Monroe Ave., Memphis, TN, 38163.

Previous observations have shown that many neurons in primary somatosensory cortex (SI) that respond to vibrotactile stimuli serving as the "go-cue" for hand movement also exhibit decreases in activity 60-80ms before movement even though the stimulus remains present (Nelson, Exp. Brain Res., Suppl. 10, 1985). These, and other observations, suggest that SI neurons may have their activity modulated by centrally originating inputs. This study sought to determine if other SI neurons which do not respond to the go-cue also changed activity prior to movement.

Rhesus monkeys flexed or extended their wrists in response to a vibrotactile cue (27,57 or 127Hz sine wave) delivered to their hands via a manipulandum after they maintained a fixed wrist position for a randomized hold time (0.5, 1.0 or 1.5s). The stimulus remained until the animal's hand moved 5 degrees from the held position in this self-paced task. Muscle activity was periodically monitored by placing EMG wires into nine forelimb muscles. Single cortical neuronal activity was monitored by conventional means. Of 619 SI task related cells, 103 neurons (23 from area 3a; 18 from area 3b; 46 from area 1; 16 from area 2) were chosen because: 1) each did not change activity for at least 60ms after stimulus onset and 2) each exhibited a significant change in activity prior to movement onset. A limited number of area 4 neurons were also recorded. Phasic activity changes in these occurred between 70-110ms before movement onset. Of the SI neurons, 65% responded to palpation of muscles or movement of a single joint, 20% had cutaneous receptive fields and the remainder were not tested. Both increases and decreases in activity were observed ranging from 20-170ms prior to movement onset. One possible source of modulating influences upon these cells is from peripheral receptors activated by changes in muscle activity prior to movement. It was determined that 40 of 103 cells changed activity after onset of forelimb muscle activity. Therefore it is possible that changes in activity for these cells were due to peripheral inputs. The remaining 63 neurons might receive inputs from other body regions that contribute to the observed activity changes prior to movement. This was not confirmed by natural stimulation. Of these neurons, (19 from area 3a; 3 from area 3b; 31 from area 1; 10 from area 2), 67% had changes of the same sign (increase or decrease) for both directions of movement; 10% had changes of different sign and the rest showed changes for one movement direction only.

These observations suggest that in addition to peripheral inputs, many SI neurons also receive central modulatory inputs that influence their activity prior to movement and do so at about the same time as presumably centrally originating influences modulate the responsiveness of other SI neurons to peripheral sensory input as previously described.

219.10 DYNAMIC TACTILE DISPLAYS EXCITE SI CORTICAL NEURONS. S. Warren, H. Hamalainen, P. Genduso*, J. Davis*, and E.P. Gardner. Dept. of Physiology and Biophysics, NYU Sch. Med., New York, NY 10016.

The sensation of motion across the skin can be elicited by sequential activation of a linear series of closely spaced probes, such as the OPTACON tactile array. These dynamic tactile displays have the advantage that one can precisely locate the specific elements activated at a particular moment, and can independently vary the spatial and temporal characteristics of the moving pattern. Humans can recognize letters of the alphabet displayed on the OPTACON, but accuracy of performance depends upon the mode of presentation and stimulus duration. We have adapted the tactile array of the OPTACON for use with a PDP-11/23 computer, to allow computer activation of each of 144 miniature probes arranged in a matrix of 6 columns and 24 rows separated by 1.1 mm. Rhesus monkeys have been trained to place their hands over the entire array, and to signal detection of probe activation using a RT paradigm. Single unit recordings were made in areas 3b, 1 and 2 of SI cortex from trained, fully alert animals. Over 70 cortical neurons have been studied with single bar stimuli swept over the tactile array at spatial periods of 1.1, 2.2 and 4.4 mm, and at temporal frequencies of 25, 50 and 100 Hz, in both distal and proximal directions. Cortical responses seemed most strongly influenced by the temporal frequency of probe activation, and the apparent direction of motion across the skin. At low stimulus frequencies, rapidly-adapting and motion sensitive neurons fired one or more impulses in a brief burst, with 15-18 ms latencies. While adaptation conferred directionality to the initial burst, responses during the remainder of the sweep appeared independent of the number of rows previously activated, or the direction from which individual rows were approached. Moderate stimulus frequencies (50 Hz) evoked one impulse/stimulus, but at 100 Hz such frequency following was degraded. Slowly-adapting neurons usually responded more vigorously to low frequency stimuli, but did not demonstrate one-for-one entrainment to the stimulus pulses. Direction sensitive neurons preferred high frequency stimulation, and showed decreased ON-direction responses as the temporal frequency declined, suggesting that slowly moving stimuli may be more vulnerable to inhibition than rapidly moving ones. These findings show that the 240 Hz rate of stimulation used clinically by the OPTACON may not be the most appropriate for obtaining good resolution of spatial detail by cortical neurons. They also demonstrate that dynamic tactile displays provide a useful means for comparing cortical responses to spatial patterns, applied at a variety of temporal frequencies, and at different receptive field locations. (Supported by USPHS Grants NS11862 and NS17973).